[SY01-1-1]

THE PROTEASOME: ASSEMBLY AND SPATIO-TEMPORAL DYNAMICS

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The proteasome is a sophisticated, 2.5-MDa, multisubunit complex that contains a catalytic core particle (CP: 14 subunits) and two terminal regulatory particles (RPs: 19 subunits); the RPs associate with the termini of the central CP at opposite orientations. One longstanding question is how the complex structure of the proteasome is organized with high fidelity. From more than 10 years of research, we proposed a novel assembly mechanism that is assisted by multiple proteasome-dedicated chaperones whose crystal structures have been determined. In addition, the proteasome is distributed diffusely in the cytoplasm of quiescent cells, but it is located predominantly in the nucleus of actively dividing cells, such as tumor cells. Intriguingly, a proteotoxic stress by amino acid-analog stimulates the proteasome accumulation to the aggresome known as aggregation of misfolded proteins and decline of ATP level causes the cytoplasmic granules of the proteasome referred as proteasome storage granules (PSGs) in the cell. Recently, we found that hyperosmotic stress induces the formation the nuclear foci of proteasomes within few seconds. The foci formation occurred in a reversible fashion and it exhibited liquid droplet-like behavior. We also observed rapid exchange of proteasomes and ubiquitin in the foci. The clearance of the foci required active proteasomes, p97 AAA-type ATPase chaperone and RAD23 shuttling factor (UBL-UBA protein), implying that this structure is a novel nuclear proteolytic center for adapting to nuclear stress. Based on aforementioned results, I discuss spatio-temporal dynamics of proteasomes in response to various environmental stresses.

Keyword: Proteasome, Ubiquitin, Proteolysis, Assembly

[SY01-1-2]

FORMYL-METHIONINE-TARGETING PROTEOLYTIC SYSTEM: THE FMET/N-END RULE PATHWAY

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Owing to the design of AUG genetic codon, protein biosynthesis begins with methionine (Met) in the cytosol of eukaryotes. In contrast, bacteria and eukaryotic organelles (mitochondria and chloroplasts), initiate the protein synthesis with formyl-methionine (fMet). Here we show, using the yeast Saccharomyces cerevisiae, that the formyltransferase Fmt1 of this eukaryote, while normally imported into mitochondria, can also mediate, in vivo, the synthesis of fMet-containing proteins by cytoplasmic ribosomes. The resulting fMet of eukaryotic proteins acts as as a specific protein degradation signal which can be targeted by the unprecedent polyubiquin-mediated and proteasome-dependent proteolytic system, termed the fMet/N-end rule pathway.

Keyword: formyl-methionine, proteolysis, ubiquitin, N-end rule, degron

[SY01-1-3]

PROTEASOMAL DEGRADATION OF POST-TRANSLATIONALLY MODIFIED TAU PROTEINS

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The proteasome is the converging point in the cell for thousands of ubiquitinated proteins and intrinsically disordered proteins that are generated from hundreds of distinct molecular pathways. Tau is one of disordred proteins degraded by proteasomes through ubiquitin-independent manners. Its intraneuronal aggregation collectively provides a nosological entity termed as tauopathies, notably including Alzheimer's disease (AD). Tau proteins undergoes a number of diverse posttranslational modifications (PTMs) and appear to be controlled by their complex crosstalks. It still remains to be elucidated which of the PTMs or their combinations have pro-aggregation or anti-aggregation properties. We have examined various consequences of tau PTMs including degradation through the ubiquitin-proteasome system. Some of our data about that enhancing proteasome activity was beneficial to delay accumulation and aggregation of tau will be presented. Our results may demonstrate that the combination of molecular events can comprise one pathologic signature event, and that targeting multiple components of the cascade could be a good strategy to alleviate the formation of pathological forms of tau.

Keyword: tau, post-translational modification, ubiquitin, proteasome, proteolysis

[SY01-1-4]

DYSREGULATED PHOSPHORYLATION OF RAB GTPASES BY LRRK2 INDUCES NEURODEGENERATION

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Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common cause of familial and sporadic Parkinson's disease (PD). Elevated kinase activity is associated with LRRK2 toxicity, but the substrates that mediate neurodegeneration remain poorly defined. Given the increasing evidence suggesting a role of LRRK2 in membrane and vesicle trafficking, here we systemically screened Rab GTPases, core regulators of vesicular dynamics, as potential substrates of LRRK2 and investigated the functional consequence of such phosphorylation in cells and in vivo. In vitro LRRK2 kinase assay with forty-five purified human Rab GTPases was performed to identify Rab family proteins as substrates of LRRK2. We identified the phosphorylation site by tandem mass-spectrometry and confirmed it by assessing phosphorylation in the in vitro LRRK2 kinase assay and in cells. Effects of Rab phosphorylation on neurodegeneration were examined in primary cultures and in vivo by intracranial injection of adeno-associated viral vectors (AAV) expressing wild-type or phosphomutants of Rab35.Our screening revealed that LRRK2 phosphorylated several Rab GTPases at a conserved threonine residue in the switch II region, and by using the kinase-inactive LRRK2-D1994A and the pathogenic LRRK2-G2019S along with Rab proteins in which the LRRK2 site was mutated, we verified that a subset of Rab proteins, including Rab35, were authentic substrates of LRRK2 both in vitro and in cells. We also showed that phosphorylation of Rab regulated GDP/GTP-binding property in cells. Moreover, in primary cortical neurons, mutation of the LRRK2 site in several Rabs caused neurotoxicity, which was most severely induced by phosphomutants of Rab35. Furthermore, intracranial injection of the AAV-Rab35 T72A or AAV-Rab35-T72D into the substantia nigra substantially induced degeneration of dopaminergic neurons in vivo.Here we show that a subset of Rab GTPases are authentic substrates of LRRK2 both in vitro and in cells. We also provide evidence that dysregulation of Rab phosphorylation in the LRRK2 site induces neurotoxicity in primary neurons and degeneration of dopaminergic neurons in vivo. Our study suggests that Rab GTPases might mediate LRRK2 toxicity in the progression of PD.

Keyword: LRRK2, Rab, phosphorylation, neurodegeneration, Parkinson's disease

[SY01-3-1]

MOUSE MODELS OF AUTISM RISK GENES FOR THERAPEUTIC DISCOVERY

Craig Powell[1]

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Mutations in synaptic genes and genes that regulate synaptic function are known to be causal for autism and intellectual disability. Our work over the past decade has aimed to elucidate pathophysiological mechanisms of autism using genetic models of autism and intellectual disabilities based on human genetic findings. I will discuss our translational work identifying novel therapeutic targets in mouse models of autism and initial efforts to bring these findings to patients with autism and intellectual disability in the clinic.

Keyword: synapse, neuron, brain, autism, genetics

[SY01-3-2]

ULTRASONIC VOCALIZATION DEFICITS IN RODENT MODELS FOR AUTISM

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Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental disorders, characterized by early-onset deficits in social behavior and communication across multiple contexts, together with restricted, repetitive patterns of behavior, interests, or activities. ASD is among the most heritable neuropsychiatric conditions with a heritability of 50-80%, and while available evidence points to a complex set of genetic factors, the SHANK gene family has emerged as one of the most promising candidates (e.g. Grabrucker et al., Trends Cell Biol, 2011). Therefore, the three SHANK genes (SHANK1, SHANK2, SHANK3) are prime targets for modeling ASD in mice and various genetic models were generated within the last few years (e.g. Jiang & Ehlers, Neuron, 2013). As the diagnostic criteria for ASD are purely behaviorally defined, the validity of mouse models for ASD strongly depends on their behavioral phenotype. Behavioral phenotyping is therefore a key component of the current translational approach and requires sensitive behavioral test paradigms with high relevance to each diagnostic symptom category (e.g. Silverman et al., Nat Rev Neurosci, 2010). While behavioral phenotyping assays for social deficits and repetitive patterns of behavior, interests, or activities are well-established, the development of sensitive behavioral test paradigms to assess communication deficits in mice is a daunting challenge. Measuring ultrasonic vocalizations (USV) appears to be a promising strategy. In the first part of my talk, I will provide an overview on the different types of USV in mice and will discuss available evidence in support of the notion that such USV serve important communicative functions, for instance as social contact calls. Briefly, three distinct types of mouse USV are typically differentiated, mainly on the basis of developmental stage and social context: (I) isolation-induced USV in pups, (II) interaction-induced USV in juveniles, and (III) and interaction-induced USV in adults, with emission rates and acoustic call features being strongly sex-dependent in adulthood (e.g. Wöhr, Neurosci Biobehav Rev, 2014). The second part of my talk will be devoted to studies on alterations in the emission of USV in Shank mouse models for ASD (e.g. Jaramillo et al., Autism Res, 2016) and I will discuss potential underlying factors, such as parvalbumin, a calcium binding protein expressed in inhibitory interneurons and strongly involved in the regulation of the excitation/ inhibition balance in the brain, repeatedly linked to ASD (e.g. Lee et al., Biol Psychiat, 2017).

Keyword: Ultrasonic vocalizations, Social Behavior, Autism, Shank, Parvalbumin

[SY01-3-3]

NMDAR DYSFUNCTION IN AUTISM SPECTRUM DISORDERS

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A large number of synaptic proteins have recently been associated with diverse neuropsychiatric disorders, including autism spectrum disorders (ASDs), schizophrenia, attention deficit hyperactivity disorder, and mood disorders. ASDs represent a group of neurodevelopmental disorders characterized by impaired social and communication deficits and restricted and repetitive interests and behaviors. Although many ASD-related genetic variations have been identified, only a small number of them have been verified for their causality by approaches including mouse genetics. NMDA receptor dysfunction is one of the emerging mechanisms thought to underlie ASDs. In this presentation, I will discuss how defects in some of the synaptic scaffolding molecules are associated with NMDA receptor dysfunctions and autistic-like behavioral abnormalities in mice.

Keyword: Synaptic, Autism Spectrum Disorder, NMDA receptor

[SY01-3-4]

MODEL SYSTEMS TO ANALYZE AUTISM SPECTRUM DISORDERS (ASDS)

Tobias Boeckers[1]

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MODEL SYSTEMS TO ANALYZE AUTISM SPECTRUM DISORDERS (ASDs) Author(s) : Tobias Boeckers Address : Ulm University, Institute of Anatomy and Cell Biology, Albert-Einstein-Allee 11, 89081 Ulm, Germany, tobias.boeckers@uni-ulm.de Shank proteins (also known as ProSAP) represent a family of postsynaptic scaffolding molecules (Shank1-3) that are thought to be involved in the regulation of excitatory synapses development, function and plasticity. These proteins are equipped with various domains for protein-protein interactions with many other synaptic proteins, including glutamate receptors, and other synaptic scaffolding and signaling molecules. Importantly, Shank has been implicated in diverse neuropsychiatric disorders, including Phelan-McDermid syndrome, autism spectrum disorders and schizophrenia. The mechanisms underlying these abnormalities collectively termed "Shankophaties". It has already been shown that ProSAP1/Shank2 knockouts mice and ProSAP2/Shank3 knockouts mice display autistic-like behavior including repetitive grooming behaviors, social interaction deficits, increased anxiety and hyperactivity. Moreover, the mutants exhibit imbalances of glutamatergic system. in search for pathomechanisms or treatment options for these neuropsychiatric diseases researchers rely on in vitro studies or animal models. Since several years now, the so-called "Yamanaka factors" can be used to reprogram cells to induced pluripotent stem cells (iPS). We established a system to generate iPS cells from patient hair keratinocytes to specifically analyze mutations leading to inherited form of autism. With respect to autism related mutations, we also found that iPS technology is a valuable tool to identify morphological alterations and developmental defects caused by autism gene mutations. Moreover, these cells can be used to test and screen new therapeutic agents in vitro. Overall, our studies show that iPS cell technology helps to define pathway alterations caused by disease specific mutations.

Keyword: Synapse, Shank, Autism, iPS

[SY01-4-1]

NRF2 AND TUMOR MICROENVIRONMENT

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Our body has an ability to sense environmental stress and induce cellular defense enzymes. NRF2 is a transcription factor essential for the coordinated induction of cellular defense enzymes and protection of tissues. Nrf2-null mice are sensitive to a wide variety of toxic electrophiles and reactive oxygen species (ROS). KEAP1 acts as a subunit of ubiquitin-E3 ligase that degrades NRF2 constitutively. KEAP1 also acts as a sensor for electrophilic and oxidative stresses. Covalent modifications of the cysteine residues of KEAP1 abrogate the ubiquitin ligase activity and stabilize NRF2, which leads to cellular NRF2 activation. KEAP1 acts as a floodgate to regulate NRF2 entry into the nucleus. This system has been referred to as the Cysteine Code. The two-site recognition and hinge-latch model has also been proposed for the KEAP1-NRF2 system. Disruption of the two-site recognition explains the mechanism how NRF2 accumulates in the nucleus escaping from the Cul3-Keap1 E3 ubiquitin ligase system. We have verified this model through structure biology, mouse genetics, thermodynamics and human disease analyses. NRF2 inducers are shown to be important for the treatment of stress-based diseases. NRF2 suppresses inflammations through repressing pro-inflammatory cytokine gene expression and also suppresses oxidative tissue damage through inducing a set of antioxidant enzyme genes. Meanwhile, many somatic missense mutations have been identified in KEAP1 and NRF2 genes of human cancers. These mutations disrupt the KEAP1-NRF2 complex and result in constitutive activation of NRF2. Subsequently, elevated expression of NRF2 target genes confers advantages on the growth of cancer cells through the metabolic reprogramming and induction of cellular defense enzymes. Thus, NRF2 inhibitor is now important for the chemo-sensitization therapy of cancers. On the other hand, NRF2 activity in the host microenvironment has also been shown to be important to repress cancer cell growth. In this sense, NRF2 inducers are also important for cancer treatment. The KEAP1-NRF2 system opens a new avenue to the understanding of regulatory processes underlying the stress response and cancer progression.

Keyword: KEAP1, NRF2, Cysteine code, somatic mutation

[SY01-4-2]

PTEN FAMILY IN CANCER AND BEYOND

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Nuclear PTEN is essential for the maintenance of chromosomal stability. PTEN contains an N-terminal phosphatase domain that dephosphorylates PIP3 and a C-terminal region with less defined function. We have generated a mouse model with heterozygous deletion of the Pten C-terminal domain of PTEN. The Pten mutant mice undergo genomic instability and develop spontaneous tumors. We also found that Pten C terminal disruption induces p53 and its downstream targets. Simultaneous depletion of p53 facilitates malignancy and promotes metastasis, suggesting that PTEN and p53 play different roles in suppression of tumor development. We have recently found that PTEN controls DNA replication progression through MCM2. PTEN also stabilizes replication forks through RPA1. We propose that PTEN is a regulator of DNA replication and protector of replication forks. Our data highlights a new mechanism by which PTEN maintains genomic stability and suppresses tumorigenesis. In our recent studies, we have revealed a mechanism of alternative protein translation, through which we identified a PTEN isoform with novel functions. A CUG codon upstream of and in-frame with the coding region of canonical PTEN initiates translation of an N-terminally extended form of PTEN, which we have designated PTENα. We found that eukaryotic translation initiation factor 2A (eIF2A) controls PTENα synthesis and a CUG-centered palindromic motif is required in this process. PTENα induces cytochrome c oxidase activity and ATP production in mitochondria. Deletion of PTENα impairs mitochondrial respiratory chain function. Our data provide insights into the mechanism by which the PTEN family is involved in multiple cellular processes. These results suggest that mammalian cells can use alternate translation initiation mechanisms to produce isoforms of protein with distinct functions.

Keyword: PTEN, genomic stability, DNA replication, RPA1, PTENα

[SY01-4-3]

ROLE OF CANCER-ASSOCIATED FIBROBLASTS IN PROGRESSION OF BREAST CANCER

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Cancer-associated fibroblasts (CAFs) constitute a major compartment of the tumor microenvironment. CAFs produce a variety of cytokines, growth factors and extracellular matrix proteins, thereby stimulating tumor progression. CAFs are distinct from normal fibroblasts for their overexpression of α-smooth muscle actin and fibroblast specific protein-1. Recent studies suggest that CAFs play an important role in proliferation and migration of cancer cells through crosstalk with them. In the present study, we investigated the role of CAFs on breast cancer progression and underlying molecular mechanisms. When MDA-MB-231 cells were treated with the conditioned medium (CM) collected from cultured CAFs, the cell viability and migration were significantly elevated. Furthermore, these cells became transformed into a more proliferative phenotype, exhibiting enhanced mRNA expression of cycin-D1, c-Myc and PCNA as well as increased phosphorylation of Akt and STAT3. In addition, MDA-MB-231 cells exhibited elevated expression of proliferative and invasive genes including MMP2 and MMP9 when with CAFs by using an indirect co-culture system. Notably, mRNA levels of fibroblast growth factor2 (FGF2), SDF1, IL-6 and IL-8 detected in CAFs were higher than those in normal fibroblasts of same patients. In contrast, FGF2 was expressed at a relatively low level in breast cancer cells. Therefore, (add comma) we focused on FGF2-FGF receptor 1 (FGFR1) signaling in the context of communication between breast cancer cells and CAFs. CAF-induced cell migration was abolished in the presence of FGF2-neutralizing antibody by using CAF-CM as well as an indirect co-culture system, and CAF-promoted FGFR1 expression was also reduced under the same conditions. In addition, treatment of MDA-MB-231 cells with FGF2 induced the phosphorylation of FRS2 and Akt. FGF2-induced cell migration and up-regulation of cyclin-D1 expression were abrogated by silencing of FGFR1. Furthermore, FGF2 promotes nuclear localization of FGFR1. Taken together, above findings suggest that secretion of FGF2 by CAFs interacts with FGFR1 of the breast cancer cells, thereby stimulating their proliferation and migration of breast cancer cells.

Keyword: Tumor microenvironment, Cancer associated fibroblasts, FGF2, FGFR1, Breast cancer

[SY01-4-4]

EXPLORING NOVEL CANCER-RELATED MICRORNAS AND THEIR DIAGNOSTIC AND THERAPEUTIC POTENTIALS IN PERSONALIZED CANCER MEDICINE (PCM)

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MicroRNAs (miRs) are endogenous small non-coding RNAs that regulate gene expression by interfering with translation and/or stability of target transcripts. In cancer cells, down-regulation of tumor-suppressor (TS)-miRs has been shown to be associated with cell proliferation, epithelial and mesenchymal transition (EMT), invasion/metastasis, and chemo-resistance. In order to explore novel TS-miRs for the development of miR-based cancer therapy, we conducted function-based screening using miR-libraries in cancer cell lines, and identified more than 20 novel TS-miRs in various cancers. Among those, miR-634 activates the apoptotic pathway by directly concurrent targeting of genes associated with mitochondrial homeostasis, anti-apoptosis, antioxidant ability, and autophagy. The enforced expression of miR-634 remarkably enhanced chemotherapy-induced cytotoxicity in cancer cells in vitro and in vivo, suggesting that concurrent miR-634–mediated modulation of cytoprotective mechanisms may be useful for cancer therapy. More recently, we identified miR-3140, which directly suppresses BRD4 as well as CDK2 and EGFR. BRD4 mediates transcriptional elongation of the oncogene MYC and plays a critical role in tumorigenesis in various cancers including NUT midline carcinoma (NMC). NMC is a rare and aggressive tumor typically driven by a t(15;19) rearrangement leading to the BRD4-NUT fusion gene. miR-3140 also downregulates the BRD4-NUT chimeric oncoprotein directly by binding to its coding sequences (CDS) in NMC cells. Several studies have shown that small compounds of BET bromodomain inhibitors (BETis) such as JQ1 are highly effective against various cancers, including triple negative breast cancer, pancreatic cancer, and NMC, and many clinical trials using BETi have been started, while the acquired resistance to BETi has emerged as a serious problem so far. Our identified TS-miR-3140 may be a promising candidate for the development of miR-based cancer therapy in intractable tumors including NMC and overcome resistance to BETi due to the direct repression of BRD4. Taken together, these findings provide novel insights into the application of miR-based therapeutics in precision cancer medicine (PCM).

Keyword: cancer, miR-634, NRF2, miR-3140, BRD4

[SY01-5-1]

THREE-DIMENSIONAL IMAGE ANALYSIS REVEALS CYTOSKELETAL DYNAMICS IN CELL-FREE SYSTEMS

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Understanding how complex shape emerges in vivo remains one of the major problems in developmental biology. Here, we describe two approaches to advance our understanding of shape formation at both cellular and tissue scales. First, we use three-dimensional Particle Image Velocimetry (PIV), single-cell tracking and rapid in vivo imaging to quantitatively dissect the formation of the Zebrafish somite. We demonstrate that tissue-tissue interactions are essential in shaping the somite to its final, distinctive, chevron morphology. Integrating this data within a vertex model, we provide a detailed analysis of how coordinated cell rearrangements and tissue-tissue interactions work synergistically to drive complex shape formation. Second, we look to understand how polarity emerges in cells utilising a cell-free system. Although the protein networks underlying polarity are highly conserved and found in all metazoans, it is still not clear how they establish cell polarity and how the spatiotemporal information they provide organises the cell. Using specially microfabricated chambers, we are able to extract material from both oocytes and embryos for live imaging. We are able to visualise, for example, microtubule interactions within Drosophila at very high spatial-temporal resolution. We then apply many of the above image analysis techniques to these extracts to quantitatively explore the underlying dynamics. Overall, by utilising the latest imaging, image analysis and microfabrication technologies, we are gaining deeper insight into how complex shape emerges during development on both cellular and tissue scales.

Keyword: PIV, Morphology, Modeling, Drosophila, Polarity

[SY01-5-2]

REGULATION OF ERROR-PRONE DNA DAMAGE REPAIR PATHWAYS IN BACTERIA

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DNA damage is a threat to genome integrity. Cells in all domains of life are able to repair damaged DNA efficiently by employing a combination of high and low fidelity pathways. While repair is essential for cell viability, some pathways are also potent sources for mutagenesis and can impact adaptive evolution as well as antibiotic and stress resistance. Furthermore, these repair pathways are not universally conserved, raising the possibility that survivability under stress may vary across bacterial species. The prevalence of error-prone repair pathways and their mechanisms of regulation in bacteria remain poorly understood and is the current focus of our research. In this talk, I will outline our recent work on understanding the conservation and regulation of two error-prone bacterial repair systems: non-homologous end joining and translesional-synthesis repair.

Keyword: DNA damage, Bacteria, Microscopy

[SY01-5-3]

OPTOGENETIC RECONSTITUTION REVEALS CORE FUNCTIONAL MODULES AND ARCHITECTURE OF THE CORTICAL FORCE-GENERATING MACHINERY DURING MITOSIS

Tomomi Kiyomitsu[1]

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For my PhD and HFSP fellowship studies, I examined the kinetochore and cortical force-generating machinery, respectively, both of which interact with microtubule plus-ends during mitosis to generate forces. For my career development award (CDA) projects, I have continued to study the cortical machinery and found that the cortical force-generating machinery appears to develop several molecular and structural features analogous to the kinetochore. By changing research subjects, I might be able to find general mechanisms of force generation at the dynamic plus-end of microtubules. I am grateful to have received the HFSP fellowship and CDA grant. Here, I present the most exciting findings from my CDA projects.

Keyword: Mitosis, Spindle positioning, Cortical force generator

[SY01-5-4]

CHEMICAL, GENETIC AND OPTICAL APPROACHES TO THE DECONSTRUCTION OF NEURAL CIRCUITRY

Sung-Yon Kim[1]

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Mammalian brains are staggeringly complex, with many billions of neurons forming an intricate neural network. A key question in neuroscience is how this neural network produces functional outcomes, such as cognition, emotion and bodily responses. To tackle this question, functional circuit mapping approaches attempt to map the entire anatomical connectivity ('connectome') and to identify the function of each neural circuit element ('functional connectome'). Recent breakthroughs in neuro-technologies, such as optogenetics and CLARITY, are increasingly enabling these challenging investigations. In the first part of my talk, I will illustrate how optogenetics can be used to dissect the anxiety circuitry in the extended amygdala. We identified a surprising new role for the bed nucleus of the stria terminalis (BNST) in the coordinated modulation of diverse anxiety features, and clarified the underlying circuit mechanisms for selection of features for the assembly of the anxious state. In the second part, I will discuss the promises and challenges of the chemical engineering-based CLARITY approach to neuroscience and introduce two novel techniques (termed stochastic electrotransport and SWITCH) that we have have developed to achieve easy, scalable and conservative clearing and rapid, uniform and complete staining of intact tissues, respectively. My goal is to combine cutting-edge systems neuroscience tools to comprehensively understand the circuitry underlying various emotional states, such as anxiety, fear and stress, at all structural, molecular and functional levels.

Keyword: Neural Circuitry, Tissue Processing, Optogenetics, CLARITY, Homeostasis

[SY02-1-1]

PAN-CANCER ANALYSIS OF CANCER GENOME REVEALED GENOMIC AND PROTEOMIC LANDSCAPE ASSOCIATED WITH RESPONSE TO IMMUNOTHERAPY

Jong Hwa Bhak[1]

[1]UNIST, Korea

CSCG (close species comparative genomics) is an approach to find causative highly influencing genetic variations for specific functions in biological species in a long term evolution. In comparing genomes, it is critical to select the best genetic distances among the genomes to detect certain causative geno-phenotypes associations. For examples, to detect the exact causative genetic mutation for white tigers, it is necessary to compare very closely related normal and white tiger genomes with a clear phenotypical difference. Another example is to detect the causative mutation for deep diving whales, it is necessary to select a proper background genomes which are from non-diving species. Causative mutations are thought to be naturally selected after randomized variations to be transmitted by the individuals to the next generations. Are causative mutations occur completely randomly and selected by natural means of selection? There is a possibility that individuals have possibly facilitated or predisposed to certain environment first and directed mutations that suitably fit occurred afterwards in the propagation of life. Darwinian natural selection is a generalized descriptive theory for the propagation of life. Recently, there are evidence of Lamarckian inheritance in terms of epigenomics. Evolution theory has been changing since the time of Darwin. I speculate that there are causative mutations that are directed by the functional and behavioural advancement before random variations. Such individuals could have actively sought out to select themselves to the environment. This thought-experimental speculation of directed and computed active mechanism encompassing an infinite number of ways of incorporating information to the genome and other omes is termed covolution.

Keyword: genomics, evolution, covolution

[SY02-1-2]

THE TRANSCRIPTION UNIT ARCHITECTURES IN BACTERIAL GENOMES

Byung-Kwan Cho[1]

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Over the past decade or so, dramatic developments in our ability to experimentally determine the contents and functions of genomes have taken place. In particular, high-throughput sequencing technologies are now inspiring a new understanding of the bacterial genome on a global scale. Bacterial genomes are organized by structural and functional elements, including promoters, transcription start and termination sites, open reading frames, regulatory noncoding regions, untranslated regions and transcription units. Thus, identification of those genomic elements is prerequisite for understanding the complete regulatory network of a bacterial cell. Here, we show the architecture of bacterial genomes and their gene expression patterns at the transcriptional and translational levels along with different developmental phases, by integrating genome-scale data of transcription start sites (TSSs), transcription termination sites, mRNA abundance and ribosome-protected mRNA fragment (RPF) abundance. For instance, Streptomyces strains produce various secondary metabolites including antibiotics, and many of these are used in medical and agricultural fields. The production of these compounds occurs after passing through the transition from primary metabolism to secondary metabolism, and involves complex morphological differentiation. Despite the significant attributions of Streptomyces to biotechnological area, their complex organizational structures and gene regulation mechanisms are not fully discovered. Now, we offer comprehensive information on genome architecture, and transcriptional and translational gene expression patterns that allows better understanding of bacterial genomes.

Keyword: Next-generation sequencing, RNA-seq, Ribo-seq, Term-seq, Transcription unit

[SY02-1-3]

PAN-CANCER ANALYSIS OF CANCER GENOME REVEALED GENOMIC AND PROTEOMIC LANDSCAPE ASSOCIATED WITH RESPONSE TO IMMUNOTHERAPY

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Immunotherapy has emerged as a promising anti-cancer treatment, however, little is known about the genetic characteristics that dictate response to immunotherapy. We develop a transcriptional predictor of immunotherapy response and assess its prediction in genomic data from ~10,000 human tissues across 30 different cancer types to estimate the potential response to immunotherapy. The integrative analysis reveals two distinct tumor types: the mutator type is positively associated with potential response to immunotherapy, whereas the chromosome-instable type is negatively associated with it. We identify somatic mutations and copy number alterations significantly associated with potential response to immunotherapy, in particular treatment with anti-CTLA-4 antibody. Our findings suggest that tumors may evolve through two different paths that would lead to marked differences in immunotherapy response as well as different strategies for evading immune surveillance. Our analysis provides resources to facilitate the discovery of predictive biomarkers for immunotherapy that could be tested in clinical trials. Furthermore, subset analysis of gastrointestinal cancer revealed potential mechanism for resistant to immunotherapy

Keyword: TCGA, immunotherapy, pan-cancer, cancer genome, predictive marker

[SY02-1-4]

BIG DATA RESOURCES AT BEIJING INSTITUTE OF GENOMICS AND THE BHBD INITIATIVE

Zhang Zhang[1], Wenming Zhao[1], Jingfa Xiao[1] and Yiming Bao[1]

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Genome data are increasing dramatically as the result of new technologies. Sometimes, these data are not shared with the scientific communities because of the lack of centralized databases in China and other developing countries. Other times, data are required to be deposited into international databases such as NCBI, in order to obtain accession numbers needed for publication. This could be challenging for researchers in China and other countries because of the following reasons: 1. large data size; 2. slow data transfer due to limited international internet bandwidth; 3. language barrier and technical issues in communication. To alleviate these problems, several resources/databases were established at the BIG Data Center (BIGD, http://bigd.big.ac.cn), Beijing Institute of Genomics (BIG), Chinese Academy of Sciences. One of such databases, the Genome Sequence Archive (GSA), is a data repository for archiving raw sequence reads, equivalent to SRA at NCBI. It supports data generated from a variety of sequencing platforms and provides data storing and sharing services free of charge for worldwide scientific communities. In addition to raw sequencing data, GSA also accommodates secondary analyzed files in acceptable formats. It has user-friendly web interfaces for easy data submission and provides technical support in both English and Chinese, which therefore will greatly promote genome data sharing among the scientific communities. The accession numbers provided by GSA are accepted by many journals. In addition, BIGD also hosts other resources, such as Gene Expression Nebulas, a data portal of gene expression profiles based entirely on RNA-Seq data; Genome Variation Map, a comprehensive collection of genome variations for featured species; Genome Warehouse, a centralized resource housing genome-scale data with particular focus on economically important animals and plants; Methylation Bank, an integrated database of whole-genome single-base resolution methylomes; and Science Wikis, a central access point for biological wikis developed for community annotations. In response to the appeal "Open Data in a Big Data World" by the International Council for Science, Dr. Yongbiao Xue, director of BIG, recently proposed an initiative of Open Biodiversity and Health Big Data (BHBD) to the International Union of Biological Sciences (IUBS), of which he is an executive member. This proposal was approved by IUBS. Global sharing of BHBD is able to advance scientific research and promote the fair distribution of benefits throughout the world, which yet cannot be accomplished without engagement of the entire global communities on the following three issues: - To build the principles and mechanisms for global sharing of

BHBD in accordance with laws and ethics of member countries. - To develop a big data platform for BHBD integration, translation and sharing that is publicly accessible to worldwide communities. - To promote the level of participation and influence of IUBS in global biological research. The BHBD initiative (http://bhbd-alliance.org/) will be built based on BIGD resources. Taken together, BIGD is dedicated to providing freely accessible data repositories and a variety of data resources in support of worldwide research activities. We welcome data submissions and comments/suggestions to our resources.

Keyword: database, GSA, BHBD, BIGD

[SY02-2-1]

ROLE OF MG53 IN CARDIOMETABOLIC DISEASES

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Metabolic syndrome featured by a cluster of disorders, including hypertension, hyperglycemia, dyslipidemia and central obesity, is caused by nutrient overload and physical inactivity. Metabolic syndrome increases the incidence of cardiovascular disease and type 2 diabetes (T2D), and a combination of these conditions is the leading cause of death around the world. Insulin resistance is the central pathogenic factor shared by these metabolic disorders. However, the mechanism underlying insulin resistance is not fully understood. Recently, we have identified mitsugumin 53 (MG53 or TRIM72), a striated muscle-specific E3 liagse, is a principal mechanism underlying insulin resistance and metabolic disorders, together with their cardiovascular complications. Specifically, MG53 is universally upregulated in multiple animal models and humans with insulin resistance and metabolic disorders; and overexpression of MG53 leads to severe systemic insulin resistance and full-blown metabolic syndrome. Importantly, in mg53 TG mice, skeletal muscle insulin resistance occurs before the onset of obesity and the impairment of insulin signaling in non-muscle tissues such as liver and fat. In contrast, genetic ablation of MG53 protects mice against high-fat diet-induced insulin resistance and metabolic disorders. On mechanism, MG53 acts as a novel E3 ligase to meditate the ubiquitin-dependent degradation of insulin receptor (IR) and insulin receptor substrate 1 (IRS1) in both cardiac and skeletal muscle in the setting of insulin resistance and metabolic syndrome, affording a long-sought mechanism underlying metabolic disease-associated downregulation of IR and IRS1. MG53 is also responsible for the lipid metabolic disorders in the heart through the dysregulation of PPAR- . These findings not only define MG53 as a powerful regulator of insulin sensitivity, but also establish MG53-mediated skeletal muscle insulin resistance as a central mechanism underlying defective insulin response and whole-body metabolic disorders, marking MG53 E3 ligase as an important therapeutic target for the treatment of diverse metabolic diseases and associated cardiovascular complications.

Keyword: MG53, lk

[SY02-2-2]

INVESTIGATING THE REGULATION OF ENERGY METABOLISM BY AMPK IN VIVO

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AMP-activated protein kinase (AMPK) plays a major role in metabolic regulation. AMPK responds to changes in intracellular adenine nucleotide levels, and is activated by an increase in AMP/ADP relative to ATP. In addition, AMPK is regulated in response to increased levels of calcium, and recent work has shown that changes in the level of fructose 1,6, bisphosphate plays an important role in regulating AMPK activity. Activation of AMPK increases the rate of catabolic (ATP-generating) pathways and decreases the rate of anabolic (ATP-utilising) pathways. In addition to its role in maintaining intracellular energy balance, AMPK regulates whole body energy metabolism. Given its key role in controlling energy homeostasis, AMPK has attracted widespread interest as a potential therapeutic target for metabolic diseases. We recently solved the structure of AMPK in complex with a small molecule activator, revealing important insights into the mechanism of activation of AMPK. We have generated a transgenic mouse model that allows us to express AMPK harbouring a gain-of-function mutation. Using this model, we have begun to examine the effect of AMPK activation on metabolic pathways in vivo. The use of this new model offers us the opportunity to explore the physiological role of AMPK.

Keyword: AMPK, METABOLISM, OBESITY, SIGNALLING, PROTEIN KINASE

[SY02-2-3]

ROLE OF AMP DEAMINASE IN DIABETIC CARDIOMYOPATHY

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Diabetes mellitus promotes coronary atherosclerosis and also induces changes in the myocardium per se, leading to contractile dysfunction of the heart referred to as diabetic cardiomyopathy. Multiple changes in energy metabolisms and intracellular Ca2+ regulatory mechanisms by diabetes mellitus, which depends on the phase of diabetic cardiomyopathy, induce diastolic dysfunction with or without systolic dysfunction of the left ventricle. By analysis of myocardial metabolomes, we found 2.5-fold increased AMP deaminase (AMPD) activity in a rat model of type 2 diabetes, and diastolic ventricular dysfunction upon increased ventricular afterload was correlated with reduction of tissue ATP and the adenine nucleotide pool. The protein level of 90-kDa full-length AMPD3 was increased in whole myocardial lysates and in SR fraction by 55% and 123%, respectively, in diabetic hearts, while AMPD3 mRNA levels and ubiquitination of AMPD3 were similar in diabetic and nondiabetic hearts. MicroRNA array analysis revealed downregulation (>50%) of 57 microRNAs in diabetic hearts compared to those in non-diabetic controls, among which miR-301b was predicted to interact with the 3′UTR of AMPD3 mRNA. AMPD3 protein level was significantly increased by miR-301b inhibitor and was decreased by a miR-301b mimetic in H9c2 cells. A luciferase reporter assay confirmed binding of miR-301b to the 3′UTR of AMPD3 mRNA. The findings indicate that AMPD upregulation by miR-301b-mediated transcriptional regulation plays a crucial role in diabetic cardiomyopathy.

Keyword: diabetes mellitus, heart failure, microRNA, AMP deaminase, ATP

[SY02-2-4]

GLOBAL PROTEOMICS OF SKELETAL MUSCLE AND VALIDATION LEAD TO IDENTIFICATION OF PGC-1A AND FATTY ACID METABOLISM DOWNSTREAM TO EXCHANGE PROTEIN DIRECTLY ACTIVATED BY CAMP (EPAC) IN FORCED TREADMILL EXERCISE CAPACITY

Wai-Kin So[1], Hyoung Kyu Kim[2], Yingxian Chen[1], Seung Hun Jeong[2], Patrick Ka Kit Yeung[1], Billy C.K. Chow[1], Jin Han[2] and Sookja K. Chung[1]

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Exchange protein directly activated by cAMP (Epac) mediates cAMP-mediated cell signal independent of protein kinase A (PKA). Previously, mice lacking Epac1, but not Epac2 deficient mice, displayed systemic metabolic defect, including the glucose homeostasis, which suggested a possible involvement of skeletal muscle. Firstly, we performed a global systemic proteomics and pathway bioinformatics analyses of the skeletal muscle of wild type (WT, Epac1+/+; Epac2+/+), Epac1-deficient (Epac1-/-), Epac2-deficient (Epac2-/-), and double knockout of Epac1 and Epac2 (Epac1-/-; Epac2-/-) mice. In addition, we have tested their forced treadmill exercise tolerance. In extensor digitorum longus and soleus muscles, Epac1, but not Epac2, was detected by real-time PCR and Western blot analyses. Moreover, Epac1-/- mice exhibited significantly reduced work done in the forced treadmill exercise with a shorter running distance before exhaustion and lower number of type 1 fibers compared to those of Epac1+/+ mice. Forced treadmill exercise-enhanced insulin sensitivity and glucose uptake were impaired as evidenced by the altered AMPK activation and GLUT4 expression in Epac1-/- muscle. The significantly reduced expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) in soleus muscle of Epac1-/- mice without or with exercise stress suggested the alternation of oxidative metabolism. Furthermore, expressions of genes involved in uptake and oxidation of fatty acids, ERRα and PPARδ and fatty acid content were lower in muscles lacking Epac1, suggesting a role of Epac1 in forced treadmill exercise capacity by regulating PGC-1α pathway and lipid metabolism in skeletal muscle. Taken together, Epac1 plays an important role in forced treadmill exercise capacity by regulating PGC-1α and fatty acid metabolism in addition to its role in insulin sensitivity and glucose uptake in the skeletal muscle.

Keyword: Epac, protein kinase A, exercise, skeletal muscle, PGC-1α

[SY02-2-5]

MITOCHONDRIAL METABOLIC REGULATION BY REACTIVE SULFIDE SPECIES

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Mitochondria are dynamic organelles that continuously undergo fission and fusion, which are necessary for maintaining bioenergetic homeostasis in cells. Mitochondrial fission and fusion cycle is precisely regulated by three GTP-binding proteins, dynamin-related protein 1 (Drp1), mitofusins (Mfn1 and mfn2) and optic atrophy 1 (Opa1), and these three G proteins have redox-sensitive cysteine (Cys) residues. Especially, mitochondria predominantly show tubular form in adult cardiomyocytes and are reported to be fragmented by exposure to electrophilic chemical substances. We found that electrophilic modification of Cys-624 on Drp1, caused by endogenous or exogenous electrophiles, increased Drp1 GTPase activity as well as cardiac vulnerability to mechanical stress in rodent hearts. In contrast, reactive sulfide species such as Cys persulfides that are produced in cells are likely involved in electrophile metabolism. Protein Cys persulfide detection assay revealed that endogenous Drp1 abundantly formed Cys persulfide in rat cardiomyocytes, and exposure to electrophiles such as methylmercury (MeHg) reduced Drp1 persulfide level. Supplementation of sulfur to Cys-624 by exogenous treatment with NaHS as a sulfur substrate for 24 hours completely abolished electrophile-mediated sulfur deprivation of Drp1 protein as well as exacerbation of cardiac cell injury induced by mechanical stretch. These results strongly suggest that formation of Cys persulfide on Drp1 proteins play a key role in mitochondrial quality control and bioenergetics by negatively regulating Drp1 activity.

Keyword: mitochondrial quality control, redox signaling, GTP-binding protein, reactive persulfide species, heart failure

[SY02-3-1]

TARGET IDENTIFICATION AND VALIDATION OF NATURAL PRODUCTS WITH LABEL-FREE METHODS

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Identifying protein targets of natural products and deciphering the specific mechanisms-of-action at the molecular level are crucial steps in the development of drugs to treat human diseases. A number of target protein identification methodologies including conventional affinity chromatography using labeled natural products as well as recent target identification methods with label-free natural products such as Drug Affinity Responsive Target Stability (DARTS), Cellular Thermal Shift Assay (CETSA), and Thermal Proteome Profiling (TPP) have been developed to identify the direct binding proteins of natural products. LC-MS/MS has also been used to screen and identify candidate protein targets. Here, we propose a new method combining DARTS with liquid chromatography/tandem mass spectrometry (DARTS and LC-MS/MS) to resolve the labor-related drawbacks of traditional affinity-based methods. In addition, recent advances for target identification of natural products towards functional and translational applications will be presented by introducing our case studies of "drug-target" identification and validation of FK506 and other clinical drugs.

Keyword: Target identification, DARTS, LC-MS/MS, Target validation, drug-target

[SY02-3-2]

OPTO-BIOANALYSIS: IMAGING AND CONTROLLING GPCR ACTIVITIES IN LIVING CELLS

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A current trend of bioanalysis is to deeply understand life phenomena and practically apply the knowledge to medical and pharmaceutical fields. To analyze the biological events in living cells, technologies related to fluorescence and bioluminescence imaging have been advancing rapidly in the past decade. Remarkable advancement has been achieved in the monitoring of bioactive small molecules, protein conformational changes, protein localization and dynamics, and protein-protein interactions in real-time at the level of single living cells and organisms. I herein focus on a novel design of engineered luciferases for the analysis of GPCR activities in living cells; the principle is based on complementation and reconstitution of the split-luciferase fragments when they are brought sufficiently close together. I will show the results of direct monitoring of GPCR-β-arrestin interactions in living cells. In addition, an optogenetics approach was used for the analysis of GPCR-β-arrestin interactions, in which we found a unique role of β-arrestin in GPCR trafficking: Temporally controlling the interaction between β-arrestin and GPCR reveals that the duration time of the GPCR-β-arrestin interaction determines the trafficking pathway inside the cells. I will also focus on the screening of chemical ligraries for discovering a specific GPCR inhibitors using the above technologies.

[SY02-3-3]

MOLECULAR ENGINEERING OF AN ENZYME DRUG: IMPROVING CATALYTIC ACTIVITY OF L-ASPARAGINASES USED IN THERAPY OF ACUTE LYMPHOBLASTIC LEUKEMIA

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The therapeutic effect of the clinically used enzyme drug L-asparaginase (L-ASNase) relies on the fact that cancerous cells of acute lymphoblastic leukemia (ALL) depend on the supply of the free amino acid L-asparagine (L-Asn) from the blood stream. Serum depletion of L-Asn selectively blocks protein biosynthesis and induces apoptosis of leukemic cells. The two FDA-approved enzymes, which are of bacterial origin, display nocuous side effects mainly attributed to immunogenicity and hypersensitivity reactions. This talk highlights the design and molecular evolution of human L-asparaginases aiming to improve their catalytic efficiency such that they could replace the clinically approved bacterial enzymes. A genetic selection system has been developed to identify variants of enhanced activity, and a high-throughput droplet-based microfluidic platform has been generated for miniaturization of kinetic assays that can be performed using purified enzymes or single bacterial cells. A three-step coupled-enzyme fluorescence assay allows for measuring ASNase activity on the natural substrate Asn in water-in-oil droplets of nL to pL reaction volumes, at throughput rates of up to 104 per sec. Moreover, biocompatible nanomaterials serving as microcarriers for enzyme molecules have been fabricated to enhance protein stability and extend serum half-life.

Keyword: Enzymde drug, Leukemia, L-asparaginases, Enzyme engineering, Nanoparticles

[SY02-3-4]

APPLICATION OF IFRET FOR PROTEIN DETECTION AND DRUG SCREENING

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Most fluorescence detection is accompanied with inevitable labeling process of target molecules, which may change the biological nature of the targets. Intrinsic fluorescence resonance energy transfer (iFRET) is also a FRET based on non-radiative energy transfer from fluorescence donor to acceptor. Since, however, iFRET utilizes the intrinsic fluorescence of Trp residues in (unmodified) target proteins as fluorescence donor it has been utilized to detect target proteins in a label-free manner by designing target-specific iFRET probes which can receive FRET energy from Trp. We have successfully detected native streptavidin, caspase-3 and PTP1B in vitro as well as streptavidin in cells using home-built biological UV microscope. Recently we have developed an iFRET model peptide which can monitor metal ion-induced amyloid β peptide aggregation. The probe showed increased fluorescence upon addition of divalent metal ion implying a conformational change of amyloid β peptide. It was expected from previous structural analysis that showed a close proximity between iFRET donor and acceptor, which locate C- and N-termini, respectively, upon metal coordination of the peptide. After 3 hours incubation, aggregation was detected by TEM image and the aggregates showed a time-dependent growth. Using this probe, natural product library were screened for inhibition of amyloid β peptide aggregation. The hit compounds selected in the screening revealed a common structural features and protection of amyloid β-induced neuronal cell death.

Keyword: Protein detection, FRET, drug screening, amyloid β, Intrinsic fluorescence

[SY02-3-5]

CHEMICAL APPROACH TO MANIPULATE THE PROTEIN DEGRADATION MACHINERY FOR HUMAN HEALTH

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Protein quality control through the ubiquitin-proteasome system has been increasingly recognized in recent years. Aberrant protein species and undesirable protein aggregates, if not cleared properly, compromise protein homeostasis and ultimately culminate in devastating diseases, which are collectively called as 'proteopathies'. Especially, the 26S proteasome is increasingly recognized as a versatile player for fine-tuning the substrate degradation for maintaining protein homeostasis. Also, deubiquitinating enzymes, numbering over a hundred in human proteome, exclusively reverse the ubiquitination, and thereby potentially regulate protein turnover. We recently developed potent small-molecule inhibitors selectively targeting USP14, a major deubiquitinating enzyme on the proteasome. USP14 inhibitors can remarkably enhance the proteasome activity for potential therapeutic use, and also can serve as specific tools to discover new deubiquitination biology on the proteasome. Our group primarily investigate critical deubiquitination machinery that are implicated in human pathophysiology. We plan to develop functional and chemical strategies to modulate deubiquitination reactions and thereby define them as novel regulators in maintaining protein homeostasis. This research will provide the fundamental basis for understanding cellular proteomic balance in normal and various disease states, and will also establish new druggable targets for human health.

Keyword: Proteostasis, Protein quality control, Proteasome, Deubiquitinating enzyme, Small-molecule inhibitor

[SY02-4-1]

PROTEOMICS FOR MOLECULAR MEDICINE

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High resolution mass spectrometry has already become an essential modality for discovery studies in biomedical research. Targeted approaches are being increasingly applied for rapid, high-throughput and quantitative measurements of both modified and unmodified peptides in a variety of settings. However, the application of these proteomic technologies to a clinical setting has lagged far behind. I will discuss examples where we have initiated the use of discovery and targeted studies to harness the power of mass spectrometry and apply it in a clinical setting. Current methods applied in Clinical Microbiology laboratories for microbial genus and species identification include culture, biochemical methods, 16S rRNA sequencing, and MALDI-TOF-based fingerprinting. By employing high resolution tandem mass spectrometry, we have achieved rapid diagnosis of a variety of bacteria including Mycobacterium tuberculosis, which takes about two months for culture. Direct identification of Mycobacterium tuberculosis from clinical specimens such as CSF and sputum using proteomics has not been described previously. We have also used mass spectrometry for confirming a diagnosis of malaria from urine samples demonstrating the versatility of proteomic platforms. Finally, I will discuss how mass spectrometry can be used to identify protein deposits in amyloidosis from formalin fixed paraffin-embedded (FFPE) sections used in pathology laboratories for histologic analysis. Targeted approaches can be adapted in all of these scenarios to further increase the sensitivity, throughput and robustness, which are all essential in clinical laboratories.

Keyword: Clinical, Diagnostics, Microbiology

[SY02-4-2]

PANCREATIC CANCER PROTEOMICS: FROM TUMORIGENESIS TO EARLY DIAGNOSIS

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Our presentation on the pancreatic cancer (PC) will be divided into two sections; tumorigenesis mechanism and early diagnosis. First, we will address a question of how post-translational modifications of tumor suppressors can induce abnormal cell growth. In this work, we report an identification of site-specific O-GlcNAcylation as a critical block of FOXO3 that may abrogate a part of the p53 pathway, resulting in aberrant pancreatic cancer cell growth. We found that changes in O-GlcNAcylation at Ser284 modulated p21-mediated cancer cell growth. Overexpression of either O-GlcNAcylated FOXO3 (FOX-OV) or a Ser-to-Ala mutant (S284A) in PANC-1 cells indicated that S284 O-GlcNAc acts as a critical block of the FOXO tumor suppressor and induces proliferation in PANC-1 cancer cells by stimulating the MDM2-p53-p21 axis. Furthermore, S284A mutant cells lacking S284 O-GlcNAc and FOX-OV cells exhibited opposing MDM2-p53-p21 axis expression patterns at both the mRNA and protein levels. Thus, our study provides evidence that pancreatic cells can be induced by aberrant O-GlcNAcylation at S284 of FOXO3. Second, we will explore further mechanism of action of the previously identified complement factor B (CFB) as a candidate serologic biomarker for PC. When used together with cancer antigen 19-9 (called ComB-CAN kit), CFB exhibited 90% sensitivity and 98% specificity for early-stage (I/II) PC diagnosis versus healthy donors (HD) and pancreatitis in 145 specimens. A gene ontology analysis of proteogenomic datasets from CFB-knockdown PANC1 (shCFB) versus shControl (mock) cells indicated roles for CFB in cell migration and invasion, which were supported by distinct wound patterns. Molecular assays indicated the suppression of epithelial–mesenchymal transition-related genes in shCFB cells, supporting a potential oncogenic role of CFB, and the exosomal levels of CFB were much higher in plasma from PC patients relative to HD. These combined results suggest a potential oncogenic function for CFB that might underlie its role as an early diagnostic marker of PC. (This work was supported by grants from the Korean Ministry of Health and Welfare- HI13C2098 and HI16C0257 to Y.-K.P., and JW Bioscience-Yonsei Cooperative Research Fund to Y.-K.P).

Keyword: PANCREATIC CANCER, FOXO3, BIOMARKER, PROTEOMICS, EARLY DIAGNOSIS

[SY02-4-3]

SYSTEMS GLYCOBIOLOGY IN DISEASE AND N-GLYCAN BRANCHING

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Our group has been interested in glycosyltransferases such as GnT-III, GnT-IV, GnT-Va, Vb (GnT-IX), Fut8 and St6Gal1 and sysems glycobiology approach discovered several target proteins on which these enzymatic products carry (1). Core fucose, a product of α 1, 6 fucosyltransferase (Fut8), is a cancer biomarker target (1) and is also implicated in COPD (chronic obstructive lung disease), a progressive and inflammatory airway disease due to cigarette smoking. We found that a keratan sulfate disaccharide designated L4 ([SO3--6]Galβ1-4[SO3--6] GlcNAc) showed protective effects in two murine COPD models. L4 attenuated alveolar destruction, reduced neutrophil influx and inflammatory cytokines, inactivated matrix metalloproteinase and myeloperoxidase in bronchoalveolar lavage fluid (2). A receptor for L4 was found to be C-type lectin, langerin. Bisecting GlcNAc, a GnT-III product was high in Alzheimer's disease patients (2). Analysis of knockout mice of GnT-III revealed that decreased cleavage of APP (Aβ-precursor protein) by BACE1 (β-site amyloid precursor protein cleaving enzyme-1) as well as decreased Aβ plaque. The lack of this modification directs BACE1 to late endosomes/lysosomes where it is less co-localized with APP, leading to accelerated lysosomal degradation. β-Galactoside α2,6-sialyltransferase-1 (St6gal1) was most downregulated in VATs (visceral adipose tissues) from obese mice and differentiated adipocyte model 3T3-L1 cells. Integrin-β1 was identified as one of the target proteins of St6gal 1 in adipose tissues, and phosphorylation of focal adhesion kinase (FAK) was decreased after HFD (high fat diet) feeding. St6gal1 overexpression in differentiating 3T3-L1 cells inhibited adipogenesis with increased FAK phosphorylation while KO mice showed enhanced adipogenesis. The downregulation of St6gal1 during adipogenesis was canceled by a DNA methyltransferase inhibitor, suggesting an involvement of epigenetic DNA methylation in St6gal1 silencing (4). In conclusion new findings of various glycosyltransferase functions will open a new avenue toward novel and promising druggable candidates. References: 1) Taniguchi N., Kizuka Y. Ad Cancer Res. 2015; 126:11-51. 2) Gao C., et al. Am J f Physiol Lung Cell Mol Physiol. 2017; 312(2):L268-L276. 3) Kizuka Y., et al. EMBO Mol Med. 2015 Jan 15;7(2):175-89 4) Kaburagi T., et al., J. Biol Chem. 2017, 292(6):2278-2286.

Keyword: Systems glycobiology, N-glycan, COPD, Alzheimer7s disease, Obesity

[SY02-5-1]

STRUCTURAL AND MECHANISTIC INVESTIGATION OF THE HUMAN GLUCOSE TRANSPORTERS GLUTS

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Glucose is the primary fuel to life on earth. Cellular uptake of glucose is a fundamental process for metabolism, growth, and homeostasis. The major facilitator superfamily glucose transporters GLUTs, exemplified by human GLUT1-4, are prototypes in the study of solute transport. We were able to determine the atomic structures of human GLUT1 and GLUT3 in multiple conformations during a transport cycle, which reveal the molecular basis for ligand recognition and transport. The 1.5 angstrom structure of GLUT3 in complex with glucose reveals the molecular details of substrate coordination. The crystal structure of human GLUT1 at 3.2 angstrom resolution in the inward-open conformation allows accurate mapping and potential mechanistic understanding of disease-associated mutations in GLUT1. Comparison of the GLUT structures in the outward-open, outward-occluded, and inward-open states provides insights into the alternating access cycle for GLUTs, whereby the C domain provides the primary substrate binding site and the amino terminal domain undergoes rigid-body rotation with respect to C domain. We also determined the crystal structure of XylE, an E. coli homologue of GLUT1-4. Whereas GLUT1-4 are facilitative uniporters, XylE is a proton-driven symporter. Structural comparison and biochemical analysis of GLUTs and XylE allow examination of transport mechanisms by passive facilitators versus active transporters.

[SY02-5-2]

CRYO-EM STRUCTURE OF BOVINE MITOCHONDRIAL RESPIRASOME

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The conversion of energy from nutrients into ATP is mainly carried out by the oxidative phosphorylation system (OXPHOS) within the inner mitochondrial membrane. The mammalian OXPHOS is composed of five large respiratory complexes - NADH-ubiquinone oxidoreductase (complex I, CI), succinate-ubiquinone oxidoreductase (complex II, CII), ubiquinol-cytochrome c oxidoreductase (complex III, CIII; also known as the cytochrome bc1 complex), cytochrome c oxidase (complex IV, CIV) and ATP synthase (complex V, CV). Four respiratory complexes (CI-CIV) facilitate the electron transfer reaction as well as the generation of a proton gradient across the inner membrane that is utilized to generate ATP by CV. These large respiratory complexes can be further organized into several stoichiometric supercomplexes, amongst which the respirasome containing CI, CIII dimer, and CV (CICIII2CIV) is the most prominent supercomplex. Here, we purified the respirasome from bovine heart mitochondria and determined its structure by cryo-electron microscopy (cryo-EM) at 4.16 Å resolution. Sub-region refinement after masked 3D classification of CI and CIII can further improve the resolution of their density map in which we were able to build atomic models of entire CI and CIII subunits, revealing two distinct, "open" and "closed" conformation of CI. Furthermore, we could identify four different conformations by further 3D classification and refinement of the entire respirasome, suggesting rotations around the pivot between CI and CIII2 in the inner mitochondrial membrane would be implicated in the efficient electron transfer processes. Collectively, our respirasome structures provide insights into the electron transport from NADH to cytochrome c in cellular respiration.

Keyword: Protein Structure, Cryo-EM, Mitochondrial Respirasome

[SY02-5-3]

INTEGRATIVE STRUCTURAL INVESTIGATION ON MACROMOLECULAR PROTEIN COMPLEXES

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Proteins in cells form multi-subunit protein complexes. It is imperative to investigate the structures of the complexes as a whole to understand molecular mechanisms of the complex. However, it has been challenging to investigate large protein complexes. Here, I have investigated large protein complexes including histone nuclear importin complex and Huntington's disease protein through an integrative structural approach including X-ray crystallography, electron microscopy, small X-ray scattering and integrative modeling. I will discuss the strategy to investigate large protein complexes through an integrative structural approach.

Keyword: structures, chromatin, Neurodegeneration, X-ray crystallography, cryoEM

[SY02-5-4]

COMPLEX STRUCTURE OF RNA POLYMERASE AND TFE REVEALS TFE BINDING OPENS CLAMP AND STALK DOMAINS DURING TRANSCRIPTION INITIATION

Sung-Hoon Jun[1], Jaekyung Hyun[1], Hyeongseop Jeong[1], Jeongseok Cha[2], Hoyoung Kim[2], Katsuhiko Murakami[3] and Hyun-Soo Cho[2]

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The archaeal transcription apparatus is closely related to the eukaryotic RNA polymerase II (Pol II) system. Archaeal RNA polymerase (RNAP) and Pol II evolved from a common ancestral structure and the euryarchaeal RNAP is the simplest member of the extant archaeal–eukaryotic RNAP family. In this presentation, the cryo-EM structure of the complex between euryarchaeal RNAP from Thermococcus kodakarensis and a transcription initiation factor E (TFE) at 3.8 Å resolution would be introduced. Structural comparison of RNAP-TFE and apo-RNAP shows that binding of TFE shifts the conformation of the clamp and stalk domains from a closed state to an open one by 12.6° and 3.4°, respectively. The cryo-EM structure of RNAP-TFE-promoter DNA at 3.7 Å resolution shows the structural change of TFE when the clamp and stalk are in the closed state. Together, this study reveals a structural dynamics of TFE and suggests a mechanical details of the TFE function during transcription initiation.

Keyword: RNA polymerase, transcription factor E, transcription initiation, Cryo-EM

[SY02-5-5]

SEPTIN FILAMENTS AND THE ASSEMBLY OF A MOLECULAR JIGSAW

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Septins are GTP-binding proteins involved in membrane remodelling events and diffusion barrier formation. 13 human septins form heteropolimeric filaments based on core particles composed of either six or eight monomers. The most widely studied hexameric core particle is that composed of septins 2, 6 and 7 whilst the octamer includes also SEPT9. The order of the septins along the filament is well established but much remains unknown about how the filament spontaneously forms both in vivo and in vitro. Most of our recent work has focused in two areas related to filament formation. 1) attempts to characterize the hexameric core particle using electron microscopy and employing alternative combinations of septins according to Kinoshita´s rule. We show that what has been considered the canonical arrangement of the core particle is, in fact, inside-out. 2) understanding the nature of the septin-septin interfaces necessary for stability. For this purpose we have probed the different interfaces with a wide variety of biophysical techniques including NMR, CD, SPR and protein crystallography. Recently, we have solved structures of the GDP and GTP-analog complexes for all of the members of a particular septin sub-group including SEPT3, SEPT9 and SEPT12, which are believed to occupy the terminal positions of octameric core particles. In the case of SEPT9, two monomers are observed to be squeezed together at the interface when bound to the GTP analog but drawn apart when in the presence of GDP. This squeezing is predicted to result in liberating a polybasic helix which is normally observed to be tucked into the space between the two subunits. Based on this model we predict that GTP binding and hydrolysis are important events leading to the association and disassociation of the heterofilament with the membrane, a dynamic process which may be fundamental to biological activity. This work was supported by CNPq (#550514/2011-2, 303204/2011-7) and FAPESP (2016/04658-9, 2012/00268-0, 2014/15546-1).

Keyword: septins, cytoskeleton, filamentous proteins, GTPases

[SY03-1-1]

HIF-2A REGULATION OF INFLAMMATORY ARTHRITIS

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Rheumatoid arthritis (RA) is a systemic autoimmune disorder that manifests as chronic inflammation and joint tissue destruction. However, the etiology and pathogenesis of RA have not been fully elucidated. Here, we explored the role of the hypoxia-inducible factors (HIFs), HIF-1α (encoded by HIF1A) and HIF-2α (encoded by EPAS1). HIF-2α was markedly up-regulated in the intimal lining of RA synovium, whereas HIF-1α was detected in a few cells in the sublining and deep layer of RA synovium. Overexpression of HIF-2α in joint tissues caused an RA-like phenotype, whereas HIF-1α did not affect joint architecture. Moreover, a HIF-2α deficiency in mice blunted the development of experimental RA. HIF-2α was expressed mainly in fibroblast-like synoviocytes (FLS) of RA synovium and regulated their proliferation, expression of RANKL (receptor activator of nuclear factor–κB ligand) and various catabolic factors, and osteoclastogenic potential. Moreover, HIF-2α–dependent up-regulation of interleukin (IL)-6 in FLS stimulated differentiation of TH17 cells—crucial effectors of RA pathogenesis. Additionally, in the absence of IL-6 (Il6−/− mice), overexpression of HIF-2α in joint tissues did not cause an RA phenotype. Thus, our results collectively suggest that HIF-2α plays a pivotal role in the pathogenesis of RA by regulating FLS functions, independent of HIF-1α.

Keyword: arthritis, rheumatoid arthritis, HIF-2a, inflammatory disease

[SY03-1-2]

MECHANISMS CONTROLLING INNATE IMMUNE RESPONSES TO NUCLEIC ACIDS

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Toll-like receptor 7 (TLR7) and TLR8 are endosomal sensors for viral single-stranded RNA (ssRNA) and induce innate immune responses. These TLRs also responds to synthetic small molecules such as R848 and Imiquimod. However, it remains unclear how TLR7 and TLR8 sense these two distinct ligands. Two ligand-binding sites are revealed by the structure of TLR7 and TLR8: the first site of TLR7 binds to small chemical ligands, a guanosine (G) or deoxyguanosine (dG), whereas the second site binds to uridine-containing oligoribonucleotides (U-ORN). On the other hand, TLR8 binds to uridine (U) and purine-containing ORN. We have found that TLR7 and TLR8 are synergistically activated by nucleosides and ssRNA. With ssRNA, G/dG activates TLR7 and induces cytokine production in macrophages, cDCs and pDCs. These results strongly suggest that TLR7 and TLR8 recognize nucleosides as well as ssRNA. This talk focuses on the mechanism controlling nucleoside-sensing by TLR7 and TLR8 in monocytes.

Keyword: Toll-like receptor, nucleoside, RNA, monocyte

[SY03-1-3]

CHITINASE REGULATION OF ALLERGIC INFLAMMATION AND LUNG FIBROSIS

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The GH18 gene family contain true chitinases that bind and cleave chitin (C) and chitinase-like proteins (CLPs) that bind but do not cleave the chitin polysaccharide. These C/CLPs found across species from lower life forms (archea, prokaryotes, eukaryotes) to man and dysregulated expression has been noted in a number of human diseases. However, the nature of their contributions has been enigmatic because chitin is the only documented substrate of chitinases, and chitin and chitin synthase do not exist in mammals. Mammals encode two functional chitinases, chitotriosidase (Chit1, chitinase 1) and acidic mammalian chitinase (AMCase). In recent years, there is mounting evidence to suggest that mammalian chitinases play a key role in mediating the Th2 cell-driven inflammatory responses. The genetic variation in the Chit1 gene was associated with atopy frequency and atopic biomarkers, including blood eosinophils, serum IgE, and eosinophil cationic protein (ECP), that further support a signficant role of Chit1 in allergic diseases. In addition, recent animal experiments have demonstrated significant changes in Chit1 expression at sites of inflammation, remodeling and fibrosis. To understand the exact in vivo function of Chit1 in these diseases, we developed Chit1 transgenic (Chit1 Tg) and null mutant mice (Chit1-/-) and investigated its role in allergic inflammation and lung fibrosis. These studies identified that Chit1 inhibits allergic inflammation by regulation of regulatory T cell (Treg) differentiation via enhancing TGF-β signaling. Chit1 also significantly increases the collagen accumulation in the lung via interacting with TGF-β. Our studies further revealed that TGF-β receptor associated protein 1 (Tgfbrap1) and Foxo3 are the major interacting partners of Chit1 that modulates TGF-β-stimulated Smads2/3 signaling and Smad7 expression, respectively. These studies identified a novel regulatory role of Chit1 in the development and progression of allergic and fibrotic lung diseases and its potential use as a biomarker and therapeutic target of the diseases in that TGF-β plays a significant role.

[SY03-1-4]

MITOCHONDRIAL REGULATION OF THE STING SIGNALING PATHWAY

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Mitochondria are highly dynamic organelles that constantly move within cells and alter their morphology and numbers. The morphology of mitochondria is determined by the balance between fusion and fission. These so-called mitochondrial dynamics are coupled to the metabolic function as well as reactive oxygen species (ROS) production, mitophagy, and various cellular signaling. Recently, evidence is emerging that mitochondrial dynamics and ROS play important roles in the regulation of innate immune signaling including MAVS-mediated viral RNA sensing and NLRP3-mediated inflammasome pathway. However, the role of mitochondrial dynamics in the stimulator of interferon genes (STING)-mediated DNA sensing pathway has not been investigated. Here, I'll present data showing the role of mitochondria in regulating the STING pathway activity. We found that deficiency of mitofusin 1 (MFN1), an effector molecule for mitochondrial fusion, suppressed the activation of the STING pathway, resulting in the decreased induction of interferon (IFN)-β and its target gene, ISG56, in conjunction with diminished activation of the signaling molecules downstream of STING, TBK1 and IRF3. These results highlight the crucial role of MFN1 for maintaining the competency of the STING pathway. Previous study reported that the protonophore carbonyl cyanide 3-chlorophenylhydrazone (CCCP) suppressed STING-mediated IFN-β production via disrupting mitochondrial membrane potential (MMP). However, how MMP dissipation causes the suppression of the STING pathway remains unknown. We found that that CCCP inhibits activation of STING and its downstream signaling molecules, TBK1 and IRF3, but not STING translocation to the perinuclear region. CCCP impairs the interaction between STING and TBK1 and concomitantly triggers mitochondria fission. Importantly, the knockout of the crucial mitochondria fission regulator DRP1 restored the STING activity, indicating that CCCP down-modulates the STING pathway through DRP1-mediated mitochondria fragmentation. Finally, we found that NLRP3-activating agonists, ATP and nigericin, caused mitochondria fragmentation and inhibited the STING pathway. However, the suppression of the STING pathway and mitochondria fission induced by nigericin was not dependent on NLRP3 inflammasome activation and its key element, potassium efflux, and mediated in a DRP1-independent manner. In summary, we demonstrated that mitochondria dynamics play a critical role for the competency of STING pathway and can be targeted by inflammasome activating stimuli. Our findings will help understanding of the regulatory mechanism for STING activation and the role of mitochondria

dynamics in the context of innate signaling pathway.

Keyword: Innate signaling, STING, Mitochondria, Inflammasome

[SY03-1-5]

APPLYING GENOMICS TO IMMUNO-COMPROMISED RARE DISEASE PATIENTS FOR PRECISION MEDICINE

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Rare disease patients often get little attention in research due to the small number of patients and strong genetic etiology frequently precludes readily available treatment options. However, recent genomics-driven dissection of patient genome allows geneticists to identify pathogenic variants in unprecedented scale and efficiency. In the presentation, I will introduce two instances where we were able to pinpoint genetic cause of two idiopathic immune defective patients, study their pathogenesis mechanisms, and successful application of treatment options based on the genetic discoveries.

Keyword: Whole exome sequencing, autoimmune, hyperinflammatory, rare disease

[SY03-2-1]

EXPLORING THE INTERACTION BETWEEN DROSOPHILA MELANOGASTER AND ITS NATURAL TRYPANOSOMATID HERPETOMONAS MUSCARUM

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Dipteran insects transmit serious infections to humans, often in the form of trypanosomatid parasites. To accelerate research in more difficult contexts of dipteran-parasite relationships such as tsetse-trypanosomes and sand fly-Leishmania, we have focused on the interaction of the model dipteran Drosophila melanogaster and its natural trypanosomatid Herpetomonas muscarum, the genome of which we have sequenced. Transcriptomics analysis of whole flies implicated reactive oxygen species, the Toll receptor, JAK-STAT, Imd as well as targets of the NF-κB homologue Relish in the fly response and several genes conserved in the response of trypanosomes to tsetse from the Herpetomonas perspective. Tissue-specific knock down of key components of these pathways in enterocytes (ECs), intestinal stem cells (ISCs) and the fat body influenced initial numbers, infection dynamics and time of parasite clearance. Proliferation of ISCs was triggered by the parasite and loss of Relish in those cells suppressed proliferation, while loss of Toll in ECs influenced enterocyte numbers. Both Relish in ISCs and Toll in ECs resulted in increased Herpetomonas uptake and delayed clearance. These networks of signalling from both the side of Drosophila as well as the side of Herpetomonas, imply that an evolutionary conserved mechanism for how dipterans respond to kinetoplastids may exist.

Keyword: Drosophila, Herpetomonas, Innate Immunity, Trypanosomatids

[SY03-2-2]

COMMENSALS OF TWO KINDS; PROMOTER OR ELIMINATOR OF VIBRIO CHOLERAE INFECTION

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Indigenous microbes inside the host intestine maintain a complex self-regulating community. The mechanisms by which gut microbes interact with intestinal pathogens remain largely unknown. Here, we identified a commensal Escherichia coli strain whose expansion predisposes mice to infection by Vibrio cholerae, a human pathogen. We refer to this strain as 'atypical' E. coli (atEc) because of its inability to ferment lactose. The atEc strain is extremely resistant to reactive oxygen species (ROS) and proliferates extensively during antibiotic treatments. Intestinal ROS levels are decreased in atEc-transplanted mice, favoring proliferation of ROS-sensitive V. cholerae. An atEc mutant defective in ROS degradation fails to facilitate V. cholerae infection when pre-transplanted. A species of genus Bacteroides, otherwise a dominant gut microbe, was completely eliminated upon treatment with clindamycin (CL), an antibiotic that specifically kills anaerobes. CL-treated and therefore Bacteroides-depleted mice developed cholera-like symptoms when infected with V. cholerae. Furthermore, cultivated Bacteroides cells killed V. cholerae in vitro, demonstrating antagonistic relationship between the Bacteroides sp. and V. cholerae. Together, our results suggest that enteric infection is an event that occurs depending on the composition of intestinal microbiota and the Bacteroides sp. has potential to be developed as an anti-cholera intervention.

Keyword: Microbiota, Enteric infection, Microbiome, Reactive oxygen species

[SY03-2-3]

MODULATION OF HOST IMMUNITY BY TARGETING MICROBIOME

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The intestinal microbiota may play a critical role in the modulation of human health. Dysregulation of the commensal flora, in both diversity and composition, is intimately linked to functional changes of the immune system and subsequently contributes to development of immune disorders. Although probiotics are considered as prophylactic or therapeutic modalities to restore homeostasis of the gut microbiota, efficacy of probiotics is strain specific with great variations within the same species. To identify regulatory T cell (Treg)-inducing probiotic strains, we have screened several hundred strains of bacteria. Among the several hundred strains screened, Bifidobacterium bifidum PRI1 (Bb PRI1) was selected based on its IL-10highIL-12low inducing capabilities in mixed mesenteric lymphocyte cultures. Mono-association of Bb PRI1 significantly enhanced the generation of induced CD4+Foxp3+ Helioslow Treg (iTreg) cells in a DC-dependent manner. These iTreg cells express enhanced levels of CTLA4 and IL-10 and exhibit activated Treg cell phenotypes. Cell surface polysacchaccharides (CSP) of Bb PRI1 were identified as key components for Treg induction. CSP efficiently recapitulated the activity of whole bacteria and acted via regulatory dendritic cells. Treg cells induced by Bb PRI1 or purified CSP display stable and robust suppressive capacity towards experimental colitis. Collectively our study establishes CSP as a novel functional component of Treg-inducing bacteria, underscoring potential applicability of CSP-producing probiotics as modulators of diverse hyper-immune disorders. These findings provide new insights into the molecular mechanisms by which iTreg-inducing probiotics-host interaction could establish immunological homeostasis in the gut. This study was supported from the Institute for Basic Science (IBS; IBS-R005), Republic of Korea.

Keyword: Microbiome, Immunity, Probiotics, Effector molecule, Mechanism of action

[SY03-2-4]

REGULATION OF METABOLISM AND SYSTEMIC PHYSIOLOGY IN DROSOPHILA

Carl Thummel[1]

[1]Univ of Utah, USA

An alarming rise in the incidence of diabetes and obesity over the past few decades has refocused research efforts on the regulation of metabolism and the causes of metabolic disorders such as diabetes and obesity. The Thummel lab is studying the basic molecular mechanisms by which metabolism is controlled using the wide range of genetic tools available in Drosophila. We seek to uncover fundamental aspects of metabolic regulation that are conserved through evolution with the aim of preventing and curing human disease. There are two major lines of study underway in the Thummel lab: (1) the transcriptional regulation of metabolism by nuclear receptors and (2) functions for evolutionarily conserved mitochondrial proteins. Nuclear receptors are a family of ligand-regulated transcription factors that play central roles in growth, development, and metabolism. The Drosophila genome encodes 18 nuclear receptors, compared to 48 in humans and 284 in C. elegans, providing the smallest complete set of these factors in any genetic model system. Our current research is focused on three nuclear receptors that play central roles in metabolism: dHNF4, E78, and dERR. We anticipate that, as with past studies in Drosophila nuclear receptors, discoveries stemming from our work will improve our design of mouse models for future functional studies and provide new directions for combating critical human diseases associated with nuclear receptor dysfunction, including cardiovascular disease, diabetes, and obesity. Mitochondria are dynamic and complex organelles that play a central role in all aspects of biology, including energy production, intermediary metabolism, and apoptosis. Remarkably, in spite of many efforts to define the mitochondrial proteome, one-fifth of these proteins remain largely uncharacterized. Our lab is studying the functions of key evolutionarily conserved mitochondrial proteins in close collaboration with Jared Rutter's lab in the Biochemistry Department. Much of our current work is focused on functional studies of the Mitochondrial Pyruvate Carrier (MPC) that we discovered several years ago. Current work is focused on roles for the MPC in stem cell proliferation, cell growth, and cancer. This research is supported by the NIH (R01 DK075607, R01 DK108941).

Keyword: metabolism, Drosophila, mitochondria

[SY03-3-1]

ELECTROCHEMICAL DETECTION OF PATHOGEN USING FERROCENYLNAPHTHALENE DIIMIDE

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Detection of viruses and bacteria is important to prevent an epidemic or a pandemic. This has been carried out by PCR using target-specific primers, followed by gel electrophoresis of PCR products. Although this method is a standard practice, tedious work such as the preparation of gels, electrophoresis, and stain to visualize DNA bands on the gel is needed. Detection may be facilitated considerably by omitting these manipulations. To attain this goal, we attempt to develop a new gel electrophoresis-free detection method for PCR products, in which ferrocenylnaphthalene diimides (FND) coupled with naphthalene diimides having dithiolane (NDIss) as a molecular staple of double stranded DNA1 are used (Figure 1A). The principle of this method is illustrated in Figure 1B. NDIss is the ligand enabling immobilization of double stranded DNA on the gold electrode. Double stranded PCR products will be immobilized on the electrode after treatment with NDIss through its dithiolane units bound to double stranded DNA. Since FND is concentrated on the double stranded region of DNA and generates an electrochemical signal, the electrode will give rise to an electrochemical response in an electrolyte containing FND. Only the PCR product is double stranded in this system and is best suited for this method. We tested this notion by comparing it with gel electrophoresis with a PCR product originating from a norovirus. The sensitivity of this methods is 100 times higher than that of gel electrophoresis. Although the method awaits sophistication, the observations made above are encouraging enough for us to pursue a practical way to a simple and rapid detection of PCR products. I appreciate the significant contribution made by researchers who appear the following paper.

Keyword: norovirus, pandemic, electrochemical detection, ferrocenylnaphthalene diimides (FND), molecular staple

[SY03-3-2]

HOST CELL MIMIC POLYMERSOME FOR RAPID DIAGNOSIS AND DISTINGUISH OF HIGH/LOW PATHOGENIC INFLUENZA VIRUSES BY USING VIRAL FUSION AND CELL ENTRY MECHANISM

Seungjoo Haam[1]

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Highly pathogenic avian influenza virus (HPAIV) infections have epidemically and continuously occurred to poultries and even crossed the species barrier to humans, leading to fatalities. Currently, PCR-based molecular test is the most sensitive diagnostic tool for HPAIV; however, it requires analysis in centralized diagnosis systems by a trained individual leading to delays in quarantine and isolation. In this study, we have developed a differential avian influenza virus (AIV) rapid detection method and evaluated it in vitro and using clinical specimens from HPAIV H5N1-infected animals. We demonstrated that our rapid test method reveals highly sensitive and specific detection of HPAIV/LPAIV and can be thus a useful field diagnostic tool for HPAIV outbreaks as well as for rapid quarantine control of the disease.

Keyword: Polymersome, Influenza virus, Rapid diagnosis

[SY03-3-3]

ENGINEERED PROTEIN ASSEMBLIES TO UTILIZE BIOMOLECULAR MULTIVALENCY IN BIOSENSING

Yongwon Jung[1]

[1]KAIST, Korea

Multivalency is a key principle in nature for many biological processes such as cell-cell communications, phase separation, and even simple DNA hybridization. For example, multivalent interactions between cells or with other organisms (bacteria and viruses) are governed by multiple ligand-receptor interactions on cell surfaces. Simple (and often weak) individual biomolecular interactions can be highly strengthened and diversified by employing multivalency. To study and employ multivalent bio-interactions, however, multivalent scaffold architectures that can display multivalent biomolecules in a well-defined manner must be developed. Here I will introduce several new strategies to fabricate large protein assemblies, which can be valuable assets to utilize multivalent protein interactions. In particular, modifications and applications of fluorescent proteins, avidin proteins, and cage proteins with highly interesting binding properties will be discussed. Several examples of how newly fabricated protein assemblies can be applied in bioanalytical applications will also be discussed.

Keyword: biosensor, protein engineer, multivalency

[SY03-3-4]

MICROFLUIDIC CHIP-BASED DETECTION OF PATHOGENS

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Microfluidics represent is a great platform for detecting pathogens. We demonstrate that microfluidics can improve the performance of both immunoassays and nucleic acid assays to allow better means for pathogen detection. These improvements are driven by miniaturization and proper surface chemistry. Our method is straightforward and well accommodates conventional assays such as ELISA and PCRwith improved throughput, sensitivity and stability. Combined with nanoparticles and nanomaterials, microfluidics show great promise in developing novel sensing platforms and realizing point-of-care detection of pathogens with unprecedented speed. These detection platforms also allow biosensors to be used for screening for therapeutics, e.g., nanocarriers for introducing siRNA, CRISPR/Cas, and so forth.

Keyword: MICROFLUIDICS, NANOMATERIALS, BIOCHEMICAL ANALYSIS

[SY03-4-1]

SELECTIVE AUTOPHAGY: FIGHTING DISEASE ONE PROTEIN AT A TIME

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Cells count on surveillance systems to handle protein alterations and organelle damage. Malfunctioning of these systems contribute in large extend to the abnormal accumulation of those altered components in cells and tissues in numerous diseases and in aging. Autophagy is an essential cellular process that contributes to protein quality control along with chaperones and other proteolytic pathways. Of the different types of autophagy that co-exist in mammalian cells, dysfunction of two of them, macroautophagy and chaperone-mediated autophagy, has been shown to result in major alterations of proteostasis, one of the hallmarks of aging. The better molecular characterization of the different autophagic pathways has considerably advanced our understanding of their physiological role. In addition, alterations in autophagic pathways have been linked to the pathogenesis of detrimental human pathologies, such as cancer, neurodegenerative and metabolic diseases. Chaperone-mediated autophagy (CMA) activity decreases with age and in different human age-related diseases such as neurodegenerative and metabolic disorders. We have recently developed a series of mouse models with systemic or tissue-specific blockage of CMA to gain a better understanding of the contribution of reduced CMA to the phenotype of aging. Analysis of these models has revealed that added to the previously known role of CMA as part of the cellular response to stress, this type of autophagy is also required in the regulation of important cellular processes such as metabolism of lipids and carbohydrates, cell cycle, cell reprograming and cellular differentiation. In this talk, I will describe how phenotypic characterization of these mice is allowing us to link CMA deficiency with different age related diseases.

Keyword: autophagy, lysosomes, aging, neurodegeneration, diabetes

[SY03-4-2]

MITOCHONDRIAL FUNDC1 REGULATES SELECTIVE MITOPHAGY AND PROTEOSTATIC STRESS RESPONSE

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Mitophagy, a selective process that removes damaged or unwanted mitochondria, is critical for the maintenance of mitochondrial quality and quantity. Previously, we have revealed that FUNDC1, a mitochondrial outer-membrane protein, functions as a mitophagy receptor to mediate hypoxia-induced mitophagy. FUNDC1 harbors an LC-3 –interacting region (LIR) and interacts with LC-3 to mediate mitophagy both in cultured cell systems and in (patho-)physiological settings. We revealed that the reversible phosphorylation of FUNDC1 modulates its affinity with LC-3 for subsequent mitophagy. In an effort to understand the role of p62 in FUNDC1 mediated mitophagy, we found that FUNDC1 interacts with HSC70, a multifunctional cellular chaperone, to promote delivery of cytosolic client substrates via the TOM/TIM complexes to the mitochondrial matrix where some of them are degraded by the mitochondrial LONP1 protease. Accumulation of misfolded proteins in mitochondria promotes the formation of mitochondrion-associated protein aggregates (MAPAs), which are degraded by the FUNDC1-medaited mitophagy machinery. Our results reveal that mitochondria organize the complementary autophagic degradation of the proteasomal clients, and have significant implications for understanding aging and aging-related diseases.

Keyword: mitochondrial, mitophagy, FUNDC1

[SY03-4-3]

AUTOPHAGY ENHANCER AS A NOVEL THERAPEUTIC AGENT AGAINST METABOLIC SYNDROME AND DIABETES

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Autophagy is a critical regulator of cellular homeostasis dysregulation of which is associated with diverse diseases. We screened a chemical library from Korea Research Institute of Chemical Technology for novel autophagy enhancers employing a Renilla luciferase-LC3 reporter construct. An autophagy enhancer (MSL) increased LC3-I to -II conversion without mTOR inhibition. Autophagy enhancer activated calcineurin through direct binding, and induced dephosphorylation/nuclear translocation of transcription factor EB (Tfeb), a master regulator of lysosomal biogenesis and autophagy gene expression. Autophagy enhancer accelerated intracellular lipid clearance, which was reversed by lalistat 2 or Tfeb knockout. Autophagy enhancer also attenuated IL-1β release and caspase-1 cleavage after treatment of macrophages with palmitic acid plus LPS, indicating reduced inflammasome activation. Further, autophagy enhancer reduced expression of TNF-α, IL-6 and pro-IL-1βα mRNA, which was independent of inflammasome activation. Decreases in cytokine mRNA expression were due to attenuated NF-κB activation by autophagy enhancer, which in turn resulted from enhanced calcineurin activity. Autophagy enhancer administration improved metabolic profile of ob/ob mice and ameliorated inflammasome activation, which was accompanied by induction of Tfeb target genes such as lysosomal genes, autophagy genes and mitochondrial genes. A chemically-modified autophagy enhancer with increased microsomal stability (MSL-7) improved glucose profile not only in ob/ob mice but also in mice with diet-induced obesity. Our data indicate that our novel autophagy enhancers could be new drug candidates for diabetes or metabolic syndrome with lipid overload.

[SY03-4-4]

A ROLE OF FKBP8 IN THE MITOCHONDRIAL FRAGMENTATION AND MITOPHAGY

Seung-Min Yoo[1], Shun-Ichi Yamashita[2], Hyun Joo Kim[1], Tomotake Kanki[2] and Yong-Keun Jung[1]

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Mitochondrial quality control is important to miantain healthy mitochondria and operates mitochondrial dynamics to replace mitochondrial elements necessary for maintaining normal function and selectively degrades damaged mitochondria via mitophagy under stress. Despite a great advance in understanding mitochondrial quality control, critical mediators of mitophagy and a coordination of mitochondrial fission event and mitophagy needs to be elucidated. Here we show that FKBP8 plays an essential role in the mitochondrial fission and stress-mediated mitophagy. FKBP8 was identified from functional screening with cell-based assays targeting GFP-LC3B to the mitochondria by the enforced expression of 500 cDNAs encoding mitochondrial proteins. Among members of the FKBP family, FKBP8 only affected mitochondrial morphology. Knockdown of FKBP8 expression generated enlarged and tubular forms of mitochondria. Conversely, ectopic expression of FKBP8 produced smaller and fragmented mitochondria. FKBP8-mediated mitochondrial fragmentation occurred in Drp1 knock out cells but did not further aggravate mitochondrial fragmentation in Opa1 knockout cells. N-terminal 93WLDI96 motif of FKBP8 was critical for the mitochondrial fragmentation and the binding of FKBP8 to Opa1. Interestingly, the fragmented mitochondria generated by FKBP8 was LC3-positive and degraded via lysosome, a mitophagy event. A LIR motif 24FEVL27 found in the N-terminus was essential for binding to LC3. This type of mitophagy occurred in BNIP3/NIX knockout cells but was blocked in Atg5 or FIP200 knockout cells. Upon iron stress, FKBP8 was recruited onto the budding site of mitochondria undergoing mitophagy and colocalized with LC3 on the budding region. Similarly, FKBP8 was also required for mitophagy and mitochondrial fission under hypoxic stress. Together, these results indicate that FKBP8 plays a role in mitochondrial dynamics via binding to Opa1 and recruits LC3 to mitochondria undergoing mitophagy under stress conditions

Keyword: Mitochondria, dynamics, mitophagy, FKBP

[SY03-5-1]

CHARACTERIZATION OF BROWNING-SUSCEPTIBLE WATS

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Genetic factors among inbred mice have been widely used for understanding obesity and related phenotypes. However, there have been a few reports on browning induction in subcutaneous WAT "the beige fat" among inbred strains of mice, although, activation of the beige fat can ameliorate obesity and improve glucose homeostasis through energy dissipation and heat generation. Thus, we studied browning propensity in 6 inbred strains of mice response to cold and beta3-adrenergic agonist to determine the browning difference. Next, to understand browning propensity based on genetic back ground, we analyzed RNA sequencing data obtained from cold and CL 316,243 stimulated inguinal white adipose tissue in C57BL/6J, DBA/2 and 129/svJ inbred strains of mice. UCP1, browning marker, was highly expressed in DBA/2 and 129 mice both cold and CL stimulation. However UCP1 was up-regulated only by CL stimulation in Balb/c mice, not in cold adaption. 129/svJ, DBA/2, C3H/HeN revealed higher energy expenditure and O2 consumption than other three strains after CL injection. Further, there were strain dependent gene expression pattern among C57BL/6J, DBA/2 and 129/svJ strains of mice. The genes associated with browning were upregulated in baseline and after cold and CL 316,243 stimulation at DBA/2 and 129/svJ compared to C57BL/6J mice. We accomplished transcriptomic analysis of iWAT among B6, 129 and DBA/2 mice after CL and cold challange. According to browning susceptibility, transcriptomic profiles revealed unique enriched pathway from 3 different strains of inbred mice. Our study indicates that there are different browning capacity among inbred mice and also different pathway leading to browning induced by cold and CL stimulation. Different browning capacity based on genetic factor may allow us to understand new mechanism leading to browning.

Keyword: Browning, Inbred mouse, Adipose tissue, Transcriptome

[SY03-5-2]

SPHINGOLIPID METABOLISM IN OBESITY AND HEPATOSTEATOSIS

Tae-Sik Park[1]

[1]Gachon University, Korea

Sphingolipids are implicated in etiology of chronic metabolic diseases including cardiovascular diseases and diabetes. In this study, we investigated whether de novo sphingolipid biosynthesis is associated with development of adipose tissues. SPTLC2, a subunit of serine palmitoyltransferase, was transcriptionally upregulated in adipose tissues of obese mice and during differentiation of 3T3-L1 cells. SPTLC2 knockdown suppressed expression of adipogenic genes and lipid accumulation in 3T3-L1 cells. To confirm this, we have developed adipocyte-specific SPTLC2 deficient (aSPTLC2 KO) mice that have lipodystrophic phenotype even with high fat diet feeding. The cell size and mass of adipocyte tissue were reduced dramatically and expression of adipogenic genes was downregulated. Whereas, the fatty acids destined to the adipose tissue were accumulated by increased uptake into liver and caused hepatic steatosis. aSPTLC2 KO mice fed a high fat diet did not increase the body weight but fasting glucose levels were elevated and developed systemic insulin resistance. Although adenoviral SPHK2 overexpression in liver did not recover lipodystrophic phenotype, the floxed mice showed increased fat mass. This is in part due to downregulation of S1P receptor 1 in adipose tissue of aSPTLC2 KO mice and SPTLC2-suppressed 3T3-L1 cells. Collectively, our observations suggest that tight regulation of de novo sphingolipid biosynthesis and S1P signaling plays an important role in adipogenesis and hepatosteatosis.

Keyword: sphingolipid, obesity, steatosis, sphingosine 1-phosphate, adipogenesis

[SY03-5-3]

DECONSTRUCTION ADIPOGENESIS IN VIVO WITH SINGLE CELL RNA-SEQ

James Granneman[1]

[1]Wayne State University, USA

Recruitment of brown/beige adipocytes (BA) in white adipose tissue (WAT) involves proliferation and differentiation of adipocyte stem cells (ASC) in concert with close interactions with resident immune cells. To deconvolve stromal cell heterogeneity in a comprehensive and unbiased fashion, we performed single cell RNA sequencing (scRNA-seq) of > 33,000 stromal/vascular cells from epididymal WAT (eWAT) and inguinal WAT (iWAT) under control conditions and during β3-adrenergic receptor (ADRB3) activation. scRNA-seq identified distinct ASC subpopulations in eWAT and iWAT that appeared to be differentially poised to enter the adipogenic pathway. ADRB3 activation triggered the dramatic appearance of proliferating ASC in eWAT, whose differentiation into BA could be inferred from a single time point. scRNA-seq identified various immune cell types in eWAT, including a proliferating macrophage subpopulation that occupies adipogenic niches. These results demonstrate the power of scRNA-seq to deconstruct adipogenic niches and suggest novel functional interactions among resident stromal cell subpopulations.

[SY03-5-4]

ETHNICITY SPECIFIC EXONIC VARIANTS AND ITS IMPLICATIONS IN TYPE 2 DIABETES

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Ethnicity specific genetic variations are crucial for understanding human biology and predisposition to various complex disorders. We analyzed data obtained by deep sequencing 1,303 Korean whole exomes; the data were generated by three independent whole exome sequencing projects (named the KOEX study). The primary focus of this study was to comprehensively analyze the variant statistics, investigate secondary findings that may have clinical actionability, and identify loci that should be cautiously interpreted for pathogenicity. A total of 495,729 unique variants were identified at exonic regions, including 169,380 nonsynonymous variants and 4,356 frameshift insertion/deletions. Among these, 76,607 were novel coding variants. On average, each individual had 7,136 nonsynonymous single nucleotide variants and 74 frameshift insertions/deletions. We classified 13 pathogenic and 13 likely pathogenic variants in 56 genes that may have clinical actionability according to the guidelines of the American College of Medical Genetics and Genomics, and the Association for Molecular Pathology. The carrier frequency of these 26 variants was 2.46% (95% confidence interval 1.73 – 3.46). We also investigated the association of these exonic variants with risk of type 2 diabetes (T2D) and related clinical phenotypes in Koreans. Among nonsynonymous variants, PAX4 Arg192His increased risk of T2D and GLP1R Arg131Gln decreased risk of T2D in genome-wide significance. Another variant at PAX4 192 codon, Arg192Ser was nominally associated with T2D. The catalog of identified variants, its annotation, and frequency information are publicly available. These findings should be useful resources for investigating ethnically specific characteristics in human health and disease.

Keyword: Genetics, Sequencing, Diabetes, Exome, Nonsynonymous

[SY04-1-1]

EXOSOMES AND EXTRACELLULAR VESICLES: FROM BENCH TO CLINIC

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The secretion of nano-sized lipid bilayered exosomes is a universal cellular process occurring from simple organisms to complex multicellular organisms. Recent progress in this area has revealed that exosomes, also known as extracellular vesicles and microvesicles, play multiple roles in intercellular and interspecies communication, suggesting that exosomes are NanoCosmos, i.e., extracellular organelles that play diverse roles in intercellular and interkingdom communication. This presentation focuses on the comprehensive aspects of cancer exosomes including their components (http://evpedia.info), biogenesis, and diverse pathological functions such as angiogenesis and immune modulation that should facilitate further applications, especially to develop in vitro and in vivo cancer diagnostic tools and therapeutics including our recent progress in novel mammalian and bacterial exosome-mimetic technology for targeted delivery of chemotherapeutics and siRNA. Based on the the concept of emergent properties of exosomes, future research directions to decode the complexity of intercellular communication network and the secret of life will be briefly introduced.

[SY04-1-2]

EXTRACELLULAR VESICLES AS A NOVEL THERAPEUTIC TARGET FOR CANCER METASTASIS

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Extracellular vesicles (EVs), such as exosomes and microvesicles, encapsulate several genetic information as new modulators of both intercellular crosstalk and disease pathogenesis. EVs can act as autocrine/paracrine effectors, based on evidence that they are able to transport a characteristic composition of proteins and nucleic acids, such as mRNA and microRNA. Importantly, exposure to various stressors can modify the composition of EVs to change the surrounding micro-environment through EV cell-to-cell communication. Several studies have shown that cancer-derived EVs contribute to cancer progression, including metastasis; thus, the strategy of eliminating cancer-derived EVs has been considered a promising way in which to suppress cancer malignancy. Here, we provide a novel strategy for therapeutic treatment to target cancer-derived EVs and inhibit the metastasis of breast cancer in a mouse model, establishing a rationale for further clinical investigation. Our results demonstrated the concept that inhibition of the function of cancer-derived EVs using antibodies and inhibition of cancer-specific EV-secretory molecules are effective for preventing cancer metastasis, which is applicable as therapeutic agents in clinical situations. (references) 1. Yokoi A, et al. Nat Commun, 2017 2. Katsuda T, et al. Cell Stem Cell, 2017 3. Akimoto M, et al., Nat Commun, 2016 4. Kosaka N, et al. J Clin Invest, 2016 5. Takahashi RU, et al. Nat Commun, 2015. 6. Tominaga N, et al. Nat Commun, 2015 7. Ono M, et al. Sci Signal, 7:ra63, 2014 (2015 Signaling Breakthrough of the Year) 8. Yoshioka Y, et al. Nat Commun, 2014

Keyword: Exosome, microRNA, metastasis, therapy

[SY04-1-3]

EXTRACELLULAR VESICLE SURFACE INTERACTIONS

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Extracellular vesicles are phospholipid bilayer enclosed structures that establish important interactions with both the plasma membrane of cells and molecules of the extracellular matrix. Extracellular vesicle surface interactions may impact, among others, vesicular stability, immune recognition and cellular uptake of vesicles. Proteomic analysis of extracellular vesicles isolated from complex matrices such as blood plasma show substantial amounts of plasma proteins co-isolated with extracellular vesicles. Moreover, we found that extracellular vesicle preparations isolated by currently available methods fail to purify extracellular vesicles from blood plasma without lipoproteins such as LDL. In vitro mixing of LDL with either microvesicles or exosomes resulted in a striking attachment of LDL onto the surface of isolated vesicles. LDL binding onto the surface of extracellular vesicles interfered with detection of conventional extracellular vesicle surface markers by flow cytometry. We have also demonstrated ApoB100 positive Triton sensitive (vesicular) events in the pericardial fluid of patients with coronary artery bypass surgery. In addition, we found that genotoxic stress of cells results in the release of exosomes with DNA attached onto their exofacial surface. In contrast, cell activation or apoptosis induction failed to induce such exosomal release of DNA. Using an optical biosensor we demonstrated that DNA attached onto the exofacial surface of exosomes could mediate exosomal binding to fibronectin. Our data suggest that extracellular vesicle surface interactions are not restricted to those with proteins but may also include association of extracellular vesicles with lipoproteins and nucleic acids.

Keyword: extracellular vesicles, interaction, lipoproteins, DNA, surface

[SY04-1-4]

PROTEOMIC ANALYSIS OF ACUTE MYELOID LEUKEMIA-DERIVED EXTRACELLULAR VESICLES

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Acute myeloid leukemia (AML) is a malignant disease categorized by blocking monocyte differentiation and maturation as hematopoietic cells. AML is divided into 8 subtypes according to French-American-British (FAB) classification, which mainly depends on cell maturity and differentiation. Extracellular vesicles (EV) are known to perform critical physiological and pathological functions including communication in mammalian cells. Only a few proteomic studies on subtype-specific AML have been reported. As EVs perform multifaceted pathological functions in intercellular signaling and communication, it is essential to profile and compare EV-proteome changes for understanding the pathophysiology of AML differentiation. To elucidate the proteomic characteristics of the EVs from AML, we isolated EVs from human dermal fibroblast, human bone marrow-derived mesenchyme stem cells and AML such as acute promyelocytic leukemia (HL60), acute myelomonocytic leukemia (KG-1), and acute monocytic leukemia (THP-1). Proteome profiles of isolated EVs were analyzed by using liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses. We compared each group of proteomes and observed changes in leukocyte-genesis mechanism and proteoglycan mechanism in AML that could explain differentiation of AML from the bone marrow. It is worthy to note that the commonly identified proteins were enriched in the cellular components of extracellular exosome and membrane, and engaged in the pathways of leukocyte surface antigen as well as myeloid-associated differentiation. Our study might help to understand the intracellular/extracellular of AML differentiation pathways that could explain physiological regulation factors in AML groups.

Keyword: Proteomics, Exosome, Leukemia

[SY04-3-1]

SYNAPSE SPECIFIC PLASTICITY WITHIN ENGRAM ASSEMBLIES GOVERNS THE MEMORY TRACE IDENTITY

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Memories are formed through long-term changes in synaptic efficacy, a process known as synaptic plasticity, and are stored in the brain in specific neuronal ensembles called engram cells, which are activated during corresponding events. When two memories are associated, cell ensembles corresponding to each memory overlap and are responsible for the association15. However, each memory has its own identity. How the brain stores and defines a specific memory identity when two memories interact and are encoded in the shared ensemble remains elusive. Here, we show that synapse-specific plasticity represents specific memory entities, and that synaptic plasticity between specific engram assemblies is both sufficient and crucial for information storage. In auditory fear conditioning (AFC) in mice, after complete retrograde amnesia, optogenetic stimulation of the activated ensemble terminals of auditory cortex (AC) and the medial geniculate nucleus (MGm) in the lateral amygdala (LA) failed to induce fear memory recall, indicating that the memory engram no longer existed in that circuit. Complete retrograde amnesia of a given fear memory did not affect the linked fear memory encoded in the shared ensemble. Furthermore, potentiation or depotentiation of the plasticity at synapses specific to one memory affected the recall of that memory without influencing the linked memory. Thus, sharing of engram cells underlies the linkage between memories, while synapse-specific plasticity guarantees the identity and storage of individual memories.

Keyword: memory identity, amnesia, synapse-specific plasticity, fear conditioning, engram

[SY04-3-2]

MECHANISM OF DISEASE PROGRESSION IN PARKINSON'S DISEASE

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Propagation of α-synuclein aggregates has been suggested as the root cause of pathological spreading, and thus of disease progression, in Parkinson's disease (PD). The mechanism underlying the propagation has significant implications in therapeutics, yet remains elusive. Here, we demonstrate in cell culture, nematode, and rodent models that LRRK2, a PD-linked kinase, plays a pivotal role in the propagation of α-synuclein in a kinase activity-dependent manner. The PD-linked G2019S mutation in LRRK2 that increases the kinase activity further bolsters the propagation efficiency. The function of LRRK2 in α-synuclein propagation is mediated by phosphorylation on Rab35. Constitutive activation of Rab35 overrides the reduced propagation phenotype of lrk-1 mutant nematode. Finally, in a mouse model of synucleinopathy, administration of LRRK2 kinase inhibitor reduced α-synuclein aggregates by having more α-synuclein engaged in the lysosomal degradation pathway. These results suggest that LRRK2-mediated Rab35 phosphorylation as a novel therapeutic target for modifying the disease progression.

Keyword: Parkinson disease, synuclein, LRRK2, protein aggregation, lysosome

[SY04-3-3]

AWAKENING DORMANT DOPAMINERGIC NEURONS BY BLOCKING ABERRANT TONIC INHIBITION TO ALLEVIATE PARKINSON'S DISEASE

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Pharmacologic treatment of Parkinson's disease (PD) can be divided into symptomatic and disease-modifying therapy. Until now, there is no proven disease-modifying therapy for PD. This lack of disease-modifying therapy can be partly attributed to the belief that dead neurons cannot be revived based on a long-standing concept of dopaminergic neuronal death in the substantia nigra pars compacta (SNpc) as the main cause of PD. In contrast, the concept of the presence of dormant dopaminergic neurons has not been carefully explored and the possibility of awakening of the dormant neurons as the disease-modifying therapeutic strategy against PD has not been tested. Here we show that optogenetic inhibition of SNpc neurons causes parkinsonian motor symptoms with a dramatic loss of tyrosine hydroxylase (TH), one of the two dopamine-synthesizing enzymes, without neuronal death, while optogenetic activation alone recovers motor function and TH expression in various PD animal models. This recovery effect is caused by awakening dormant dopaminergic neurons, some of which are devoid of TH expression but still express DOPA decarboxylase (DDC), another enzyme in dopamine biosynthetic pathway. The TH loss is caused by reduced dopaminergic neuronal firing under aberrant tonic inhibition, which is attributed to excessive GABA synthesized by monoamine oxidase-B (MAO-B) in reactive astrocytes. Genetic deletion or pharmacological inhibition of MAO-B to block astrocytic GABA synthesis recapitulates the therapeutic effect of optogenetic activation. Consistently, SNpc of postmortem PD patients shows a significant population of TH-negative/DDC-positive (TH-/DDC+) neurons surrounded by numerous GABA-positive glia. Our study proposes that awakening dormant dopaminergic neurons by blocking excessive astrocytic GABA synthesis could be an effective therapeutic strategy against PD.

Keyword: Parkinson's disease, glia, GABA, tonic inhibition, MAO-B

[SY04-3-4]

INHIBITORY BASAL GANGLIA INPUT INDUCES EXCITATORY MOTOR SIGNALS IN THE THALAMUS

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The basal ganglia are a brain structure that controls complex movements. During low dopamine states, such as Parkinson disease (PD), the basal ganglia more strongly inhibit their target neurons. For the past three decades, scientists have assumed that this stronger inhibition caused the motor problems of PD patients. To test this assumption, we used optogenetic technology to directly activate basal ganglia inhibitory output and then examined the response of target neurons in the thalamus, a part of the brain also involved in movement in mice. Surprisingly, the target neurons in the thalamus exhibited a paradoxical increase in activity in response to the inhibition. This rebound excitation produced aberrant muscular rigidity and tremor that is very similar to the symptoms of PD patients. Eliminating this rebound firing caused the motor symptoms to be completed cured in a mouse model of PD, proving that the rebound firing causes the motor problems experienced by PD patients. This study overturns three decades of consensus on the provenance of Parkinsonian symptoms as a breakthrough, both for understanding how the brain normally controls movement of our body and for understanding how this control goes awry during PD and related dopamine-deficiency disorders.

Keyword: Thalamus, Parkinson disease, basal ganglia, dopamine

[SY04-3-5]

NEURAL CIRCUIT MECHANISMS FOR MODULATION OF SENSORY PROCESSING AND INTEGRATION

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Sensory perception in the real world requires proper integration of different modality inputs. The process of multisensory integration is not uniform. It varies from individual to individual and changes at different behavioral states of the animal. What factors affect the multisensory integration? How does the mammalian brain reconstruct a multisensory world at different states? Here I present our recent findings on neural circuit mechanisms for modulation of sensory processing and integration. We found that the posterior parietal cortex receives converging inputs from the primary visual and auditory cortices and plays a critical role in audio-visual integration. We further found that the circuits in the posterior parietal cortex can be modulated by the locomotion. We suggest that the multisensory information is not a simple, fixed signal in the brain. Multisensory processing is dynamically modulated in the mammalian brain and leads to a unique experience of perception.

Keyword: Multisensory perception, Vision, Audition, Modulation, Locomotion

[SY04-3-6]

NMDA RECEPTOR CHANNEL GLUN2D SUBUNIT AS A NEW TARGET MOLECULE FOR MEDICINES FOR MENTAL DISORDERS

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N-methyl-D-aspartate (NMDA) receptor channels play crucial roles in various brain functions, such as cognition, memory, learning, neural development, and pain. Ketamine, a noncompetitive NMDA receptor antagonist, increases locomotor activity in rodents and causes schizophrenia-like symptoms in humans. Low dose of ketamine reportedly ameliorates anti-depressant resistant depression. Although activation of the dopamine (DA) pathway is hypothesized to mediate these effects of ketamine and another noncompetitive NMDA receptor antagonist phencyclidine (PCP), the precise mechanisms by which these drugs induce their effects remain to be elucidated. We found that acute and repeated administration of ketamine and PCP did not increase locomotor activity in mice lacking GluN2D, a subunit of NMDA receptor. GluN2D knockout mice did not show impairment of prepulse inhibition by PCP, either. We investigated the effect of PCP on extracellular levels of DA (DAex) in the striatum and prefrontal cortex (PFC) using in vivo microdialysis and locomotor activity in mice lacking GluN2A or GluN2D subunit and found that PCP significantly increased DAex in wildtype and GluN2A knockout mice, but not in GluN2D knockout mice, in the striatum and PFC. Furthermore, DNA array experiments revealed that PCP-induced fos expression was abolished in GluN2D knockout mice. These results suggest that PCP and possibly ketamine enhance dopaminergic transmission, increases locomotor activity, and induces fos expression by acting at GluN2D. GluN2D may be a new target for pharmacotherapy of dependence, schizophrenia and depression.

Keyword: NMDA, Ketamine, Phencyclidine, Knockout mouse, Mental disorders

[SY04-5-1]

HIF-1 AND TUMOR PROGRESSION

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Malignant solid tumors contain hypoxic regions due to an imbalance between uncontrolled proliferation of cancer cells and insufficient formation of a vascular network. Accumulating evidence has suggested that a hypoxia-responsive transcription factor, hypoxia-inducible factor 1 (HIF-1), functions not only in cellular adaptive response to hypoxia but also in malignant progression of cancers. However, gene networks responsible for the activation of HIF-1 have not yet been fully elucidated, which makes it difficult to develop novel therapeutic strategies for cancers. Recently, we succeeded in identifying isocitrate dehydrogenase 3 (IDH3), ubiquitin C-terminal hydrolase-L1 (UCHL1), and lymphocyte antigen 6 locus E (LY6E), as novel activators of HIF-1, and reported that they function in reprogramming of glucose metabolic pathway, distant metastases, and angiogenesis of cancers, respectively. Moreover, we revealed that hypoxic tumor cells predominantly survive radiation therapy, migrate toward functional blood vessels in a HIF-1-dependent manner, and eventually cause tumor recurrence. In my talk, I would like to highlight the functions of HIF-1-related gene networks in malignant progression of cancers and tumor recurrence after radiation therapy, and provide rational basis to exploit them as novel targets for the diagnosis and treatment of cancers.

Keyword: hypoxia, HIF-1, cancer, malignant progression, microenvironment

[SY04-5-2]

HYPOXIA SUFFOCATES HISTONE DEMETHYLASES TO CHANGE HISTONE CODE AND GENE EXPRESSION

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Hypoxia changes the expression of the hypoxia-responsive genes through two main pathways. First, hypoxia activates transcription factors (TF) such as Hypoxia-inducible Factor (HIF). Second, hypoxia decreases the activity of DNA, RNA and histone demethylases that require O2 and α-Ketoglutarate (α-KG) as substrates. Especially Jumonji C domain-containing histone demethylases (JMJDs) affect gene expression through their regulation of the active or repressive histone methylations. We conducted profiling of H3K4me3, H3K9me3, and H3K27me3 under both normoxia and hypoxia identified 75 TFs, whose binding motifs were significantly (P

Keyword: Hypoxia, Histone, methylation, JMJDs

[SY04-5-3]

SENESCENCE AND HYPOXIA IN TUMOR MICROENVIRONMENT

Jae-Seon Lee[1]

[1]Inha University, Korea

Senescence has become regarded as a general biological program of terminal growth arrest, since a variety of treatments have been shown to trigger premature senescence. DNA-damaging agents including ionizing radiation (IR) and chemotherapeutic drugs prematurely induce senescent phenotypes in cancer cells. Recent studies suggest that induction of premature senescence is a promising treatment for solid cancers. However, senescent cancer cells exhibit characteristic senescence-associated secretory phenotype (SASP), which has beneficial and detrimental effects on the tumor microenvironment. We are focused to IR- and chemotherapeutic drug-induced premature senescence in cancer cells. Our final research goal is to contribute to increase the efficacy of cancer therapy via the modulation of cancer cell senescence. We are identifying novel senescence regulatory genes and elucidating their molecular action mechanisms. For example, we found novel roles of Wig1 and PAPSS2 in cancer cell senescence. RNA binding protein Wig1 acts as a guidance protein to dictate association of RNA-induced silencing complex (RISC) with p21 target mRNA. Thus, we demonstrated that fine-tune of p21 level by Wig1 is essential for the prevention of cellular senescence. We also revealed that undersulfation of heparin sulfate induced by PAPSS2 depletion leads cancer cell senescence through the activation of FGFR1-AKT-p53-p21 signal transduction cascade. In addition, we are trying to consider various aspects of tumor microenvironment, which are related with cancer cell senescence. We are analyzing the effect of hypoxia on cancer cell senescence and the relationship between endothelial cell senescence and hypoxia/metastasis. Our findings may aid to considering cancer cell senescence as promising therapeutic strategy for the cancer treatment.

Keyword: senescence, cancer therapy, hypoxia

[SY04-5-4]

MEDIATOR COMPLEX: MEDIATING TRANSCRIPTION AND CANCER DEVELOPMENT

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Cell fate changes, including cellular programming and reprogramming are largely directed by transcription factors. The distinct subunits of Mediator complex specifically interact with different transcription factors, to relay the environmental and developmental signaling for fine-tuning the temporal and spatial gene expression that determine the cell fates. Studies from us and others showed that the Mediator complex plays important roles during transcription initiation, elongation, termination, RNA processing, and possibly epigenetic regulation. We show now that Mediator is indeed an epigenetic regulator. By a screening assay, we identified H2B mono-ubiquitination (H2Bub) as a Mediator MED23-dependent histone modification. And MED23 depletion reduced recruitment of H2Bub modifier RNF20/40 due to their interaction in vivo and in vitro. Whole-genome-sequencing analysis revealed that MED23 couples with H2Bub toregulate transcriptional activation. Furthermore, MED23-depletion led to H2Bub deficiency and facilitated myogenesis. Considering that differentiation and reprogramming (dedifferentiation) are opposite processes of cell fate determination, we went on to discover that MED23 and H2Bub coupling could reprogram somatic cells into tumor cells. We first observed that the levels of both H2Bub and MED23 are increased in cancer tissues than in normal tissues. Combined microarray and ChIP-seq analysis, we then revealed that the genes controlled by MED23-H2Bub are enriched in tumorigenesis-related pathways, suggesting that MED23-dependent H2Bub could be an important novel mechanism in cancer development.

Keyword: Mediator Complex, MED23 subunit, Transcription Regulation, Epigenetics, Cancer Development

[SY05-1-1]

THE LYSOSOMAL AMPK ACTIVATION PATHWAY - GLUCOSE SENSING AND IMPLICATIONS

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Glucose is not only the main source for energy, but also a precursor for the synthesis of many biomolecules. On entering glycolysis, glucose is converted in three steps to fructose-1,6-bisphosphate (FBP), which is then split into triose phosphates by FBP aldolases. Glucose deprivation activates AMP-activated protein kinase (AMPK), a sensor known to play a pivotal role in adaptive responses to energy stress, but it had been unclear how glucose deprivation is sensed and signals to activate AMPK and inactivate mTORC1. We found that glucose shortage induces AXIN/LKB1 to translocate to the lysosome and activates AMPK, concurrently inactivating mTORC1. We have now found that it is the aldolase that senses the availability of FBP/glucose and directly links to AMPK activating complex on the lysosome in a manner independent of energy status change. This coupling of AMPK activation with mTORC1 inactivation may have profound implications for calorie restriction and drug development. The seminar will also talk about our latest findings that point to a device in transmitting a metabolic status, which is analogous to an electric capacitor formed between the ER and lysosome involving a family of "local" calcium channels.

Keyword: glucose, AMPK, lysosome

[SY05-1-2]

CONTROL OF SYSTEMIC ENERGY METABOLISM BY CRTC2-DEPENDENT TRANSCRIPTIONAL PATHWAY

Hye-Sook Han[1], Byeong Hun Choi[1], Jun Seok Kim[1], Geon Kang[1] and Seung-Hoi Koo[1]

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Liver plays a crucial role in controlling energy homeostasis in mammals, although the exact mechanism by which it influences other peripheral tissues has yet to be addressed. Here we show that CREB regulated transcriptional co-activator (CRTC) 2 is a major regulator of whole body energy metabolism. We observed that liver-specific knockout of CRTC2 in mice lowered blood glucose levels with improved glucose, insulin, and pyruvate tolerance under high fat diet conditions. Surprisingly, liver-specific CRTC2 knockout mice displayed increased energy expenditure with smaller lipid droplets in brown and white adipose depots. Close investigation revealed that both plasma and hepatic FGF21 levels were higher in liver-specific CRTC2 knockout mice, which is largely due to the reduced miR-34a and the resultant induction of SIRT1 and PPARα in the liver. Indeed, miR-34a promoter contains CREB binding sites and was transcriptionally controlled by CREB/CRTC2. Finally, we observed that expression of miR-34a reversed the metabolic changes by CRTC2 knockout in the liver, which was restored by concomitant expression of FGF21 via adeno-associated virus-mediated delivery. These data collectively suggest that CREB/CRTC2 negatively regulates the SIRT1/PPARα/FGF21 axis via an induction of miR-34a. Thus, reducing CRTC2 activity in the liver could be beneficial in the control of both glucose and lipid homeostasis in obese individuals.

Keyword: cAMP, CRTC2, FGF21, miR-34a, metabolic disorder

[SY05-1-3]

BRAIN FOXO1 REGULATES ENERGY HOMEOSTASIS & MOOD BEHAVIORS

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Dopaminergic (DA) neurons are involved in integration of neuronal and hormonal signals to regulate energy homeostasis and psychiatric behaviors. However, the factors responsible for the regulation of energy balance and mood behaviors in dopaminergic neurons are required to be addressed. My group recently found that forkhead transcription factor O1 (FoxO1) is involved in the regulation of energy balance together with mood behaviors in DA neurons. Specifically, FoxO1 is highly expressed in DA neurons and mice lacking FoxO1 specifically in the DA neurons (FoxO1 KODAT) show markedly increased energy expenditure and interscapular brown adipose tissue (iBAT) thermogenesis accompanied by reduced fat mass and improved glucose/insulin homeostasis. In addition, FoxO1 KODAT mice exhibited enhanced leptin sensitivity as well as less anxiety and depression-like behaviors. Therefore, this presentation will cover our recent findings in which functional roles of FoxO1 in regulation of energy balance and mood behaviors in DA neurons.

[SY05-1-4]

REGULATION OF HYPOXIA RESPONSES BY FLAVIN ADENINE DINUCLEOTIDE-DEPENDENT MODULATION OF HIF PROTEIN STABILITY

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Oxygen deprivation induces a range of cellular adaptive responses that enable to drive cancer progression. We have recently reported that lysine-specific demethylase 1 (LSD1) upregulates hypoxia responses by demethylating RACK1 protein, a component of hypoxia-inducible factor (HIF) ubiquitination machinery and consequently suppressing the oxygen-independent degradation of HIF-1alpha (Yang et al., 2017, EMBO J). This ability of LSD1 is attenuated during prolonged hypoxia, with a decrease in the cellular level of flavin adenine dinucleotide (FAD), a metabolic cofactor of LSD1, causing HIF-1alpha down-regulation in later stages of hypoxia. Exogenously provided FAD restores HIF-1alpha stability, indicating a rate-limiting role for FAD in LSD1-mediated HIF-1alpha regulation. Transcriptomic analyses of patient tissues show that the HIF-1 signature is highly correlated with the expression of LSD1 target genes as well as the enzymes of FAD biosynthetic pathway in triple-negative breast cancers, reflecting the significance of FAD-dependent LSD1 activity in cancer progression. Together, our findings provide a new insight into HIF-mediated hypoxia response regulation by coupling the FAD-dependence of LSD1 activity to the regulation of HIF stability.

Keyword: EPIGENETIC, METABOLITE, CANCER, HYPOXIA

[SY05-1-5]

NUCLEAR RECEPTOR CROSSTALK CONTROLS HEPATIC LIPID HOMEOSTASIS TO PROTECT AGAINST NAFLD

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Dysregulation of hepatic lipid metabolism results in the development of hepatic steatosis, contributing to the chronic insulin resistance and steatotic hepatitis. The hepatic metabolic pathways are governed by highly dynamic transcriptional networks of orphan nuclear receptors (ONRs), including PPARs, farnesoid X receptor (FXR), and liver X receptor (LXR). ONRs are ligand-activated transcription factors with no defined ligands. Many ONRs are expressed in tissues involved in metabolism, such as skeletal muscle, adipose tissue, and liver, and play critical roles in the regulation of metabolism. Genetic studies have shown that many ONRs regulate nutrient metabolism and physiology of obesity and type II diabetes. Given that numerous synthesized ligands for ONRs are used for developing putative drugs for human metabolic diseases. ONRs are emerging as therapeutic targets for the treatment of metabolic diseases. Previously, we have reported that RORα, a member of ONRs, possesses tumor suppressive function by transrepressing canonical Wnt/β-catenin signaling leading to inhibition of colon cancer growth and by increasing p53 stability upon DNA damage response. RORα is known to regulate cerebellum development. The staggerer (sg) mice, natural Rorα spontaneous mutant mice, display ataxia and severe cerebellar atrophy. Moreover, ROR functions to regulate circadian rhythm as a key regulator of the cyclic expression of BMAL1 together with REV-ERBs. The RORα/REV-ERB feedback loop controls the circadian expression pattern of BMAL1, indicating that RORα plays a key role in the core circadian clock. In addition, sg mice show lower expression levels of genes involved in lipid metabolism, including apolipoprotein A-1 (apoA1) and apolipoprotein C-III (apoCIII). Thus, sg mice exhibit less body weight gain compared with wild-type (WT) mice. Given that sg mice have huge cerebellar defects, it is still possible that physiological changes observed in sg mice are indirect effects. Thus, the physiological roles of RORα to control transcriptional networks to modulate lipogenesis and gluconeogenesis still remain unclear. We report that RORα plays a key role to control hepatic lipid metabolism to protect against diet-induced obesity and hepatic steatosis, using liver-specific Rorα- deficient mouse model. High-fat diet (HFD)-fed liver-specific Rorα deficient mice (ROR LKO mice) show severe metabolic defects, including hepatic steatosis, obesity, and insulin resistance, although no physiological changes have been observed with control diet (CD). Genome-wide transcriptome analysis reveals that PPARγ signaling is remarkably

elevated in RORα LKO mice. RORα specifically recruits HDAC3 to the PPARγ target promoters to suppress PPARγ transcriptional activity. Finally, PPARγ antagonism by using PPARγ antagonist GW9662, largely ameliorates body weight gain and hepatic steatosis in HFD-fed RORαLKO mice, indicating that dysregulated PPARγ signaling is a critical metabolic cue, leading to metabolic defects in HFD-fed RORαLKO mice. Together, our data demonstrate that RORα controls PPARγ signaling to protect against hepatic metabolic homeostasis and obesity in response to HFD.

[SY05-2-1]

T CELL MEDIATED ANTIMICROBIAL MECHANISMS IN LEPROSY AND TUBERCULOSIS

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We have discovered three distinct antimicrobial pathways against intracellular mycobacteria in humans. First, we demonstrated that some CD8+ CTLs deliver the antimicrobial protein granulysin into infected MΦ and thus kill intracellular mycobacteria. Furthermore, we found that some "tri-cytotoxic T cells" (T-CTLs), a subset of CD8+ CTLs that express granzyme B, perforin, and granulysin, express the NK receptor NKG2C and have antimicrobial activity against M. leprae-infected MΦ, representing one subpopulation of antimicrobial CTLs (amCTLs). Second, we found that IL-26, an antimicrobial protein secreted by Th17 cells, kills extracellular bacteria, can enter MΦ, colocalized with M. leprae and reduces bacterial viability. Third, we discovered that CD4+ Th1 cells secrete IFN-γ, which triggers the vitamin D-dependent induction of the antimicrobial proteins cathelicidin3. All three pathways involve the antimicrobial proteins that exist in humans but not mice, such that these human antimicrobial mechanisms cannot be studied in in mice.

[SY05-2-2]

IMMUNOPATHOGENESIS OF SEVERE FEVER WITH THROMBOCYTOPENIA VIRUS

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Severe Fever with Thrombocytopenia virus (SFTSV) listed in the World Health Organization Prioritized Pathogens is an emerging Phlebovirus in the Phenuiviridae family. The virus was discovered in China in 20103 and has since spread to other countries in East Asia. SFTS has fatality rates ranging from 12% to as high as 30% in some areas and induces immunopathogenic disease with a characteristic thrombocytopenia that is remarkably similar to viral hemorrhagic fevers. Infected ticks, most frequently Haemaphysalis longicornis, are the major source of human SFTSV infection, however, human-to-human transmission by direct contact has been reported. Due to the lack of therapies and vaccines against SFTSV infection, there is a pressing need to understand the pathogenesis of SFTSV to develop effective vaccines and antiviral agents. Previous studies have shown that the virally encoded nonstructural protein (NSs) blocks type-I interferon (IFN) induction and is thought to facilitate disease progression. Here, we report that SFTSV NSs plays an essential role in viral immunopathogenesis. Specifically, SFTSV NSs targeted the TPL2 kinase complex to robustly induce expression of immune suppressive genes, specifically IL-10 cytokine. Combined use of viral reverse genetics, a TPL2 kinase inhibitor and Tpl2-/- mice showed that the NSs-mediated activation of TPL2 signaling pathway led to the robust production of IL-10 cytokine. While SFTSV-WT infection of anti-IFNAR1 antibody-treated wild-type mice led to rapid weight loss and death, 100% of anti-IFNAR1 antibody-treated Tpl2-/- mice survived an infection. In fact, the administration of TPL2 inhibitor substantially improved the survival of anti-IFNAR1 antibody-treated wild-type mice infected with SFTSV. Our study demonstrates that SFTSV NSs targets the TPL2 signaling pathway to induce expression of immune suppressive genes including IL-10 as means to dampen the host defense and promote viral pathogenesis and that TPL2 signaling pathway is a potential therapeutic target to treat SFTSV-infected patients.

Keyword: SEVERE FEVER WITH THROMBOCYTOPENIA VIRUS (SFTSV), TPL-2, IL-10, THERAPY, NSS

[SY05-2-3]

HOST-MICROBE INTERACTION IN THE INTESTINE

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Intestine is a unique tissue, where many commensal bacteria, called microbiota, inhabit. Therefore, intestinal mucosa is protected from microbiota as well as pathogenic bacteria by several types of barriers. One of these barriers is constructed by mucus layers, composed of inner and outer mucus layers in the colon. Microbiota is present in the outer mucus layer, whereas there is no microbiota in the inner mucus layer. Separation of microbiota from the intestinal epithelial cells contributes to prevention of intestinal inflammation. Indeed, invasion of bacteria into the colonic epithelial surface was shown in several mouse models of intestinal inflammation. However, the precise mechanisms by which the inner mucus layer is free of microbiota in the colon remain unknown. Ly6/PLAUR domain-containing protein 8 (Lypd8), which was selectively expressed on the uppermost layer of colonic glands, was a highly glycosylated GPI-anchored protein and secreted into the colonic lumen, particularly the inner mucus layer. In mice lacking Lypd8, bacterial free space in the inner mucus layer disappeared and they were highly susceptible to intestinal inflammation. On the intestinal epithelial cell layer of the colon of the mutant mice, flagellated bacteria such as Escherichia, Helicobacter and Proteus were present. Depletion of these bacteria by antibiotics restored the bacterial free space in the inner mucus layer and ameliorated the intestinal inflammation of the mutant mice. Lypd8 bound to bacterial flagella and suppressed motile activity of flagellated bacteria. Thus, Lypd8 mediates segregation of microbiota from the intestinal epithelial layer in the colon, and thereby contributes to the prevention of intestinal inflammation. We will also discuss the role of Lypd8 in infection of pathogenic enteric bacteria.

Keyword: intestine, epithelia

[SY05-2-4]

STRUCTURE, FUNCTION AND TARGET VALIDATION STUDIES OF THE MALARIA PARASITE PROTEASOME

Leann Tilley[1], Stanley Xie[1], Eric Hanssen[1], Jess Bridgford[1], Tuo Yang[1], David Gillet[1], Sabine Ottilie[2], Christopher Tsu[3], Elizabeth Winzeler [2] and Larry Dick[3]

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Artemisinin and its derivatives rapidly reduce the parasite burden in Plasmodium falciparum infections, providing prompt therapy for severe infections. Unfortunately, their usefulness is threatened by the emergence of drug resistance. We have demonstrated that artemisinins kills parasites via a two-pronged mechanism, causing protein unfolding and functionally damaging the parasite proteasome. The consequent accumulation of proteasome substrates, i.e., unfolded and polyubiquitinated proteins, activates the Unfolded Protein Response and underpins DHA-mediated killing. We showed that proteasome inhibitors work synergistically with artemisinins to overcome resistance and screened a library of 131 boronate proteasome inhibitors for inhibitory activity against purified human and P. falciparum 20S proteasome. We have identified a series of potent parasite-active compounds that show selective inhibition of the growth of P. falciparum. To further validate the target, we selected P. falciparum for resistance to the clinically used boronate proteasome inhibitor, bortezomib. Whole genome sequencing revealed mutations in the proteasome beta5 binding site. In addition, we have undertaken substrate profiling and structural studies of the plasmodium proteasome in complex with the PA28 activator to guide the design of more selective inhibitors.

Keyword: malaria, drug, proteasome, resistance, target validation

[SY05-2-5]

AUTOPHAGY AND MYCOBACTERIAL INFECTION

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Autophagy is an essential housekeeping process through lysosomal degradation of protein aggregates and damaged organelles, thus maintaining intracellular homeostasis against various stress conditions. It is now becoming clear that autophagy is crucial in host defense against infection with intracellular bacteria, including Mycobacterium tuberculosis, through enhancement of phagosomal maturation. Recently, increasing efforts have been made to develop several autophagy-targeting agents as potential host-directed therapeutics to overcome drug resistance issues in a variety of infectious diseases including human tuberculosis. In this talk, our current research of identifying host factor(s) to activate antibacterial autophagy will be briefly introduced in terms of controlling intracellular bacterial infection. Our conceptual framework may be applied to identify additional drug-affected targets when seeking to treat intracellular bacterial infectious diseases.

Keyword: autophagy, mycobacterial infection, intracellular bacterial infection, host defense, innate immunity

[SY05-3-1]

OBSERVING NEURAL ACTIVITY IN THE AUTISTIC BRAIN IN VIVO AND GENETIC RESCUE OF AUTISTIC BEHAVIORS

Long Li[1], Bo Yuan[1], Zilong Qiu[1] and Xiaoqun Wang[1]

[1]Institute of Neuroscience, Chinese Academy of Science, China

Duplications of MECP2-containing genomic segments led to severe autistic symptoms in male. Transgenic mice overexpressing MeCP2 exhibit autistic-like behaviors. Neural circuits underlying social defects in MECP2 transgenic (MECP2-TG) mice remain unknown. To observe neural activity of MECP2-TG mice in vivo, we performed calcium imaging by implanted microendoscope in the hippocampal CA1 regions of MECP2-TG and wild type (WT) mice. We identified neurons whose activities were tightly associated with social interaction, which activity patterns were compromised in MECP2-TG mice. Strikingly, we rescued the social-related neural activity in CA1 and social defects in MECP2-TG mice by deleting the human MECP2 transgene using the CRISPR/Cas9 method during adulthood. Our data points to the neural circuitry responsible for social interactions and provides potential therapeutic targets for autism in adulthood.

Keyword: autism, mecp2, microendoscopy, social cells, crispr/cas9

[SY05-3-2]

PARMACOLOGICAL TARGETING OF EXCITATORY/INHIITORY IMBALANCE IN AUTISM SPECTRUM DISORDER

Chan Young Shin[1]

[1]Konkuk University, Korea

Autism spectrum disorder (ASD) is neurodevelopmental condition in which two core domains of symptoms such as social communication deficits and restricted/repetitive behaviours are defining features. Excitatory-inhibitory neuronal imbalance (E/I imbalance) theory of ASD is one of the most promising hypothesis as exemplified by the effectiveness of MK801 and memantine on animal models of ASD. We also identified agmatine, an endogenous neuromodulator with antagonistic effects against NMDA receptors, effectively suppressed social and other behavioral deficits in prenatally valproic acid (VPA)-injected animals. We also investigated the role of AMPA receptors in the modulation of ASD-like behaviour in experimental animal models. Prenatally VPA-exposed mice have increased GluR1 expressions in the prefrontal cortex along with increased amplitude of miniature excitatory postsynaptic currents (mEPSCs) while the CNTNAP2 KO mice have decreased GluR1 expressions with reduced mEPSCs. Interestingly, the social behavior deficits were normalized by the administration of AMPA receptor antagonist and agonist for VPA mice and CNTNAP2 KO mice, respectively. However, repetitive and hyperactive behaviors were not changed. Injecting AMPA agonist or antagonist in wildtype ICR mice induced dysregulation of the social behaviors in mice without inducing repetitive or hyperactive behaviors. These results suggest that modulating NMDA or AMPA pathway might provide plausible target of ASD therapeutics.

Keyword: AMPA, NMDA, autism spectrum disorder, social behavior, comorbid symptoms

[SY05-3-3]

MODELING AUTISM USING HUMAN NEURONS IN A DISH

Hyunsoo Shawn Je[1]

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Autism and autism spectrum disorders (ASDs) affect millions of individuals worldwide. Many genetic risk factors have been identified, but underlying pathophysiological mechanisms of ASD is not known. Here, we focus on Angelman syndrome (AS), a subset of ASDs, which results from the loss-of-function in UBE3A, a gene that encodes for ubiquitin protein ligase E3 (UBE3A). By using induced human neurons derived from isogenic UBE3A KO human embryonic stem cells engineered with CRISPR/CAS9, we have identified a novel channelopathy mechanisms of AS and developed a potential therapeutic approach to ameliorate neuronal dysfunction in human AS model in a dish.

Keyword: Autism, Angelman syndrome, UBE3A, ion channel, human embryonic stem cell

[SY05-3-4]

GENETIC STUDY OF PATIENTS WITH AUTISM SPECTRUM DISORDER AND ITS CLINICAL IMPLICATIONS

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[1]Seoul National University, Korea

Autism spectrum disorder (ASD) has strong genetic components, which complex interplay of multiple genetic variants contributes to its pathogenesis. ASD has high degree of heterogeneity of phenotypes in human subjects, including social behavior, communication, cognitive style, language development and comorbid symptoms, leading the genetic research of ASD to be more complicated. The objective of this presentation is to introduce the process and results of genetic studies of ASD in Korean population, focusing on the consideration about the relations between genotypes and phenotypes. 1) The strategy of collecting thorough phenotypes of human subjects with ASD and the results of candidate gene studies focusing on quantitative relationship of specific alleles and severity of symptoms will be presented. 2) Possible approach with single genetic syndrome will be discussed, in the context of discovery of novel candidate gene. 3) Current development of next generation sequencing will be presented, including Korean ASD whole genome sequencing project. 4) Other preliminary approaches for finding genetic biomarker of ASD will be discussed, including research for mitochondrial genes, translation of findings from animal model studies, and approaches with brain organoids. It is expected that this presentation would provide an opportunity to discuss about the prospect for human genetic studies of ASD as one of the most complex neurodevelopmental disorders.

Keyword: autism spectrum disorder, complex phenotype, human subjects, genetics

[SY05-4-1]

REGULATION AND FUNCTION OF DNA METHYLATION IN STEM CELLS

Salvatore Oliviero[1]

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DNA methylation is one of the most studied epigenetic modifications and is essential for gene regulation and mammalian development. DNA methylation on promoters is associated with gene repression, while its presence on the gene body marks active transcription. The methylation pattern is established through an extremely orchestrated mechanism that implicates de novo methylation, maintenance of the methylated cytosine, and demethylation. In mammals, DNA methylation occurs mainly at the cytosine that precede the guanine (CpG). DNA methylation is catalyzed by the well-characterized DNA methyltransferase family enzymes (DNMTs), including the DNMT3A and DNMT3B that together with DNMT3L are responsible for the de novo methylation and DNMT1 that is the mediator of the maintenance of the DNA methylation pattern through cell replication. DNA methylation is an epigenetic modification considered to be rather stable, although the active mechanism of DNA demethylation mediated by ten-eleven translocation methylcytosine dioxygenase (TET) and thymine DNA glycosylase (TDG) proteins have been well characterized. In our laboratory, we developed high-throughput methods to map genome-wide DNA methylation, intermediate products of DNA demethylation, and measured the regulation of transcription resulting from these changes. In stem cells, the promoters of developmental genes are not expressed but not methylated as their DNA will become methylated only later according to the specific cell developmental differentiation. We found that the promoter hypomethylation of developmental promoters in stem cells is due to the interaction between the Polycomb PRC2 complex, bound to the promoters, with DNMTs and TET enzymes. These interactions ensure epigenetic plasticity trough cell differentiation. The rules that govern the dynamics of DNA methylation/demethylation have not yet been fully clarified and represent a key need to deeply understand both normal development and diseases as DNA methylation is deregulated in many diseases including cancer. We observed that DNA methylation is an epigenetic feature more dynamic than previously thought. We found that the promoters of highly expressed genes are subjected to active DNA methylation and demethylation mediated by the interplay between the DNA methyltransferases DNMT1 and DNMT3A with the TET1/2 and TDG proteins. Alteration of this regulation, due to TET1 downmodulation in colon tumours, is a key player in tumour growth. The function of DNA methylation within the gene body has remained elusive, we recently found that intragenic DNA methylation, mediated by DNMT3B, protects the gene body from RNA

Polymerase II spurious entry and cryptic transcription initiations. Thus, intragenic DNA methylation is mechanism by which the cell prevents errors in the transcription initiations increasing in this way the accuracy of promoter regulation.

[SY05-4-2]

RPD3L HDAC LINKS H3K4ME3 TO TRANSCRIPTIONAL REPRESSION MEMORY

Taesoo Kim[1]

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Transcriptional memory is critical for faster reactivation of necessary genes and depends on their preceding active states. However, whether transcriptional repression also displays "memory" of the prior transcriptionally inactive state remains unknown. Here we show that transcriptional repression of approximately 540 genes in yeast occurs much more rapidly if the genes have been previously repressed during carbon source shifts. This novel transcriptional response has been termed transcriptional repression memory(TREM). Interestingly, Rpd3L HDAC targeted to active promoters induces TREM. Mutants for Rpd3L exhibit increased acetylation at active promoters and a significant delay of TREM and RNA PolII dissociation. Surprisingly, the interaction between H3K4me3 and Rpd3L via the Pho23 PHD finger is sufficient to induce histone deacetylation and TREM by Rpd3L. Therefore, we propose that an active mark, H3K4me3 enriched at promoters instructs Rpd3L HDAC to induce histone deacetylation and TREM.

Keyword: Transcriptional memory, TREM, Rpd3L HDAC, H3K4me3

[SY05-4-3]

ANALYSIS OF CHROMATIN ORGANIZATION DELINEATES REGULATORY PROGRAMS OF HUMAN CARDIOMYOCYTE DIFFERENTIATION

Bing Ren[1]

[1]*University of California, San Diego, USA*

Eukaryotic chromatin forms hierarchical structures to regulate gene expression. Yet, how chromatin architecture dynamically reorganizes to modulate gene regulatory programs during development remains to be elucidated. Here, we comprehensively interrogate the dynamic chromatin architecture during key developmental stages of human cardiomyocyte differentiation using human embryonic stem cells as a model system. We define activating and inhibitory chromatin loop interactions that predict genetic consequences of non-coding genetic variants associated with cardiac-related traits/diseases. We also reveal dynamic reorganization of chromatin domains during cardiomyocyte lineage-specification.

Keyword: Enhancer, Chromatin Organization, Transcription, Stem cells, lineage-specification

[SY05-4-4]

REGULATION OF CHROMATIN ARCHITECTURE BY CHD4 IN EMBRYONIC STEM CELLS

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The positioning of the nucleosome by ATP-dependent chromatin remodelers provides the fundamental chromatin environment for the regulation of diverse cellular processes acting on the underlying DNA. Recently, genome-wide nucleosome mapping has revealed more detailed information on the chromatin-remodeling factors. Using genome-wide approaches, we analyzed total RNA transcriptomes, mRNA transcriptomes, histone modifications ChIP-seq, nucleosome positioning, ATAC-seq and Hi-C profiles by knockdown of chromatin remodeler in mouse embryonic stem cells. In this talk, I will describe how chromatin remodeler regulates nucleosome occupancy and how these activities are linked to 3D architecture of embryonic stem cell to maintain pluripotency. Finally, I will discuss the recent progress about the potential relevance of 3D architecture for regulating chromatin stability in mouse embryonic stem cells.

Keyword: Chromatin loop, Transcription, Stem Cell, chd4, Chromatin Remodeler

[SY05-4-5]

BIOCHEMICAL CHARACTERIZATION OF THE HUMAN H3K4 METHYLTRANSFERASE COMPLEXES

Jaehoon Kim[1]

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Methylation on lysine 4 of histone H3 (H3K4) is one of the prominent histone modification marks that correlate strongly with active transcription in eukaryotes. Accumulating studies in metazoans have implicated misregulation of H3K4 methylation in the pathogenesis of cancer and in developmental defects, further emphasizing the importance of understanding the regulation of H3K4 methylation. In budding yeast, a single H3K4 methyltransferase (Set1) complex can methylate all H3K4 in an H2B ubiquitylation-dependent manner. However, presence of at least six H3K4 methyltransferase (SET1A, SET1B, MLL1, MLL2, MLL3 and MLL4) complexes in mammalian cells has complicated functional characterization of each complex because of their possible redundant and non-redundant roles. To understand the roles of H3K4 methyltransferases in transcriptional regulation in mammalian cells, we have deployed biochemical analyses with purified human H3K4 methyltransferase complexes. By taking advantage of an in vitro histone methyltransferarse assay employing a reconstituted human H3K4 methyltransferase complexes and a recombinant chromatin template containing fully ubiquitylated H2B, we found that a subset of human H3K4 methyltransferase complexes exhibit H2B ubiquitylation-dependent H3K4 methylation activity. In addition, we demonstrate different subunit compositions and subunit interaction networks in human H3K4 methyltransferase complexes. Our studies establish minimal components of the H3K4 methyltransferase complexes required for H3K4 methylation and provide a mechanistic basis for H3K4 methylation in mammalian cells.

Keyword: Chromatin, H3K4 methylation, H2B ubiquitylation, Transcription

[SY05-5-1]

STRUCTURE DYNAMICS AND KINETICS OF FOLDING AND RECOGNITION IN PROTEINS BY NMR

Christian Griesinger[1]

[1]MPIbpc, Germany

Structure dynamics and kinetics of folding and recognition in proteins by NMR S. Prathihar1, C. Smith1,2, J. Reddy1, T.M. Sabo1, L. Wong1, L. Russo1, J. Kühn3, S. Pirkuliyeva3, D. Lee1, T.M. Sabo1, S. Ryzanov1,4, L. Antonschmidt1,4, A. Martinez Hernandez5, H.Y. Agbemenyah6, S. Shi7, A. Fischer6, G. Eichele5, D. Lee1, S. Becker1, A. Leonov1,4, R. Benz8, M. Zweckstetter1,4, J. Wienands3, A. Giese7, and C. Griesinger1,4 1Dept. for NMR-based Struct. Biology, Max-Planck Institute for Biophysical Chemistry; 3Institute of Cellular and Molecular Immunology, Georg August University of Göttingen, Göttingen; 4DFG-Center for the Molecular Physiology of the Brain, Göttingen; 5Genes and Behavior Dept., Max-Planck Institute for Biophysical Chemistry, Göttingen; 6 European Neuroscience Institute Göttingen;, 7 Center for Neuropathology and prion research, LMU, Munich, Germany; 8 Jacobs University of Bremen, Germany Kinetics of protein dynamics will be discussed on examples of folded on unfolded proteins (1). Protein recognition will be described with a new mathematical method to distinguish conformational selection and induced fit (2) which includes a concept for the measurement. Further, the role of partially disordered proteins in droplet formation is investigated. The adaptor protein SLP65 which interacts with CIN85 (3). The two proteins are essential for B cell activation. The protein is found to be mainly unstructured and its various segments entertain different functions or interact with membranes, SH3 domains and forming coiled coils. Based on the structures, a molecular lego will be described that reduces the SLP65/CIN85 interaction to its absolutely necessary essentials. The two proteins can perform phase separation which is related to function. We are additionally interested in a class of IDPs that are important in neuro- and cellular degeneration, which form oligomers and fibrils. Interference with these aggregates specifically on the oligomer level proves to be a valid concept for treatment of devastating diseases such as Parkinson's, Alzheimer's, Creutzfeldt Jacob disease and Type II diabetes mellitus (4). Suprising links to some cancers can be identified which will be also be discussed in the lecture (5). (1) C. Smith et al. Proc. Natl. Acad. Sci. USA. 113, 3296-74 (2016); C. Smith et al. Angew. Chem. Int. Ed. 54, 207-10 (2015) (2) Paul, F. and T.R. Weikl, Plos Computational Biology, 2016. 12(9). (3) M. Engelke et al. Science signaling: 7 (339) ra79 (2014); J. Kühn et al. Science signaling: 9 (434) ra66 (2016) (4) C.W. Bertoncini et al. PNAS 102, 1430-1435 (2005); P. Karpinar et al. EMBO J 28, 3256-3268 (2009); J. Wagner et al. Acta Neuropath. 125, 795-813 (2013);

A.A. Deeg, Biochim. Biophys. Act. 1850 (9), 1884-1890 (2015); S. Shi, J. Neuropath. Exp. Neurol. 74(9) 924-933 (2015); J. Wagner et al. Act. Neuropath.130, 619-631 (2015) (5) E. Turriani, et al. Proc. Natl. Acad. Sci. USA 114, E4971-E4977 (2017)

[SY05-5-2]

GENOMIC AND BLOOD BIOMARKER APPROACHES IN DIFFERENTIATING VARIOUS NEURODEGENERATIVE DISEASES

Seong Soo An[1], Van Giau Vo[1], Eva Bagyinszky[1], Kyuhwan Shim[1], Angelo Jamarlan[1], Sungmin Kang[2], Young Chul Youn[3] and Sangyun Kim[4]

[1]Gachon University Medical School, Korea, [2]PeopleBio Inc., Korea, [3]Chungang University Hospital, Korea, [4]Seoul National University Bundang Hospital, Korea

Several neurodegenerative diseases could present overlapping clinical phenotypes of diseases. The development of differentiate diagnoses would be crucial in the clinical setting. Hence, in our efforts to tackle the differential diagnosis of dementia patients, genetic and biomarker profiling analyses were developed for various neurodegenerative diseases. Majority of disease-causing gene mutations were uncommon in the general population, where dominant variations could be easily identified in certain neurodegenerative disorders. The development of molecular, next generation sequencing (NGS) and cytogenetic techniques allowed to identify multiple genetic mutations leading to diseases. The accurate multivariate diagnosis of diseases would be essential for appropriate treatment of patients, genetic counseling and prevention strategies. Many genetic studies have clearly indicated the molecular mechanisms underlying the etiology and pathogenesis of most neurodegenerative disorders. Our analysis could discern several types of neurodegenerative diseases associated with abnormal gene functions in Alzheimer's disease (AD), front temporal dementia (FTD), amyotrophic lateral sclerosis (ALS), prion disease, and Parkinson's disease (PD). After the identification of the mutations, their functional ramifications were probed with bioinformatics tools including protein structure predictions. Secondly, a reliable blood-based assay will dramatically aid in proper diagnosis and monitoring of disease progress. Alzheimer's disease (AD) multimer detection system (MDS) was initially developed for the detection of prion oligomers in the blood for prion diseases, such as scrapie in mouse, hamster, and sheep, Bovine Spongiform Encephalopathy in cattle, Creutzfeldt-Jakob disease in human. Recently, MDS was developed and applied for measuring amyloid-β oligomers (AβOs) in the blood for AD. Synthetic Aβ was spiked into both the blood plasma of patients with AD and elderly normal controls, and the induced dynamic changes of AβOs from plasma of patients with AD was observed over time. A large number of samples could be differentiated by MDS with high sensitivity and specificity, suggesting it as a simple, noninvasive, and accessible blood-based assay for AD diagnosis. In conclusion, we would like to suggest the need to develop and perform both genetic and biomarker analyses for better understanding of the clinical manifestations, diagnosis and

prognosis of various neurodegenerative diseases.

Keyword: genome, biomarkers, dementia

[SY05-5-3]

STRUCTURAL & KINETIC STUDIES OF OLIGOMERIC AGGREGATES OF ALPHA-SYNUCLEIN

Daniel Fortunati[1], Anna Hastings[1], Ji Yoon Kim[1], Soyoung Min[1], Igor Klyubin[1], Chiara Rotella[2], Khizar Sheikh[3], Michael Rowan[1], Brian Rodriguez[2], Kenneth Hun Mok[1] and Young-Ho Lee[4]

[1]Trinity College Dublin, Ireland, [2]University College Dublin, Ireland, [3]Bruker, Ireland, [4]Osaka University, Japan

Amyloid aggregates of the protein α-synuclein are a hallmark of a family of neurodegenerative diseases known as Lewy body pathologies, which include Parkinson's. Recent evidence suggests that early oligomeric forms may have more of a pathogenic effect than mature fibrils. [1-3] In this study, high performance liquid chromatography (HPLC), together with an array of spectroscopic techniques, was used to investigate the formation of α-synuclein oligomers in real time. The main advantage of HPLC over other methods being its ability to quantify the size distribution of a heterogeneous mix of molecules as individual measurements rather than as a population averaged value. The hydrodynamic radii of α-synuclein at different stages of aggregation were also calculated under native and denaturing conditions. The results obtained were cross-verified using atomic force microscopy (AFM) and transmission electron microscopy (TEM). The effects on long term potentiation of the α-synuclein oligomers produced were studied using in vivo electrophysiology experiments on rats, and the oligomers but not the monomers of α-synuclein were found to have a significant baseline effect on long term potentiation. 1. Danzer, K.M., et al., Different species of alpha-synuclein oligomers induce calcium influx and seeding. J Neurosci, 2007. 27(34): p. 9220-32. 2. Fusco, G., et al., Structural basis of membrane disruption and cellular toxicity by α-synuclein oligomers. Science, 2017. 358(6369): p. 1440-1443. 3. Chen, S.W., et al., Structural characterization of toxic oligomers that are kinetically trapped during α-synuclein fibril formation. Proc Natl Acad Sci U S A, 2015. 112(16): p. E1994-2003.

Keyword: Protein misfolding, Alpha-synuclein, Neurodegeneration, HPLC, AFM

[SY05-5-4]

INTRINSICALLY DISORDERED PROTEIN REGIONS AS TARGETS: SMALL MOLECULE BINDING TO C-MYC

Steven Metallo[1]

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Intrinsically disordered (ID) proteins lack stable structure, existing instead as ensembles of rapidly interconverting conformations. In eukaryotes, ID proteins are prevalent and overrepresented in major disease pathways, however, their lack of a stable structure has meant these proteins were not considered potential drug targets. We have found that short, linear segments in the dimerization region of monomeric, disordered Myc can bind to small molecules. Using mutagenesis of one of these binding sites on Myc, we find that both hydrophobic and hydrophilic amino-acids affect binding. Over half of the residues in the binding site are important for binding affinity, allowing us to provide a potential model for how specificity can be achieved without structure. We also find that disordered regions outside the binding site influence the binding of small molecules to Myc as well as the self-association of Myc.

Keyword: IDP, c-Myc, Protein-Protein interaction, Protein-binding, inhibitor

[SY05-5-5]

THE MDM2-INHIBITION MECHANISM BY A PRE-STRUCTURED P53 RESCUE MOTIF IN A HYBRID TYPE DISORDERED PROTEIN SUSP4

Kyou-Hoon Han[1]

[1]KRIBB, Korea

The mdm2-inhibition mechanism by a pre-structured p53 rescue motif in a hybrid type disordered protein SUSP4 Kyou-Hoon Han Korea Research Institute of Bioscience and Biotechnology/UST Daejeon, Korea Many intrinsically unstructured/unfolded proteins (IUPs) contain transient local secondary structures even though they are "unstructured" in a tertiary sense. These local secondary structures are named "pre-structured motifs (PreSMos)" [1] and in fact are the specificity determinants for IUP-target binding, i.e., the active sites in IUPs. Using high-resolution NMR we have delineated a PreSMo active site in the intrinsically unfolded mid-domain (residues 201-300) of SUMO-specific protease 4 (SUSP4) [2]. This 29-residue motif which we termed a p53 rescue motif can protect p53 from mdm2 quenching by binding to the p53-helix binding pocket in mdm2(3-109). Our work demonstrates that the PreSMo approach is quite effective in providing a structural rationale for interactions of p53-mdm2-SUSP4 and opens a novel avenue for designing mdm2-inhibiting anticancer compounds. References: [1] Lee, S. H., et al., (2012) Understanding pre-structured Motifs (PreSMos) in intrinsically unfolded proteins: Curr. Protein. Pept. Sci., 13, 34-54 [2] Kim, D., et al (2017) The Mechanism of p53 Rescue by SUSP4: Angew. Chem. Int. Ed., 56, 1278 –1282

Keyword: p53, IUP, PreSMo, SUSP4, NMR

[SY06-1-1]

CHALLENGES & OPPORTUNITIES IN THE COMMERCIALIZATION OF ACADEMIC SCIENCE

Juan Carlos Lopez[1]

[1]*Haystack Science, USA*

For the past few years, there has been a strong drive to commercialize the discoveries that emerge from academic institutions. In a short time, venture investors and pharmaceutical companies have explored several models looking for the best way to interact with academia. At the same time, governments have become increasingly active in this space, promoting the creation of biotechnology ecosystems as engines of economic growth. But despite these developments, the process whereby new discoveries reach the clinic remains very inefficient. In this talk, I review the current state of play in this field, the obstacles that prevent progress, and possible ways in which the relationship between academia and investors can be improved.

Keyword: Translational research

[SY06-1-2]

CONVENING THE ACADEMIC INDUSTRIAL INTERFACE---A NATURE EDITOR'S PERSPECTIVE

Andrew Marshall[1]

[1]*Nature Biotechnology, USA*

A discussion of the challenges in taking a discovery out of an academic lab and commercializing it, with Nature SciCafes and Nature Masterclass program as Nature initiatives in this space. Numerous problems face faculty working in an academic institution that lack the necessary infrastructure to facilitate transfer and commercialization of biomedical research projects. Many of the same issues are encountered across Europe, Asia and even in the United States outside of Boston and the Bay Area.

Keyword: TRANSLATION, TECHNOLOGY TRANSFER, INDUSTRY, ACADEMIA, COMMERCIALIZATION

[SY06-2-1]

MITOCHONDRIAL DYNAMICS AND INNATE IMMUNITY IN CHRONIC HEPATITIS B AND C

Aleem Siddiqui[1]

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Establishment of hepatitis B and C virus infection associated chronic hepatitis is facilitated by the weak and narrowly focused immune response. Innate immunity, the first line of defense, as well as adaptive immune response is crippled paving the ways for chronic hepatitis. Mitochondrial dynamics is tightly regulated in response to alterations in cellular physiology and its dysfunction that occurs during chronic hepatitis B and C. Our work shows that HBV and HCV induce mitochondrial fission and mitophagy. Hallmark of mitophagy is the mitochondrial translocation of Parkin protein, implicated in Parkinson's disease. Inhibiting of mitophagy via Parkin silencing caused a robust cytochrome C release and an increase in apoptotic signaling molecules. These results clearly implicate that modulation of mitochondrial dynamics and induction of mitophagy by HBV/HCV is required for maintenance of persistent chronic infection. Our results also revealed that HBV-induced Parkin translocation to mitochondria inactivates antiviral mitochondrial signaling protein (MAVS) and inhibits interferon (IFN) synthesis, thus crippling innate immunity, the first line of defense. Our work explains the molecular mechanism by which HBV inactivates IFN synthesis. We found that MAVS is massively ubiquitinated both by Parkin, an E3 ubiquitin ligase and mitochondrially-recruited LUBAC (linear ubiquitin), which together abrogate downstream signaling events leading to suppression of IFN synthesis. These studies provide a unique insight into the underlying mechanisms of mitochondria-mediated liver injury that contributes to the chronic hepatitis and ultimately paves the way to the onset of hepatocellular carcinoma.

Keyword: Hepatitis virus, Mitochondrial dynamics, Parkin, MAVS, Innate immunity

[SY06-2-2]

ENHANCING THE IMMUNOGENICITY OF THE ANTIGEN USING ANTIGEN-TARGETING STRATEGY TO M CELLS IN MUCOSAL IMMUNE COMPARTMENTS

Sae-Hae Kim[1], Ju Kim[1], Jisang Park[1] and Yong-Suk Jang[1]

[1]Chonbuk National University, Korea

Mucosa-associated lymphoid tissue (MALT) is defined as solitary organized mucosa-associated lymphoid follicles and is subdivided by anatomical regions. MALT is characterized as lacking afferent lymphatics. Consequently, it only takes up exogenous antigens through its follicle-associated epithelium, which contains enterocytes, goblet cells, and microfold (M) cells. Among the MALT, the gastrointestinal mucosa maintains a tolerogenic microenvironment to protect the body from unwanted induction of the immune response to continuously exposed commensal microorganisms and food antigens. Considering that 90% of infections occur in mucosal areas, it is conceivable that using mucosal vaccination to establish protective immunity in this frontline of pathogen infection could offer great advantages in current vaccination strategy. However, the number of currently available oral vaccines is very limited compared to the number of parenteral vaccines. This limited availability of oral mucosal vaccines is closely related with the lack of an effective antigen delivery system and a strong adjuvant to stimulate immunity due to the intrinsic nature of the mucosal immune system, which has a low efficiency in antigen delivery into the inductive site and a tendency to induce oral tolerance. We have concentrated our efforts to elaborate efficient antigen delivery system to M cells to establish the strategy for effective mucosal vaccine development. Various antigen-targeting strategy with adjuvant activity which includes peptide ligands, adhesion molecule, and antimicrobial peptide against various viral antigens will be discussed. (This study was supported by the research fund from Korea Research Institute of Chemical Technology, CEVI-2016-3-1 and by the National Research Foundation (NRF) funded by the Korean Ministry of Science, ICT, & Future Planning, 2014K1B1A1073861 & 2016R1A2B2010096.)

Keyword: Adjuvant, Antigen, M cell, Mucosal Immunity, Vaccine

[SY06-2-3]

VIRUS-LIKE PARTICLES EXPRESSING RECOMBINANT CHIMERIC PROTEIN AS A VACCINE PLATFORM AGAINST EMERGING AND NEW-EMERGING VIRUSES

Jae Min Song[1]

[1]Sungshin Women's University, Korea

Virus-like particles (VLPs) are artificial protein structures with the similar overall structure to their corresponding native viruses. VLPs are able to provide distinctive three-dimensional conformation which is especially important for the presentation of conformational epitopes. First-generation VLP approaches have yielded successful vaccines against HPV and several veterinary diseases. However, these approaches offer limited rational design potential and cannot be applied to all enveloped viruses. To overcome these, we proposed hybrid VLP technology expressing recombinant chimeric protein created through the joining of transmembrane and/or cytoplasmic domain of influenza HA and antigenic protein from other viruses. Recombinant chimeric proteins display in the surface of VLPs that budded and released from a host cell with influenza M1 protein as a core protein in VLP particles. The development of many hybrid VLPs as platforms for foreign antigen display has further broadened their potential applicability both as prophylactic and therapeutic vaccines. In this presentation, we provides an overview on the design and use of VLPs for the development of new generation vaccines against emerging and new-emerging viruses.

Keyword: Virus-like particles, vaccine, platform

[SY06-2-4]

CRISPR SCREENING IDENTIFIES HOST FACTORS ESSENTIAL FOR VIRAL INFECTION

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Pooled CRISPR screens based on lentiviral systems have been widely applied to identify the effect of gene knockout on cellular phenotype. Although many screens were successful, they also have the limitation that genes conferring mild phenotypes or those essential for growth can be overlooked as every genetic perturbation is incorporated in the same population. Arrayed screens, on the other hand, incorporate a single genetic perturbation in each well, and could overcome these limitations. However, arrayed screens based on siRNA-mediated knockdown were recently criticized for low reproducibility caused by incomplete inhibition of gene expression. To overcome these limitations, we developed a novel arrayed CRISPR screen based on a plasmid library expressing a single guide RNA (sgRNA) and disrupted 1,514 genes, encoding kinases, proteins related to endocytosis, and Golgi-localized proteins, individually using 4,542 sgRNAs (3 sgRNAs per gene). This screen revealed host factors required for infection by coxsackievirus B3 (CVB3) from Picornaviridae, which includes human pathogens causing diverse diseases. Many host factors that had been overlooked in a conventional pooled screen were identified for CVB3 infection, including entry-related factors, translational initiation factors, and several replication factors with different functions, demonstrating the advantage of the arrayed screen. This screen was quite reliable and reproducible, as most genes identified in the primary screen were confirmed in secondary screens. Moreover, ACBD3, whose phenotype was not affected by siRNA-mediated knockdown, was reliably identified. We propose that arrayed CRISPR screens based on sgRNA plasmid libraries are powerful tools for arrayed genetic screening and applicable to larger-scale screens.

Keyword: CRISPR, Enterovirus, Host factor, screening, arrayed

[SY06-3-1]

TWENTY YEARS OF GROWTH, DEVELOPMENT AND CONTROVERSY

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Resveratrol (3,4',5-trihydroxy-trans-stilbene) was first isolated in 1939 by Takaoka from Veratrum grandiflorum O. Loes (root of the white hellebore). It can be speculated the trivial name resveratrol was created as a conjunction based on its chemical structure and the plant source used for isolation: a resorcinol derivative or polyphenol in resin, occurring in Veratrum species, and containing hydroxyl groups (ol). Subsequently, sporadic reports appeared in the literature, most of which were descriptive in nature. However, spurred by our seminal paper published early in 1997, resveratrol became a household word and the subject of intensive investigation. Now, in addition to being the focus of over 18,000 research papers and hundreds of review articles, resveratrol has inspired monographs, conferences, symposia, patents, chemical derivatives, etc. In addition, several commercial products are marketed under various tradenames, some in combination with other natural products. Once resveratrol was brought to the limelight, early research tended to focus on pharmacological activities related to the cardiovascular system, inflammation, and carcinogenesis/cancer development. However, over the years, the horizon has greatly expanded. At least 117 human clinical trials have been or are being conducted to explore the potential of mediating an array of biological responses, including effects on the aging process, diabetes, neurological dysfunction, obesity, endometriosis, respiratory infections, etc. Of course, some controversy has been generated, as is often the case with substances receiving such colossal attention. For example, some discrepancies exist between in vivo studies with animals and clinical investigations, or between clinical studies. This is likely due to factors such as disparate doses (ca. 5 to 5,000 mg/day), varying experimental settings, and subject variation. Molecular targets are numerous and a common mechanism is elusive or nonexistent. In essence, the compound may be viewed as promiscuous. However, since the safety profile is pristine, and use as a dietary supplement is prevalent, these features are not viewed as a disadvantage. Given the history of resveratrol, it is reasonable to advocate for additional development and further clinical investigations. Topical preparations seem especially promising, as do conditions that respond to anti-inflammatory action. Although the ultimate fate of resveratrol remains an open question, thus far the compound has inspired innovative scientific concepts and enhanced public awareness of preventative health care. This is quite phenomenal considering the structural simplicity of resveratrol, as well as common occurrence in the diet of humans, particularly as a result of grape

consumption.

Keyword: Resveratol, Natural Products, Chemoprevention

[SY06-3-2]

ENGINEERED BIOSYNTHESIS OF MEDICINAL NATURAL PRODUCTS

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Meroterpenoids are hybrid natural products that are partially derived from terpenoids, and those from fungi exhibit an extremely wide range of structural diversity and biological activities. Recent advances in genome sequencing technologies and development of tools for biosynthetic studies have allowed the discovery of many biosynthetic gene clusters for fungal meroterpenoids and intensive researches at genetic and enzymatic level. We have been working on the meroterpenoids derived from a simple aromatic precursor, 3,5-dimethylorsellinic acid (DMOA), and discovered several fascinating enzymes that catalyze drastic structural rearrangement which dramatically increase structural complexity of the molecules. For example, multifunctional, non-heme iron dependent oxygenases are the key components in the austinol and the paraherquonin pathway, in which the enzymes are responsible for the construction of the spiro-lactone and cycloheptadiene moiety, respectively. On the other hand, the terretonin biosynthesis involves a cytochrome P450 and an isomerase, which work collaboratively to perform the unprecedented ring expansion reaction to afford the terretonin scaffold. This presentation will focus on molecular basis for the unusual ring reconstruction reactions in fungal meroterpenoid biogenesis.

Keyword: Biosynthesis, Natural Product, Enzyme

[SY06-3-3]

DESIGN IDEA OF 'ULTRA HYPOTOXIC MULTIDRUG' BASED ON THE 'EFFICACY THEORY' OF TRADITIONAL CHINESE MEDICINES (TCMS)---- 'EFFECTIVE FORM', 'ADDITIVE EFFECT', AND 'TOXICITY SCATTERING EFFECT 'OF PHARMACODYNAMIC SUBSTANCES OF TCMS

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We provide here a traditional Chinese medicines (TCMs) efficacy theory [1]: 'A TCMs exerts its effect through both the additive effects of numerous effective forms (EFs, including con-stituents or/and their metabolites) on the same target and the synergistic effects based on the overall action of the additive effects on individual targets, and the TCMs weakens its toxicities through their toxicity scattering effects.' Since a TCMs may include more than 1000 constituents and each constituent may produce over 100 metabolites in vivo after administration, we consider that numerous EFs among incalculable constituents and their metabolites could work together; when the quantity of molecules of a target is larger than that of the drug molecules, the molecules of different EFs could combine with the molecules of the target successively, to exert the additive effects; when the target molecules are mostly occupied, the TCMs begins to work. The additive effects and the toxicity scattering effects of the EFs are resulted from the same efficacy centres and not identical toxic centres among the structures of different EFs. Based on the assumption that all EFs possess different structures and thereby their toxicities are more or less different from one another and based on the assumption that each active compound can act on the same target, we can design a combination drug that is composed of over 10 active compounds, and as a result the amount of each compound can be reduced to below 1/10, and therefore their toxicities will be reduced to 1/10 or less. In addition, 3-5 groups (over 10 compounds for each) which can act on different targets of the same disease, can be chosen to form a much bigger combination with over 30-50 compounds, which should have great advantages of high efficiency of multiple targets and significantly reduced toxicity and this is called ultra hypotoxic multidrug.

Keyword: Bioactive compound, Additive effects on the same target, Synergistic effects among individual targets, Toxicity scattering effects, Ultra hypotoxic multidrug

[SY06-3-4]

DISCOVERY OF NEW BIOACTIVE NATURAL PRODUCTS FROM SYMBIOTIC BACTERIA IN INSECT ECOSYSTEMS

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Symbiotic bacteria in insects have recently drawn a significant attention as an untapped source of new bioactive compounds, which may play protective roles for insect hosts. Secondary metabolites from symbiotic bacterial strains in diverse insects were explored based on LC/MS chemical analysis. Chemical profiling and dereplication of metabolites prioritized the strains with novel chemistry. New bioactive natural products were discovered from symbiotic bacteria such as the dung beetle (Copris tripartitus), the silkworm (Bombyx mori), the carrion beetle (Nicrophorus concolor), the carpenter ant (Componotus japonicus) and other insects. These new compounds include diverse structural classes such as polyketides, non-ribosomal peptides, glycolipids, and even surprisingly flavonoids. Their bioactivities were explored for inhibitory activities against human pathogenic and entomopathogenic microbes and a few disease-relevant enzymes. The discovery of structurally, biologically, and biosynthetically interesting secondary metabolites from insect symbionts demonstrates that studying insect symbionts in search for new bioactive compounds and deciphering their natural and clinical functions could be a new promising strategy in natural product-based drug discovery.

Keyword: natural products, insect, bacteria, symbiosis, structure elucidation

[SY06-4-1]

MYC CANCER GENE CLOCKS OUT FOR LUNCH

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The MYC oncogene is commonly altered in human cancers through gene amplification. We have documented that the MYC protein behaves as a transcription factor, which regulates thousands of genes involved in cell metabolism, growth, and proliferation. Unlike, the normal proto-oncogene that is under tight regulation, deregulated expression of oncogenic MYC drives constitutive biosynthesis through induction of ribosome and mitochondrial biogenesis, and heightened anabolic metabolism. This constitutive drive for biomass accumulation renders MYC-driven cells dependent on glucose and glutamine, whose withdrawal resulted in cell death. As such, drug-like small molecules have been developed for anti-cancer treatment, such as glutaminase and lactate dehydrogenase inhibitors. These inhibitors contextually inhibit tumor xenograft growth. The vulnerability of tumors to metabolic interventions is likely dependent on the genetic alterations of the cancer and its metabolic re-wiring. Further, host metabolism oscillates diurnally and is regulated by the circadian clock. Peak host metabolism could result in metabolic toxicity depending on the time of day of treatment. In this regard, we found that the MYC oncogene disrupts the molecular clock and metabolism in favor of tumor growth, such that timing of metabolic inhibition could spare normal tissue toxicity while maintaining an anti-tumor effect. When MYC clocks out for lunch, the timing of treatment or chronotherapy could result in better efficacy and diminished toxicity.

Keyword: MYC, oncogene, metabolism, circadian clock, drug development

[SY06-4-2]

TRACING THE INTERPLAY BETWEEN AMINO ACID METABOLISM AND LIPID DIVERSITY IN CONTROLLING TUMOR GROWTH

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Metabolism is central to virtually all cellular functions and contributes to a range of diseases. A quantitative understanding of how biochemical pathways are dysregulated in the context of diseases such as cancer and metabolic syndrome is necessary to identify new therapeutic targets. To this end we apply stable isotope tracers, mass spectrometry, and metabolic flux analysis (MFA) to study metabolism in mammalian cells, animal models, and human patients. Using these approaches we have characterized how proliferating and differentiated cells regulate flux of glucose and amino acids into lipid biosynthetic pathways. We are particularly interested in understanding how amino acid and lipid metabolism are coordinated to meet these needs. Transport of pyruvate into mitochondria is a critical step in these processes, and activity of the mitochondrial pyruvate carrier (MPC) strongly influences cancer growth in vivo and as tumor spheroids. Using metabolomics and lipidomics approaches we have identified mechanisms through which MPC flux impacts the production of toxic sphingolipids. Modulation of serine availability and sphingolipid metabolism strongly impact cancer cell growth, providing mechanistic insights into how amino acid metabolism can influence lipids and mitochondrial function.

Keyword: TUMOR METABOLISM, ANCHORAGE-INDEPENDENT GROWTH, SPHINGOLIPIDS, SERINE, METABOLIC FLUX ANALYSIS

[SY06-4-3]

TUMOR MOLECULAR SUBTYPING AND METABOLIC CHARACTERISTICS

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Recent molecular classification of solid cancers has identified subtypes which have distinct biological drivers reflected on transcriptome profiles. A deeper understanding of the molecular basis of cancer subtypes might lead to new classes of therapies that selectively target aberrant molecular mechanisms that are crucial for the survival and proliferation of cancer cells. Moreover, identifying metabolic characteristics specific to molecular subtypes will facilitate to find actionable metabolic vulnerabilities leading to a more rationalized approaches to current standard of care.

Here I will discuss molecular subtyping of large scale transcriptome analysis that identifies distinct subtypes of solid cancer (GC) and its clinical implication in precision medicine. Careful assessment of GC patients' molecular tributes to assign each patient into appropriate subgroup will guide molecular information driven precision cancer care. Moreover, metabolic characteristics specific to each molecular subtype might identify unprecedented opportunities to target "chemorefractory" subtype based on their metabolic vulnerabilities to improve survival and to achieve durable responses.

Collectively, identification and characterization of metabolic subtypes related to tumor molecular subtypes may contribute to broadening the current understanding of cancer biology and implementation of precision cancer medicine.

[SY06-4-4]

METABOLIC VULNERABILITIES OF LUNG SQUAMOUS CELL CARCINOMA

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Adenocarcinoma (ADC) and squamous cell carcinoma (SqCC) are the two predominant subtypes of non-small cell lung cancer (NSCLC), a set of diseases distinct in their histological, molecular, and clinical presentation. However, targetable metabolic signatures specific to individual NSCLC subtypes remain unknown. Our integrative analysis of human NSCLC tumor samples, patient-derived xenografts, the KrasG12D; Lkb1-/- murine model of NSCLC, NSCLC cell lines, and The Cancer Genome Atlas (TCGA) has uncovered markedly elevated expression of the GLUT1 glucose transporter in lung SqCC, which augments glucose uptake and fuels glycolytic flux as core metabolic features. We demonstrate that a critical reliance on glycolysis renders lung SqCC vulnerable to inhibition of GLUT1-mediated glycolytic metabolism, while lung ADC exhibits significant glucose independence. Clinically, elevated GLUT1-mediated glycolysis in lung SqCC strongly correlates with distinctly high 18F-FDG uptake in PET scan and poor prognosis. Our findings reveal previously undescribed metabolic heterogeneity of NSCLC subtypes and implicate significant potential for the development of diagnostic, prognostic, and targeted therapeutic strategies for NSCLC, including the potential to exploit the unique glycolytic reliance of lung SqCC, a cancer for which existing treatments have proven to be clinically insufficient.

Keyword: LUNG CANCER, GLUCOSE, METABOLISM, GLUT1

[SY06-5-1]

DONGHUN AWARD; DIVERSE REGULATORS OF VASCULAR PATTERNING AND DYSFUNCTION

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[1]Yonsei University, Korea

Patterning of new vascular structure is a coordinated multi-step process that involves sprouting and morphogenesis of endothelial cells (ECs) and requires the formation of asymmetric EC phenotypes and their dynamic interconversion. These processes are precisely controlled by a large number of genes and specific gene expression within developing vessel is indispensable for establishing functional vascular network. In order to identify novel genes, which are potentially involved in regulating angiogenesis and vascular patterning, we have set up in vitro EC differentiation model and analyzed gene expression profile during the differentiation by employing DNA microarray and mRNA sequencing methods. We have isolated a number of genes, which have unique expression patterns in ECs. Among these genes, Yap1 and Snail, which are differentially expressed by cell to cell contact and cell to ECM interaction, regulated endothelial sprouting by transcriptional regulation of angiopoietin-2 and VEGFR3, respectively. We have also identified Clec14a as a novel regulator of VEGFR2 and VEGFR3 signaling in ECs. These findings suggest usefulness of our system in revealing spatial and temporal involvement of various genes during vascular patterning and offer new opportunities for treating human diseases associated with vascular abnormality.

Keyword: vascular, endothelial cell, angiogenesis

[SY06-5-2]

DI AWARD; PROTEIN TRANSDUCTION TECHNOLOGY : APPLICATION OF CLINICAL PROTEIN THERAPY FOR HUMAN DISEASE

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Gene therapy and protein therapy are based on the delivery of target genes or proteins into cells instead of traditional chemotherapy to treat human diseases. According to the recent findings that abnormal activity of cellular proteins is responsible for the generation of a variety of human diseases, it become a major concern that development of therapeutic agents against fatal human diseases could be achieved by regulating the biological activity of these proteins. Nevertheless peptides or proteins are superior to other chemicals in the selectivity and efficacy of their action mode, utilization of these peptides/proteins as the therapeutic drugs is severely limited due to the difficulty of delivery into cells. To solve this delivery problem and improve protein therapy, the specific aim of this study is to develope a novel Protein Transduction Technology (PTT) by which a variety of biologically active proteins could be delivered into cells directly and efficiently for clinical therapy of human diseases. This Protein Transduction Technology is based on the previous findings that exogenous HIV-1 Tat (Transactivator) protein was able to transduce through the plasma membrane and to reach both the cytosol and nucleus in the cell. A basic domain of Tat protein spanning from residue 49 to 57 has been identified to be responsible for this translocation activity and this 9 amino acid sequence is called Protein Transduction Domain (PTD). Other various PTDs were identified such as the 3rd helix of the Antennapedia protein homeodomain, PEP-1 etc... This Protein Transduction Technology could be utilized as a reliable general technique for efficiently delivering a variety of biologically functional target proteins into large numbers of living cells or organisms for the treatment of various human diseases including Neurological Diseases(Brain Ischemia, Parkinson's Disease, Epilepy etc), Atopic Dermatitis(AD), Dry Eye, Diabetes etc...

Keyword: Protein Transduction Technology, Protein Transduction Domain, Protein Therapy, Human Diseases, Delivery System

[SY06-5-3]

MOOSA AWARD; MOLECULAR AND INFLAMMATORY SIGNALINGS LEADING TO BREAST CANCER PROGRESSION

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Elevated expression and aberrant activation of Ras have been implicated in breast cancer aggressiveness. H-Ras, but not N-Ras, induces breast cell invasion. A crucial link between lipid rafts and H-Ras function has been suggested. The present study sought to identify the lipid raft protein(s) responsible for H-Ras-induced tumorigenicity and invasiveness of breast cancer. Through a comparative proteomic analysis of lipid raft proteins, we identified a lipid raft protein flotillin-1 as an important regulator of H-Ras activation and invasion in triple-negative breast cancer (TNBC) cells. Our findings provide insight into the molecular basis of Ras isoform-specific interplay with flotillin-1, leading to tumorigenicity and aggressiveness of breast cancer. C-reactive protein (CRP), a major human acute-phase protein synthesized in response to various inflammatory stimuli, has been associated with breast cell invasion and breast cancer risk. The process of invasion and metastasis of cancer cells involves the adherence of cells to the extracellular matrix via integrin as a receptor for matrix molecules. The present study investigated the role of CRP in the adhesive phenotype of breast cells and the underlying mechanisms. Here, we show for the first time that CRP induces adhesion of MCF10A human breast epithelial cells through activation of integrin alpha2 signaling. The present study elucidates the molecular basis of the crucial link between CRP, integrin alpha2 and FcγRI pathways in MCF10A breast cells and MDA-MB-231 TNBC cells, thereby providing useful information on CRP-induced aggressiveness of breast cells in the inflammatory microenvironment.

Keyword: signaling, Ras, invasion, inflammation, breast cancer

[SY07-1-1]

METABOTROPIC GLUTAMATE RECEPTORS IN CELL TRANSFORMATION AND TUMORIGENESIS

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Overexpression or aberrant expression of G-protein-coupled receptors (GPCRs) has been detected in many cancer cell types and contributes to tumor cell growth by paracrine or autocrine signaling, maintaining an activated state that leads to enhanced cellular proliferation via down- stream effector proteins. In particular, metabotropic glutamate receptors (mGluRs) have been shown empirically to be the predominant mediators of glutamatergic signaling in many cancers. The mechanisms by which mGluRs modulate peripheral cell transformation and tumor growth are postulated to be either ectopic expression of wild- type mGluRs, increased proliferative signals arising from receptor overexpression, mutations, or expression of polymorphic variants. Among Group I mGluRs, mGluR1 has been shown to induce the neoplastic transformation first in a transgenic mouse model with spontaneous development of metastatic melanoma in vivo. Molecular analyses revealed a classical case of insertional mutagenesis led to unscheduled expression of mGluR1. Subsequently, it was demonstrated that mGluR1 could induce the neoplastic transformation in other cell types including baby kidney epithelial cells and mammary epithelial cells in addition to melanocytes, suggesting that ectopic expression of a normal GPCR is sufficient to induce malignant cell transformation in the absence of known mutation(s). In mGluR1-transformed cells, excess extracellular levels of the natural ligand, glutamate was detected, suggesting the existence of autocrine loops, a characteristic of oncogenic GPCRs. Two major signaling cascades, MAPK and PI3K/AKT were found to be constitutively activated in mGluR1-transformed cells. Pretreatment of mGluR1-expressing tumor cells with mGluR1 antagonist followed by mGluR1 agonist treatment did not activate the MAPK pathway, suggesting that MAPK activation is dependent upon functional mGluR1 receptor. Similar results were founds by genetic means by silencing RNA to mGluR1, which also led to the absence of MAPK stimulation, again points to the requirement of functional receptor for mediating cell transformation. Results from cultured cell studies suggest that ectopic expression of mGluR1 in cells led to the establishment of autocrine loop with high levels of extracellular glutamate to ensure constitutive activated receptor and upregulated glutamate signaling to participate in neoplastic transformation in vitro and tumorigenesis in vivo. Because decreasing the levels of available glutamate would most likely dampen the glutamatergic signaling and reduce the oncogenic activity of mGluR1. We tested this notion by using riluzole, an inhibitor of glutamate release, in mGluR1-expressing melanoma cells.

Riluzole is FDA approved for the treatment of amyotrophic lateral sclerosis (ALS). We shoed that treatment of mGluR1-expressing tumor cells with riluzole led to cell cycle arrest followed by apoptosis. Clinical trials with riluzole in melanoma patients showed stable disease in about 35% of patients, Currently we are testing other potential reagents to combine with riluzole for the treatment of cancer.

Keyword: melanocytes, transformtion, glutamatergic, melanoma, tumorigenesis

[SY07-1-2]

REGULATION AND RAS PROTEIN STABILITY VIA THE WNT/BETA-CATENIN SIGNALING

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From normal to a malignant state of cellular growth involves multiple gene mutations, and their interactions play important roles in the pathogenesis of cancer. In this "Cell Fate Control" session, I will present data and will discuss interactions between several popular gene mutations in both Wnt/β-catenin and Ras-ERK pathways such as APC and KRAS mutations, respectively, in the colorectal tumorigenesis and pathological outcomes including the cancer stem cell activation. The Wnt/β-catenin and the Ras-ERK pathways, two major transforming pathways, interact during the tumorigenesis. However, molecular mechanisms and cooperative roles of these two pathways are poorly understood. Both APC and KRAS mutations, which occurs as high as 90% and 40-50% of human colorectal cancer (CRC) patients, synergistically promote cellular transformation and tumor growth. One key event in this crosstalk is the stabilization of RAS, especially oncogenic mutant KRAS, by APC loss. The high increment of both β-catenin and RAS levels in CRC patient tissues implies pathological significance of the stabilization of RAS. Epidermal growth factor receptor (EGFR), a direct transcriptional target of the Wnt/β-catenin signaling pathway, is also overexpressed in human CRC, and plays a role in the synergistic tumorigenesis. Therefore, inhibition of both the Wnt/β-catenin and EGFR-RAS-ERK pathways, especially by reducing levels of the proteins elevated in CRC, could be an ideal approach for the treatment of human CRC. This concept for an ideal therapeutic approach has been proved by small molecules destabilizing both β-catenin and RAS by activation of GSK3β via targeting the Wnt/β-catenin pathway. GSK3β activated by the small molecules induce phosphorylation and subsequent polyubiquitin-dependent proteasomal degradations of both β-catenin and Ras and subsequent transcriptional suppression of EGFR overcome current limitation of the insensitiveness of the EGFR targeting therapies such as cetuximab attributed by a K-Ras mutation of patients.

[SY07-1-3]

THERAPEUTIC EFFECTS OF NOVEL TAU ANTIBODY

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Although various pathogenic molecules and mechanisms have been suggested to be involved in Alzheimer's disease (AD), the two indispensable pathogenic molecules of AD are β-amyloid (Aβ) and tau. In the meantime, majority of the mechanism studies of AD pathogenesis were focused on the Aβ, which leads big pharmaceutical companies to develop therapeutic drugs targeting Aβ. However, all the clinical trials targeting Aβ have been failed up to now, hence, tau, another inevitable pathogenic molecule, has received attention as a real treatment target for AD. To develop therapeutic antibody targeting tau, we first screened protective effects in tau-P301L transgenic mice by active immunization with several tau peptides of different residues and modifications. Among these tau peptides, we found one epitope with specific residues and modifications to show the best memory improving effects. We then screened and developed the antibody to target this epitope with high affinity and high specificity. We further confirmed the therapeutic effects of this antibody in tau-P301L transgenic mice by passive immunization. This antibody improved memory impairments and AD-related pathology in tau-P301L mice. We anticipate this antibody to be a novel pipeline for the AD therapeutics.

Keyword: Tau, Alzheimer, dementia, antibody

[SY07-1-4]

MICRORNAS REGULATING CANCER CELL SENSITIVITY THROUGH MAINTAINING GENOME STABILITY

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Homologous recombination (HR)-mediated repair of DNA double-strand break (DSB)s is restricted to the post-replicative phases of the cell cycle. Initiation of HR in the G1 phase blocks non-homologous end joining (NHEJ) impairing DSB repair. Completion of HR in G1 cells can lead to the loss-of-heterozygosity (LOH), which is potentially carcinogenic. We conducted a gain-of-function screen to identify miRNAs that regulate HR-mediated DSB repair, and of these miRNAs, miR-1255b, miR-148b*, and miR-193b* specifically suppress the HR-pathway in the G1 phase. These miRNAs target the transcripts of HR factors, BRCA1, BRCA2, and RAD51, and inhibiting miR-1255b, miR-148b*, and miR-193b* increases expression of BRCA1/BRCA2/RAD51 specifically in the G1-phase leading to impaired DSB repair. Depletion of CtIP, a BRCA1-associated DNA end resection protein, rescues this phenotype. Furthermore, deletion of miR-1255b, miR-148b*, and miR-193b* in independent cohorts of ovarian tumors correlates with significant increase in LOH events/chromosomal aberrations and BRCA1 expression.

Keyword: microRNA, Homologous Recombination, DNA repair, Cell cyle, Cancer

[SY07-2-1]

AN OVERVIEW OF THE PATHOGENIC MECHANISMS OF AUTOIMMUNE DISEASES

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Autoimmune diseases are characterized by chronic immune activation without foreign pathologic processes such as infections, cancer, trauma or chemicals. Autoimmune diseases comprise a wide range such as rheumatoid arthritis, lupus, psoriasis, and inflammatory bowel disease based on clinical presentations. The pathogenic mechanisms are quite diverse even within the same disease category. Furthermore, different diseases share similar clinical presentations and/or immunologic abnormalities. I will review recent progress on the pathogenic mechanisms of autoimmune diseases addressed as below. 1. What are the autoantigen(s) in RA? 2. What is the role of B cells in RA? 3. What is the role of Interferon alpha in lupus? 4. What is the role of complement in lupus?

Keyword: autoimmune disease, immunology, rheumatoid arthritis, lupus

[SY07-2-2]

TRANSCRIPTION FACTOR NFAT IS ESSENTIAL TO RHEUMATOID ARTHRITIS

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As seen in the success of JAK3 inhibitors in the treatment of rheumatoid arthritis (RA), the major issues in the post-genome era may be the identification of the key signalling pathway regulating distinct sets of genes. Nuclear factor of activated T-cell (NFAT) is one of the key transcription factors regulating innate immunity as well as adaptive immunity, and its dysfunction has been implicated in the pathogenesis of autoimmune diseases, including rheumatoid arthritis (RA). The NFAT family consists of NFAT1 to 4 and NFAT5. NFAT1 to 4, but not NFAT5, are all activated by calcineurin and have a conserved N-terminal domain containing calcineurin docking sites. Defects in intracellular calcium homeostasis have been identified in the lymphocytes and synoviocytes of RA patients, which may be associated with deregulated calcineurin–NFAT1 to 4 signalling in RA. Thus, cyclosporine and tacrolimus, calcineurin inhibitors, have been successfully used for RA treatment and are now being tested for efficacy in a diverse range of other autoimmune pathologies. Interestingly, NFAT5, osmosensitive transcription factor independent of calcineurin, is also activated in synovial tissues and cells of RA patients. Recent data indicate that high salt-induced NFAT5 is crucial for Th17 cell generation, increasing autoimmunity. NFAT5 also confers rheumatoid macrophages and synoviocytes apoptotic resistance under isotonic conditions and promotes chronic arthritis in mice, suggesting that it may be a new therapeutic target for RA. In this symposium, I propose critical roles of NFAT1 to 4 and NFAT5 in rheumatoid inflammation in the context of recent therapeutic developments.

Keyword: Transcription factor, NFAT1 to 4, NFAT5, pathogenesis, rheumatoid arthritis

[SY07-2-3]

THE ROLE OF CTLA-4 IN T REGULATORY CELL FUNCTION AND IMMUNE HOMEOSTASIS

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CD28 and CTLA-4 are related receptors predominantly expressed on T lymphocytes. Both molecules interact with the same ligands, CD80 and CD86, expressed on antigen presenting cells. However, the functions of the molecules are completely opposite. CD28 is the major co-stimulatory molecule on T cells, while CTLA-4 is a major inhibitor of T cell function and is critical for maintenance of T cell tolerance. One mechanism for CTLA-4 inhibition is the recruitment of phosphatases to the TCR that inhibit signaling. CTLA-4 has a higher affinity for CD80/CD86 than CD28 and may also inhibit T cell responses by ligand competition by depriving T cells of co-stimulatory signals. Other studies suggest that ligand binding may not be essential for the delivery of negative signals by CTLA-4 and that the level of expression of CTLA-4 plays a critical role. Most importantly, CTLA-4 functions as one of the critical "immune checkpoint inhibitors" to suppress anti-tumor responses. The use of anti-CTLA-4 has transformed the treatment of several human tumors including melanoma, non-small cell lung cancer and bladder cancer. One side effect of anti-CTLA-4 therapy is the induction of organ-specific autoimmunity probably secondary to a general lowering of the threshold for T cell activation. While CTLA-4 is only expressed in conventional T cells after activation, it is constitutively expressed in Foxp3+ T regulatory cells (Treg). Some studies suggest that CTLA-4 is critical for the suppressive function of Treg as selective deletion of CTLA-4 from Treg results in the rapid development of autoimmune disease. CTLA-4 is also capable of removing CD80/CD86 from the surface of dendritic cells by a process of trans-endocytosis ultimately resulting in the degradation of CD80/CD86 within the Treg. Surprisingly, Deletion of CTLA-4 from Treg in the adult mouse resulted in enhanced Treg proliferation in vivo and increased Treg suppressor function. In contrast, we have observed that the homeostatic proliferation of Treg in vivo can be markedly enhanced by treatment of mice with anti-CTLA-4. The enhanced proliferation of Treg in this model was accompanied by enhanced proliferation of memory phenotype CD4+ and CD8+ T cells consistent with a loss of suppressor function. Taken together, CTLA-4 has a profound effect on the function of both conventional CD4+ and CD8+ T cells as well as Foxp3+ Treg. While inhibition of CTLA-4 function is now part of the standard armamentarium used in cancer immunotherapy, further studies are needed to define potential mechanism whereby modulation of CTLA-4 function can be used to enhance Treg function and treat autoimmune diseases.

Keyword: CTLA-4, T regulatory cell, immune homeostasis, tumor immunity, suppression

[SY07-2-4]

BIOMATERIAL DESIGNS THAT ENABLE MOLECULAR AND CELLULAR THERAPIES TO TREAT AUTOIMMUNE DISEASES

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Systemic lupus erythematosus (SLE) is an autoimmune disease with symptoms involving inflammation and tissue damage in various tissues and organs. The loss of self-tolerance or aberrant activation in adaptive immune cell subsets including CD4 helper T cells and B cells is critically responsible for the production of autoantibody and related tissue inflammation. Meanwhile, innate immune cells such as dendritic cells (DCs) get involved in SLE pathology by producing inflammatory cytokines to stimulate the autoreactive lymphocytes. Typical treatment options are designed to result in the general attenuation of inflammatory immune responses by chronic use of immunosuppressive drugs which is far from ideal due to their toxicity, and side effects, not to mention non-specific suppression of whole immune protection. In this presentation, molecular and cellular therapies using biomaterials that are designed to regulate selective immune compartments for SLE treatment will be discussed. First, a new strategy to serve as a novel molecular therapy that modulates a specific immune activation targeting TLR7 pathway will be discussed. It is now well established that self-nucleic-acid antigens (DNA and RNA) and their immune complexes are one of the most pathogenic autoantigens in lupus patients. Autoimmune reactions against single-stranded RNA recognized by TRL7 inside certain tissue-specific immune cells play a major role in SLE pathology. Thus we attempt to develop a new strategy of regulating immune cell subsets involved in SLE pathology using targeted gene silencing via small interfering RNA (siRNA) delivered by polymeric drug-carriers. The size-dependent biodistributions of micro- and nanoparticles were investigated at both the organ level and cellular level. In particular, nanoparticles (NPs) demonstrated a remarkable tropism toward pDCs in the kidneys. After confirming the efficacy of siRNA therapeutics delivered by the nanoparticles in vitro, this strategy was also tested in vivo using a murine SLE model (MRL-lpr/lpr mice). After weekly administrations of siRNAs loaded onto NPs via tail vein for 8 weeks, the immunohistrochemistry of kidney samples, as well as the monitored proteinurea levels, demonstrated a significant reduction in pathophysiologic symptoms in the treated groups. Second, a biomaterials-based strategy to provide a synthetic niche to prepare antigen-specific regulatory B cells (B10 cells) will be discussed. B10 cells have been identified as potent negative regulators of antigen-specific T-dependent autoimmune inflammation. The therapeutic potential of these B10 cells were previously

demonstrated by transferring these cells into mice with pre-established autoimmune disease. However, there are a couple of significant hurdles in the translation of this strategy into human trials. One is that the ex-vivo expansion of B10 cells requires a co-culture with genetically modified xenogenic cells, and the other is that the manufactured B10 cells are not antigen-specific. We recently reported that full arrays of germinal center reactions can be recapitulated ex vivo in a stroma-free synthetic niche. Here I will discuss a new strategy to leverage this synthetic niche in the production of therapeutically relevant antigen-specific B10 cells. With further optimization, these novel molecular and cellular strategies may open up opportunities for more selective and more effective treatments of various autoimmune anomalies.

Keyword: Biomaterial, SLE, siRNA, nanoparticle, B10 cell

[SY07-3-1]

CLONAL EVOLUTION OF GLIOBLASTOMA UNDER THERAPY

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Glioblastoma (GBM) is the most common and aggressive primary brain tumor. To better understand the evolutionary process of GBM, we have analyzed longitudinal genomic and transcriptomic profiles from 114 patients. Our results demonstrated a highly branched evolutionary pattern in which 63% of patients experienced expression-based subtype changes. The branching pattern, together with estimates of evolutionary rate, suggest that relapse-associated clones typically existed years before diagnosis. In conclusion, our study sketches the main routes of GBM evolution under therapy, identifying a highly branched process with specific alterations and evolutionary patterns associated with treated tumors.

Keyword: Clonal evolution, glioblastoma, GBM

[SY07-3-2]

MOLECULAR CHARACTERIZATION OF CHOLANGIOCARCINOMA

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We have been focusing on genomic studies of Asian-centric cancers, particularly cholangiocarcinoma, which is associated with chronic liver fluke (opisthochris viverrini) infection and cancers (eg., urothelial and hepatocellular carcinoma) associated with herbal carcinogen, i.e., aristolochic acid. Cholangiocarcinoma (CCA) is a deadly hepatobiliary malignancy with limited treatment options. Our recent integrated genomic and epigenomic analysis across 489 CCAs, discovered four CCA clusters with distinctive genomic alterations of therapeutic relevance. Cluster 1 and 2 are enriched in ERBB2 amplifications - Cluster 1 exhibiting CpG island hypermethylation and being associated with liver-fluke infection, while Cluster 2 exhibits increased Wnt/β-catenin gene expression. Clusters 3 and 4 comprise mostly fluke-negative tumors - Cluster 3 exhibits elevated copy-number alterations and PD-1, PD-L2 expression, while Cluster 4 displays IDH1/2 and BAP1 mutations, FGFR and PRKA-related gene rearrangements, and CpG shore hypermethylation. Analysis of non-coding promoter mutations revealed a pervasive modulation of PRC2-related transcription factor binding sites. These results define CCA taxonomy of clinical relevance, proposing a strategy for clinical stratification of CCA. Finally, besides our cholangiocarcinoma works, I will also give an update on our precision medicine efforts in Singapore by focusing on the progress of our recently formed Singhealth/Duke-NUS Precision Medicine Institute (PRISM). To date, genotyping by whole-genome sequencing and phenotyping (eg., daily activity, biochemical and cardiovascular profiles) have been performed on a cohort of healthy subjects. I will report on some of the preliminary results and discuss their implications.

Keyword: Cholangiocarcinoma, Hepatobiliary, Epigenomic, Sequencing, Precision Medicine

[SY07-3-3]

K-MASTER: KOREAN CANCER PRECISION MEDICINE MATCH TRIALS

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Current molecular diagnostics in cancer patients is based on tumor biopsies, which, however, face serious limitations which can lead to false decisions. Individual tumors consist of diverse subpopulations, and the small amount of tissue obtained by needle biopsies may not represent the most aggressive subclones. Moreover, some tumor entities such as lung cancer are located at remote sites and a needle biopsy can be very difficult and at high risk. Thus, the mere analysis of the resected primary tumor alone (current standard practice in oncology) may provide misleading information with regard to the characteristics of metastases, the key target for systemic anticancer therapy. Distant metastases can harbor unique genomic characteristics not detectable in the corresponding primary tumor of the same patient and metastases located at different sites show a considerable intra-patient heterogeneity. In 2017, Korean Precision Medicine Enterprise was launched. Korean Precision Medicine Enterprise are composed of Cancer Diagnosis & Treatment (K-MASTER) Enterprise and Precision-Hospital Information System (P-HIS) Enterprise. The research objective of K-MASTER Enterprise is developing a global precision medical cancer diagnosis and treatment method by establishing a comprehensive integration platform with the best possible integration of the molecular diagnosis and new targeted agents, which can be used in the near term. Also, K-MASTER aims to operate and expand the development of a large scale genomic and clinical data warehouse, combined with various solution developments for precision medicine of cancer patients. The details of Korean Precision Medicine Enterprise project, which is an innovative initiative of Precision Medicine in Korea, will be discussed.

Keyword: Precision Medicine, Cancer, Genomics, Clinical trial

[SY07-3-4]

COMBINATORY USE OF DISTINCT SINGLE-CELL RNA-SEQ ANALYTICAL PLATFORMS REVEALS THE HETEROGENEOUS TRANSCRIPTOME RESPONSE

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A cancer is a complex cellular ecosystem. In a cancer, not a single individual cell is identical, located in varying micro-environmental conditions and interacting with different cells. Even though cancer cells are developed by clonal evolution, diverse features are frequently observed for each individual single cell. Particularly, it is important to understand the cancer cell diversity, when emergence of drug resistant cells and metastasizing cells are studied. It should be of substantial relevance if further in-depth knowledge could be obtained. Recent developments of the single cell sequencing technologies have opened a possibility for such an analysis. Several commercial platforms have become available; one platform physically separates a single cell by microfluidics, while another platform utilizes the micro-droplet technology. Each of the current platforms appeared to have its inherent advantages and disadvantages. In this lecture, technical aspects of the representative methods will be discussed, also exemplifying their application by showing recent results world-wide and from our own group. Particular focus is put on newly discovered two distinct transcriptional modules; one associated with the Aurora kinase gene and the other with the DUSP gene. These modules are aberrantly regulated in a minor population of cells and may thus contribute to the possible emergence of dormancy or eventual drug resistance within the population.

Keyword: SINGLE-CELL RNA-SEQ, CANCER

[SY07-3-5]

CLINICAL UTILITY OF INTEGRATED CLINICAL AND GENOMIC DATA FOR PRECISION MEDICINE

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Personalized cancer treatment using individual genome information is the goal of precision oncology. High-quality genome data of patient with full annotation on somatic variants in each patient is required for precision cancer medicine clinic. However, the actionability based on somatic variants could cover just a small fraction of cancer patients depending on cancer types. We have screened more than 6,000 patients with colon, stomach, lung, and breast cancer by panel sequencing, which is also annotated with thousand clinical variables through hospital information system. Statistical analysis of integrated clinical and genomic datasets could extract significant genomic and clinical features for each cancer type. We will discuss about a predictive model for clinical outcome based on machine learning methods with genomic and clinical features.

Keyword: precision medicine, genomic, big data, cancer, machine learning

[SY07-4-1]

THE INTERNATIONAL MOUSE PHENOTYPING CONSORTIUM (IMPC): A LARGE SCALE HEARING LOSS SCREEN REVEALS AN EXTENSIVE UNEXPLORED GENETIC LANDSCAPE FOR AUDITORY DYSFUNCTION

Steve Brown[1]

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The developmental and physiological complexity of the auditory system is likely reflected in the underlying set of genes involved in auditory function. In humans, over 150 non-syndromic loci have been identified, and there are more than 400 human genetic syndromes with a hearing loss component. Over 100 non-syndromic hearing loss genes have been identified in mouse and human, but we remain ignorant of the full extent of the genetic landscape involved in auditory dysfunction. The International Mouse Phenotyping Consortium (IMPC) is generating and comprehensively phenotyping a null mutant for every gene in the mouse genome. Currently mutants for over 7000 genes have been produced (over one-third of the coding genome), the majority of which have been phenotyped across a diverse array of phenotyping platforms (www.mousephenotype.org) providing a comprehensive functional catalogue of the mouse genome. As part of the IMPC phenotyping pipeline, we undertook a hearing loss screen in a cohort of 3006 mouse knockout strains. In total, we identify 67 candidate hearing loss genes. We detect known hearing loss genes, but the vast majority, 52, of the candidate genes were novel. Our analysis reveals a large and unexplored genetic landscape involved with auditory function.

Keyword: Mouse genetics, Mouse mutants, Hearing loss, Mouse functional genomics

[SY07-4-2]

ESTABLISHING THE TONOTOPIC ORGANIZATION OF THE MAMMALIAN COCHLEA

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Sound frequency discrimination begins at the cochlea, the peripheral auditory organ responsible for detecting and relaying sound information from the environment to the brain. The mechanosensory hair cells residing in the cochlea are tuned to respond to different frequencies of sound depending on their location along the cochlear duct. Hair cells located in the basal region of the cochlear duct are more sensitive to high frequency sounds and their counterparts toward the apex progressively to lower frequencies. Such functional and spatial arrangement is known as the tonotopic organization. However, how the tonotopic organization is established during development is still unclear. Recent studies suggest that the tonotopic organization is established by a temporal cascade of molecular events that is initiated by an increasing base-to-apex gradient of Sonic hedgehog (Shh) signaling both in avians and mammals. In the chicken, Bmp7 appears to be a key downstream target of Shh in mediating the tonotopic organization. However, the downstream mediators of Shh have been elusive in the mammalian cochlea. Here, we provide evidence that Follistatin (Fst), an antagonist for Bmp/TGFβ signaling, play an essential role in mediating the Shh signaling to facilitate the tonotopic organization of the mammalian cochlea.

Keyword: cochlea, hearing, inner ear, tonotopy

[SY07-4-3]

NEXT-GENERATION SEQUENCING FACILITATES THE IDENTIFICATION OF HEARING LOSS GENES IN HUMAN

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Hearing loss affects approximately 1/500~1/1000 newborns and is extensively genetically heterogeneous. Non-syndromic hearing loss (NSHL) accounts for 70% of hearing loss cases. To date, more than hundred genes have been linked to NSHL and explain about half of the clinical cases. Recent developments in sequencing technology has rendered identification of causative mutation in this high number of genes feasible. We have recruited individuals with NSHL in Yonsei University Hearing Loss cohort and provided them with molecular diagnostics using targeted gene panel sequencing or whole exome sequencing (WES). Interestingly, we found some cases in which hearing loss may be medically treatable. In addition, as a significant proportion of NSHL cases are still molecularly unsolved, we utilize WES to discover novel genes which, if mutated, cause NSHL. As genes identified by WES rely on a single family in many cases, they need further genetic confirmation. In this regard, mouse models with hearing loss phenotype are useful to find possible causative genes. I will show our recent findings and discuss the direction of future research using next-generation sequencing.

Keyword: non-syndromic hearling loss, whole exome sequencing

[SY07-4-4]

RESTORATION OF HEARING FUNCTION IN A MOUSE MODEL OF CONGENITAL HEARING LOSS USING GENE THERAPY

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Mutations in the SLC26A4 gene encoding an anion exchanger known as pendrin cause autosomal recessive sensorineural hearing loss associated with enlargement of the vestibular aqueduct. Interestingly, mutation spectrums of the SLC26A4 gene associated with deafness are shown to be different between Asian and Caucasian populations, and some of the mutations show high prevalence in a certain population indicating a founder effect. Upon the completion of human genome project and development of next generation sequencing techniques, identification of genetic mutations in patients displaying Mendelian inheritance have been greatly accelerated, and genotype-phenotype correlations have become much better understood. With these recent advancements, increasing attention has been paid to the gene therapy for treating human genetic disorders. However, considering the heterogeneity of the SLC26A4 mutations, any single strategy for gene therapy would not be an effective way to treat hearing loss in DFNB4 or Pendred syndrome. Instead, different strategies should be applied depending on the type of mutations identified in each individual. Here, I briefly summarize current progresses of our laboratory for developing gene therapy techniques that can be applied to restore hearing loss caused by different types of SLC26A4 mutations.

Keyword: hearing loss, gene therapy, mouse, gene, mutation

[SY07-5-1]

THE DICKKOPF1-CKAP4 PATHWAY, A NOVEL CANCER SIGNALING, REPRESENTS A THERAPEUTIC TARGET FOR CANCER THERAPY

Akira Kikuchi[1], Hirokazu Kimura[1], Katsumi Fumoto[1], Yoshihito Osugi[1], Ryota Sada[1] and Hideki Yamamoto[1]

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Dickkopf 1 (DKK1) is a secretory protein and antagonizes oncogenic Wnt signaling by binding to the Wnt coreceptor low-density lipoprotein receptor-related proteins 6 (LRP6). DKK1 is also suggested to regulate its own signaling to promote cancer cell proliferation, however the underlying mechanism of DKK1-induced cell proliferation has remained to be clarified. We found that cytoskeleton-associated protein 4 (CKAP4), which is a type II single-span transmembrane protein, functions as a novel DKK1 receptor. The cytoplasmic region of CKAP4 was modified with palmitate, which was necessary for the localization of CKAP4 to lipid rafts in the cell surface membrane. DKK1 bound to CKAP4 through the site (CRD1) different from the binding site (CRD2) to LRP6 and activated AKT through phosphatidylinositol 3-kinase in lipid rafts, resulting in normal and cancer cell proliferation. Then, CKAP4 was moved to non-lipid rafts and internalized via a clathrin-dependent manner to desensitize DKK1 signaling. Both DKK1 and CKAP4 were frequently expressed in tumor lesions of pancreatic, lung, and esophageal cancers and their simultaneous expression was correlated with poor prognosis. Knockdown of CKAP4 or DKK1 from cancer cells inhibited AKT activity and decreased their xenograft tumor formation abilities. Wild-type DKK1 rescued the phenotypes, but the CRD1-deleted DKK1 mutant did not, demonstrating that the binding of DKK1 to CKAP4 is important for tumor formation. The anti-CKAP4 antibody inhibited the binding of DKK1 to CKAP4 and AKT activity, thereby suppressing cancer cell-induced xenograft tumor formation. Thus, the DKK1-CKAP4 axis may represent a novel therapeutic target for cancers expressing both DKK1 and CKAP4.

Keyword: DKK1, CKAP4, cancer, antibody therapy, Wnt

[SY07-5-2]

STABILIZATION OF CYCLIN DEPENDENT KINASE 4 BY METHIONYL-TRNA SYNTHETASE IN P16-NEGATIVE CANCER

Nam Hoon Kwon[1], Peter Goughnour[1], Min Chul Park[1] and Sunghoon Kim[1]

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Although abnormal increase in the level or activity of cyclin dependent kinase 4 (CDK4) occurs pervasively in cancer, the underlying mechanism is not fully understood. Here we show that methionyl-tRNA synthetase (MRS) specifically stabilizes CDK4 by enhancing the complex formation of CDK4 with chaperon protein. A knockdwon of MRS reduced the CDK4 level, resulting in G0/G1 cell cycle arrest. The effects of MRS on CDK4 stability were more prominent in the tumor suppressor p16-negative cancer cells due to the competitive relationship of the two proteins in the binding to CDK4. Suppression of MRS reduced cell transformation and the tumorigenic ability of a p16-negative breast cancer cell line in vivo. Further, the MRS levels showed positive correlation with those of CDK4 and the downstream signalling, at high frequency in the p16-negative human breast cancer tissues. This work revealed unexpected functional connection between the two enzymes for protein synthesis and cell cycle.

[SY07-5-3]

STRUCTURAL DETERMINANTS FOR THE PATHOBIOLOGICAL PERFORMANCE OF THE HELICOBACTER PYLORI ONCOPROTEIN CAGA

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Chronic Helicobacter pylori infection in the stomach mucosa is causally associated with the development of gastric cancer. With 951,000 new cases and 723,000 deaths in 2012, gastric cancer is the third leading cause of cancer-related deaths worldwide. Moreover, East Asian countries such as Japan, Korea and China have the highest incidences of gastric cancer, accounting for more than half of all new gastric cancer patients worldwide each year. In Japan, virtually all of the gastric cancer patients are associated with H. pylori infection, highlighting the importance of H. pylori eradication for the prevention of gastric cancer in East Asian countries. Evidence accumulates that the H. pylori virulence factor CagA play a critical role in the neoplastic transformation of gastric epithelial cells. CagA has a molecular weight ranging between 120- and 145-kDa due to its C-terminal sequence polymorphisms. CagA is delivered from H. pylori into host gastric epithelial cells via a bacterial syringe-like structure termed the type IV secretion system. Once inside the epithelial cells, CagA aberrantly interacts with host proteins via its polymorphic and intrinsically disordered C-terminal tail, which contains two repeatable protein-binding motifs, the Glu-Pro-Ile-Tyr-Ala (EPIYA) motif and the CagA multimerization (CM) motif. Upon tyrosine phosphorylation by host kinases such as the Src family kinases and c-Abl, the EPIYA motif acquires the ability to interact with and thereby deregulates Src homology 2 domain-containing phosphatase 2 (SHP2), a bona fide pro-oncogenic phosphatase that stimulates cell proliferation and at the same time promotes cell motility. CagA also binds to the polarity-regulating serine/threonine kinase partitioning-defective 1 (PAR1) via the CM sequence, causing junctional and polarity defects through the PAR1 kinase inhibition. The magnitude for the pathobiological action of individual H. pylori CagA has been linked to the qualitative (sequence) and quantitative (repeat number) polymorphisms in these two binding motifs (EPIYA and CM motifs). Recent crystal structure analysis in conjunction with SPR-mediated quantitative binding studies between CagA and SHP2 or PR1 have shed light on the structural mechanisms how the C-terminal polymorphisms determine the magnitude for the pathogenic action of individual H. pylori CagA, which may underlie the high incidence of gastric cancer in East Asian countries.

[SY07-5-4]

LACTATE-INDUCED METABOLIC SIGNALING IN HYPOXIA AND CANCER

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Animal cells frequently reprogram their metabolism in response to genomic alterations or environmental changes. Cells exposed to a low oxygen condition (hypoxia) typically produce high levels of lactate as the result of the up-regulation of glycolysis when they reprogram the metabolism to adapt to the stressful condition. Long been considered as a waste product of carbohydrate metabolism, lactate is recently gaining much attention thanks to the studies on cancer metabolism, which indicated that lactate is actively implicated in many biological processes by way of an alternative fuel, an agent modulating tumor microenvironment, or a signaling molecule. Hypoxia inducible factors (HIFs) play key roles in the regulation of hypoxia responses but there are many hypoxia-associated phenomena that are not explained by HIF alone. In our attempts to identify novel regulators of hypoxia responses we discovered a lactate-dependent cell signaling system, which is mediated by an oxygen- and lactate-regulated protein, NDRG family member 3 (NDRG3). Oxygen negatively regulates NDRG3 expression at the protein level via the PHD2/VHL system, while lactate, produced in excess under prolonged hypoxia, binds to NDRG3 and blocks its proteasomal degradation by inhibiting VHL-mediated ubiquitination. The stabilized NDRG3 protein then promotes angiogenesis and cell growth by activating Raf-ERK pathway to help cells cope with hypoxia. Inhibiting cellular lactate production abolished the NDRG3-mediated responses. The hypoxia-associated NDRG3 protein induction was found to occur in diverse cell types. Our study indicates that HIF-1 and NDRG3 form an oxygen-dependent regulatory chain for hypoxia responses, divided into two chronological phases that are functionally coupled with each other using lactate as the critical link. Thus, lactate produced by the HIF-1-mediated glycolytic activation at the early phase of hypoxia functions as a metabolic signal to induce NDRG3-mediated kinase signaling at the later phase. The NDRG3-Raf-ERK axis provides a genetic basis for the lactate-induced hypoxia signaling, which can be exploited for the development of therapies targeting hypoxia-induced diseases. It is noteworthy that, in addition to the functions of lactate signaling system in hypoxia responses and tumor development, its physiologic roles in immune responses, lipid metabolism, etc as well as the relevant therapeutic implications may also be elucidated in the future.

Keyword: Lactate, Hypoxia, Cancer, NDRG3, Cell signaling

[SY07-5-5]

SPECIFIC INHIBITORS OF PHOSPHOLIPASE C EPSILON (PLC EPSILON) DISPLAY ANTI-TUMOR AND CANCER-PREVENTIVE EFFECTS AS WELL AS ANTI-INFLAMMATORY EFFECT

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It is well known that chronic inflammation plays crucial roles in the pathogenesis of various diseases including cancer. Phospholipase Cε (PLCε) was discovered by us in 2001 as a novel class of PLC regulated by not only Ras and Rap1 small GTPases but also RhoA small GTPase and heterotrimeric G protein α and β1γ2 subunits. It is expressed in non-immune cells such as epithelial cells and fibroblasts but not in immune cells. Analysis of the in vivo function of PLCε by applying various mouse models of inflammation and carcinogenesis to PLCε knockout and transgenic mice demonstrated that PLCε plays pivotal roles not only in inflammation but also in carcinogenesis by augmenting cancer-associated inflammation in the microenvironment. Moreover, two genome-wide association studies identified PLCε (PLCE1) as a predisposing gene for gastric and esophageal carcinomas, supporting the crucial role of PLCε in human carcinogenesis. Recently, we showed that PLCε mediates lysophosphatidic acid-induced protein kinase D activation and enhances proinflammatory cytokine expression from non-immune cells including cancer cells through NF-κB activation, thereby recruiting and activating immune cells to initiate inflammation. These results proved universal and crucial roles of PLCε in carcinogenesis and inflammation, introducing PLCε as a candidate molecular target for the development of anti-cancer, cancer preventive and anti-inflammatory drugs. Here we report the development of a high-throughput screening (HTS) system for PLCε-specific inhibitors using a fluorogenic substrate and the discovery of several compounds capable of inhibiting PLCε at the IC50 value of about 1 μM without affecting other PLC classes through HTS of 68,000 compounds. One of them, Compound H, effectively inhibits the NF-κB-dependent inflammatory cytokine expression in cultured human cancer cells and, moreover, not only inhibits the malignant progression of intestinal tumors formed in APCMin mice but also alleviates inflammatory colitis induced by dextran sulfate administration. Furthermore, Compound H displays anti-proliferative and anti-metastatic activity in vivo toward a xenograft of human colorectal cancer cells. These results suggest that specific inhibitors of PLCε may become promising anti-cancer and anti-inflammatory drugs.

[SY08-2-1]

SEC16: A STRESS RESPONSE PROTEIN REQUIRED FOR STRESS ASSEMBLY FORMATION AND CELL SURVIVAL

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Cell compartmentalization is achieved by membrane-bound organelles, such as those of the early secretory pathway. Newly synthesized proteins in the ER are packaged into COPII coated vesicles budding at ER exit sites (ERES) and transported to the Golgi. The large conserved hydrophilic protein Sec16 is a key ERES factor that orchestrates COPII vesicle budding. In addition to membrane-bound compartment, membrane-less compartments that are generated by phase separation also exist. Interestingly, phase separation can be triggered by stress and one of the best studied stress assemblies are stress granules. Their formation is a consequence of protein translation inhibition and accumulation of untranslated mRNAs imposed by many stress. We show that the stress of amino-acid starvation leads to the formation another stress assembly, the Sec bodies. Sec body formation parallels the inhibition of protein transport in the early secretory pathway, and results from the coalescence of ERES components (Sec16 and COPII subunits). Interestingly, Sec bodies co-form with stress granules. They share biophysical properties and are both cytoprotective and pro-survival. I will present data showing that under amino-acid starvation, Sec16 is sufficient to drive Sec body formation and is required for stress granule formation. This findings not only link the inhibition of protein transport and protein translation but also link membrane-bound and membrane-less cellular organization.

Keyword: Sec16, stress, higher order assembly, amino-acid starvation, PARP

[SY08-2-2]

SIGNALING AT THE ER-PM JUNCTIONS

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Specificity and fidelity of cell signaling is achieved by assembling signaling protein complexes at membrane contact sites (MCSs) that are formed between the ER and all other cellular membranes. MCSs are formed by tether proteins that are commonly anchored at the ER, have domains that span the space between the ER and the target membranes and motifs at the end of the spanning domains that interact with the target membranes. At the plasma membrane, MCSs formed between the ER and the plasma membrane (PM) are the ER/PM junctions. The identity and role in cell signaling of the tethers forming the ER/PM junctions are not well understood. This presentation will discuss the function of two ER/PM junction tethers in assembling Ca2+ and cAMP signaling complexes at the junctions and in mediating synergy in Ca2+/cAMP signaling to control the function of ion channels and transporters.

Keyword: MCSs, Ca2+, cAMP

[SY08-2-3]

MECHANISMS OF PROTEIN RETENTION AND SORTING IN THE GOLGI

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[1]Hkust, Hong Kong

The Golgi apparatus functions as the cell's protein sorting hub but also the site of post-translational modifications of both proteins and lipids. These post-translational modifications involve the sub-compartment-specific sequential enzymatic modifications by glycosyltransferases. Cells must therefore deploy mechanisms to ensure that Golgi resident proteins retain their compartment distributions while at the same time facilitating the sorting and transportation of non-resident proteins. My laboratory studies the mechanisms of protein retention in the Golgi in the budding yeast Saccharomyces cerevisiae. We are particularly interested in how two classes of type II membrane proteins are retained – the SNAREs and the glycosyltransferases. We (and others) have established that in yeast, Golgi localization is a dynamic process in which SNAREs and glycosyltransferases are retrieved from the Golgi to the ER in a COPI-dependent manner. I will present some of our more recent findings on the mechanisms that yeast cell employ to ensure these Golgi-localized membrane proteins retain steady-state localization.

Keyword: Golgi, Vps74 / GOLPH3, COPI, glycosyltransferase, SNAREs

[SY08-2-4]

UNCONVENTIONAL PROTEIN SECRETION OF TRANSMEMBRANE PROTEINS

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Most eukaryotic secretory or membrane proteins reach the plasma membrane through the conventional endoplasmic reticulum (ER)-Golgi pathway. However, evidence suggests that many cytosolic and signal-peptide containing proteins also can reach the cell surface via a route that bypass the ER-Golgi. These pathways are collectively referred to as unconventional protein secretion (UPS) and include both soluble cargos (Type I, II, and III) and integral membrane proteins (Type IV). A number of transmembrane proteins including CFTR, CD45, voltage-gated potassium channel Kv4, the yeast protein Ist2, the Drosophila melanogaster α integrins, Mpl, and pendrin are known to reach the cell surface via UPS under specific conditions. Understanding the UPS pathways is important not only to elucidate the mechanisms of intracellular trafficking pathways, but also has important ramifications for human health because many proteins undergo UPS are associated with human diseases. For example, our group recently found that the selective activation of the UPS pathway would be a potential therapeutic strategy for the treatment of diseases arising from the transport defects of misfolded proteins, such as cystic fibrosis and Pendred syndrome. In this talk, I will introduce the molecular machineries involved in the type IV UPS of transmembrane proteins, particularly those related to human diseases, and discuss the potential for new therapeutic strategies for the treatment of UPS-associated diseases.

Keyword: protein secretion, unconventional, transmembrane, CFTR, Pendrin

[SY08-3-1]

TARGETING MEMBRANE BINDING DOMAINS AS A NEW STRATEGY FOR DEVELOPING INHIBITORS TARGETING ONCOGENIC FORMS OF PI 3-KINASE

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PI 3-kinases are a small family of lipid kinases that play a central role in mediating the effects of many cell surface receptors. Pathways regulated include growth, cell division, gene expression, protein translation, metabolism and others. Thus it is not surprising that activation of PI 3-kinase signalling is observed in many cancers. In fact the alpha isoform of of PI 3-kinase (PIK3CA) is one of the most commonly mutated genes in cancer with common hotspot mutations being seen at E545 and H1047 positions which result in constitutively active forms on the enzyme. As a result there has been intense efforts to develop inhibitors of PI 3-kinases and PIK3CA in particular, including our own drug PWT33597. These are generally highly efficacious in pre clinical models but all suffer from issues of dose limiting toxicities when used clinically, this being linked with the fact that PI 3-kinases play such a pivotal role in normal cell function. The PI 3-kinase inhibitors do, however, show hints of activity in patients with tumours driven by PIK3CA mutations implying there may be some degree of oncogene addiction in these tumours that could be exploited. This has lead to some interest in using PI 3-kinase inhibitors as part of combination therapies and there remains some interest in understanding how they may modulate the immune system and thus potentiate the actions of checkpoint inhibitors. However, the most obvious way to overcome the issues of on target toxicity will be to find drugs that would selectively target only the oncogenic forms of the enzyme. One strategy for developing such drugs is to identify compounds that bind outside the catalytic pocket and may act allosterically. In this regard we have focussed on the common H1047 mutations in PI 3-kinase as these these mutations in the C-terminal of the molecule act to increase binding to the plasma membrane and so make substrate more available to the enzyme. In an effort to identify drugs that regulate this interface we have established novel membrane binding assays suitable for drug discovery and will report on the identification of drugs that selectively modulate the interaction of PI 3-kinase with membranes and will discuss the implications of this for developing a new geneation of PI 3-kinase inhibitors.

Keyword: Drug, PI 3-kinase, Cancer

[SY08-3-2]

GLP-1R: FROM A SMALL MOLECULAR AGONIST TO THE DETERMINATION OF 3-D STRUCTURES

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Glucagon-like peptide-1 receptor (GLP-1R) belongs to the class B family of G-protein coupled receptors (GPCRs). It exerts crucial roles in glucose homeostasis, and hence, is a validated target for the treatment of type 2 diabetes and obesity. The non-peptidic GLP-1R agonist Boc5, discovered via high-throughput screening campaigns against a compound library and structurally modified subsequently by means of medicinal chemistry, was shown to mimic a full spectrum of physiological actions of the native peptide hormone (GLP-1) both in vitro and in vivo. However, its druggability is hampered by poor oral bioavailability and difficulties in chemical synthesis. This led us to initiate structural biology studies of the receptor. Upon stabilization by negative allosteric modulators (NAMs), two crystal structures of the human GLP-1R 7-transmembrane domain (7TMD) were recently determined in an inactive conformation, revealing a common binding pocket for the NAMs present in both glucagon-like peptide-1 and glucagon receptors. Molecular modeling and mutagenesis experiments indicate that agonist positive allosteric modulators (agoPAMs) target the same general region, but in a distinct sub-pocket which may facilitate the formation of an intracellular binding site that enhances G-protein coupling. In addition, the structural determinants of GLP-1 binding to the 7-TMD of GLP-1R were also identified. The structure of human GLP-1R in complex with the G protein-biased peptide, exendin-P5, and Gs protein was recently determined at 3.3 Å global resolution. At the extracellular face of the receptor, there was a distinct organization of extracellular loop 3 and proximal transmembrane segments between the exendin-P5 structure and the published GLP-1 bound GLP-1 receptor. At the intracellular face, there was a 6-degree difference in the angle of the G s- 5 helix engagement between the receptors that was propagated across the G protein heterotrimer and variations in the rate and extent of conformational reorganization of the Gs protein. This novel structure provides novel insights into the structural basis of biased agonism. Allosteric modulation provides high selectivity, broad mimicry and less over-activation in terms of pharmacological properties. Based on the structural information, new efforts are being made to discover PAMs targeting the GLP-1R, with an ultimate goal of developing novel small molecule therapeutics to control the spread of metabolic disorders.

[SY08-3-3]

SMALL MOLECULES THAT CONTROL LIPID HOMEOSTASIS

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Sterol regulatory element-binding proteins (SREBPs) are transcription factors that control lipid homeostasis. Our laboratory has been discovering its small-molecule modulators with the goal of understanding and controlling lipid homeostasis in mammalian cells. SREBP activation is regulated by a negative feedback loop in which sterols bind to SREBP cleavage-activating protein (SCAP), an escort protein essential for SREBP activation, or to Insigs (ER anchor proteins), sequestering the SREBP-SCAP-Insig complex in the ER. We screened a chemical library of endogenous molecules and identified 25-hydroxyvitamin D (25OHD) as an inhibitor of SREBP activation. Unlike sterols and other SREBP inhibitors, 25OHD impairs SREBP activation by inducing proteolytic processing and ubiquitin-mediated degradation of SCAP, thereby decreasing SREBP levels independently of the vitamin D receptor. Vitamin D supplementation has long been proposed to reduce the risk of metabolic diseases, but the mechanisms are unknown. Our results suggest a previously unrecognized molecular mechanism of vitamin D-mediated lipid control that might be useful in the treatment of metabolic diseases. By taking an advantage of the new mechanism, we were able to design synthetic "neo-vitamin Ds" for dissecting the multiple functions of vitamin D and for providing pharmaceutical lead molecules.

Keyword: Vitamin D, SREBP, Chemical Library, Chemical Biology, Drug Discovery

[SY08-3-4]

NEXT-GENERATION MTORC1 INHIBITOR FROM THE STUDY OF AMINO ACID SENSING MECHANISM

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Amino acids not only serve as building blocks for protein synthesis but also control cellular signaling and metabolism. Intracellular amino acid availability is sensed by mechanistic target of rapamycin complex 1 (mTORC1), which is a key regulator of protein synthesis, autophagy, and cell growth/differentiation, and is implicated in several human diseases such as cancer, diabetes, and neuronal diseases. Therefore, understanding of amino acid sensing mechanism is critical for developing new strategies to treat human diseases. Leucyl-tRNA synthetase (LRS) senses intracellular leucine concentration and is translocated to the lysosomal membrane and forms specific interaction with RagD to function as GTPase-activating protein (GAP) of RagD for mTORC1 activation. Furthermore, LRS initiates the Rag GTPase cycle through the GTP hydrolysis of RagD, while Sestrin2, which is known to be the other leucine sensor, acts as an "OFF" switch of Leucine-dependent Rag-mTORC1 signaling by controlling GTP hydrolysis of RagB. Based on these studies, we developed new mTORC1 inhibitor that specifically block the leucine-sensing function of LRS by interfering with its interaction with RagD, without affecting its catalytic activity. This new mTORC1 inhibitor effectively suppresses the activity of cancer-associated MTOR mutants and the growth of rapamycin- or mTOR kinase inhibitor-resistant cancer cells. These findings may offer a therapeutic candidate for controlling tumor growth that avoids resistance mechanisms to existing mTOR inhibitors resulting from cancer-associated MTOR mutations.

Keyword: amino acid sensing, mTORC1, Leucyl-tRNA synthetase, mTORC1 inhibitor, Cancer

[SY08-3-5]

REGULATION OF CANCER METABOLISM THROUGH DUAL INHIBITORS OF HYPOXIA INDUCIBLE FACTOR-1A AND MALATE DEHYDROGENASE

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Selection of the most appropriate point of therapeutic intervention to modulate HIF (Hypoxia Inducible Factor) pathway is an important factor in pharmaceutical development. As part of our ongoing efforts to identify small-molecule HIF-1 inhibitors, we have carried out a high-throughput screening of synthetic chemical libraries by using cell-based reporter assay in various cancer cells, including HCT116 cells. The activity of HIF-1 was monitored using a luciferase reporter gene under the control of HRE (hypoxia responsive element). This resulted in the identification of several hits and we further conducted phenotype-based structure-activity relationship (SAR) study on this series identified aryloxyacetylamino benzoic acid analogue (LW6) potently inhibited HIF-1α accumulation by degrading HIF-1α without affecting the HIF-1α mRNA levels during hypoxia. Further, we identified that malate dehydrogenase 2 (MDH2) is a direct target of LW6 using a chemical biology approach. Based on this finding, we performed MDH-driven SAR studies on a series of (aryloxyacetylamino)benzoic acids and identified selective MDH1, MDH2, and dual inhibitors. We hypothesized that dual inhibition of MDH1 and MDH2 might be a powerful approach to target cancer metabolism, and selected methyl-3-(3-(4-(2,4,4-trimethylpentan-2-yl)phenoxy)propanamido)-benzoate (16c) as the most potent dual inhibitor. Kinetic studies revealed that compound 16c competitively inhibited MDH1 and MDH2. Compound 16c inhibited mitochondrial respiration and hypoxia-induced HIF-1α accumulation. In xenograft assays using HCT116 cells, compound 16c demonstrated significant in vivo antitumor efficacy. This finding provides concrete evidence that inhibition of both MDH1 and MDH2 may provide a valuable platform for developing novel therapeutics that target cancer metabolism and tumor growth.

Keyword: Hypoxia Inducible Factor, Drug Discovery, Chemical Probe, Malate Dehydrogenase

[SY08-4-1]

DECIPHERING HUMAN B CELL REPERTOIRE WITH NEXT-GENERATION SEQUENCING TECHNOLOGY

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Next-generation sequencing (NGS) has allowed a massive increase in capacity to sequence genomes at relatively low cost and in a short time frame. It has revolutionized multiple aspects of biological research and is actively being adopted into profiling human B cell receptor (BCR) repertoires. Several NGS platforms are currently available, with average read lengths of 75 bp to 8,500 bp and different error rates.

Using NGS, we successfully constructed database of human BCR repertoire from convalescent patients who recovered from SFTS (severe fever with thrombocytopenia) and MERS (middle east respiratory syndrome). Afterwards, we developed algorithms for analyzing the diversity, enrichment pattern, accumulation of somatic hyper-mutation in BCR repertoire. Through in silico analysis we selected clones of interest and prepared recombinant antibodies using a mammalian transient expression system. Their reactivity to viral coat proteins and virus-neutralizing capability was confirmed in in vitro and in vivo experiments. Currently we are investigating the clinical value of BCR repertoire profiling in autoimmune disease patients including Neuromyelitis Optica (NMO).

Keyword: NEXT GENERATION SEQUENCING, REPERTOIRE, B CELL, ANTIBODY

[SY08-4-2]

THE NEXT FRONTIER OF CANCER IMMUNOTHERAPY BY BISPECIFIC ANTIBODY

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Immune checkpoints have become the major cancer therapies as powerful and promising strategy to stimulate antitumor T cell activity. Cancer immunotherapy using immune checkpoint blockade created a major paradigm shift in the treatment of advanced-stage cancers. Recent clinical trials have demonstrated significant response rates with anti-CTLA-4 and anti-PD-1 or PD-L1 antibodies in patients with late stage melanoma and non-small cell lung cancer. The combination strategy of immunotherapeutic antibodies for treatment of cancer patients has shown benefits over single agent treatment. For example, combining anti-PD-1 antibodies to anti-CTLA-4 treatment in advanced melanoma patients has increased objective response rates. Bispecific antibodies (BsAbs) are an alternative option for the treatment of certain types of cancer. These BsAbs are composed of parts of two different monoclonal antibodies, allowing for dual binding and specificity to two different antigens. Recent progress in bispecific antibody technologies and the clinical success of a first generation bispecific T-cell engager (BiTE) antibody against CD19 resulted in major breakthrough for the next generation of T-cell redirecting bispecific antibodies. ABL Bio is developing novel bispecific antibody, which can redirect effector T cells for the targeted killing of tumor cells. ABL Bio's BsAb binds to target antigen on tumor cells in tumor microenvironment and stimulate signal on T cells by inducing IFN-gamma. The present study shows that BsAb can induce T-cell activation and proliferation in the presence of target tumor cells, redirect potent T-cell mediated killing of target tumor cells in vitro system, and inhibit growth of tumors in vivo.

Keyword: Cancer, Immunotherapy, Bispecific, Antibody, Combination

[SY08-4-3]

DEVELOPMENT OF NEXT-GENERATION ANTIBODY THERAPEUTICS AGAINST INFECTIOUS DISEASES

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Therapeutic antibodies have shown clinical success in the treatment of many diseases. By using extraordinarily large human antibody libraries, we have identified a number of potent germline-like mAbs against emerging and chronic infectious diseases, including MERS, H7N9 influenza, Zika, HBV, HIV, etc. We have also been working on the development of novel antibody constructs (from 14 kDa to 180 kDa) as the next-generation safer, cheaper, and more potent antibody-based therapeutics.

Keyword: HUMAN MONOCLONAL ANTIBODY, GERMLINE-LIKE, MERS-COV, H7N9 INFLUENZA VIRUS, ZIKA VIRUS

[SY08-4-4]

TARGETING INTRACELLULAR ONCOGENIC KRAS MUTANTS BY CYTOSOL-PENETRATING ANTIBODY

Yong-Sung Kim[1]

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Oncogenic Ras mutants, and most frequently KRas mutants (86% of Ras-driven cancers), are found in ~25% of human cancers and are high-priority anticancer drug targets. Despite 30 years of effort to develop drugs that directly target oncogenic KRas mutants, no effective pharmacological inhibitors for these mutants are clinically available, mainly because of the lack of suitable surface binding pockets for small molecules. Therapeutic antibodies have been developed that target only extracellular, but not cytosolic, proteins. To address current unmet clinical needs, our group has worked for several years to develop a cytosol-penetrating IgG-format antibody that can reach the cytosolic space of living cells owing to its endosomal escaping ability after receptor-mediated endocytosis. Using this innovative antibody technology, we have developed antibodies that directly targets intracellular oncogenic KRas mutants to block the function from outside of cells after systemic administration. Because the KRas targeting antibody holds many desirable features of the conventional IgG antibody, it shows great potential for development as a first-in-class anticancer antibody. In this talk, I will present an innovative antibody technology that directly targets intracellular oncogenic KRas mutants to block the function from outside of cells after systemic administration. Our studies also demonstrate the feasibility of developing antibody therapeutics that directly target cytosolic proteins involved in disease-associated protein-protein interactions.

Keyword: KRas, therapeutic antibody, cytosol-penetrating antibody

[SY08-5-1]

MATURATION OF AUTOPHAGOSOMES IN CELLS AND VERTEBRATES

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Macroautophagy (autophagy) requires more than 20 autophagy-related (ATG) genes, most of which are conserved in vertebrates. Most of these ATG genes are required for the formation step. By contrast, many questions remain in the maturation steps. We have identified the autophagosomal SNARE proteins syntaxin 17 and YKT6 and the tethering complex HOPS, all of which are required for fusion with lysosomes. More recently, we performed a CRISPR-based genome-wide knockout screen using a novel autophagic flux probe and found another factor required for autophagosome maturation. We have also investigated physiological functions of autophagy in not only mice but also zebrafish. We found that phenotypes of zebrafish lacking upstream and downstream ATG genes are different; zebrafish lacking upstream genes such as FIP200, ATG13, and ATG2a/b die ~10 days post fertilization (dpf), whereas those lacking downstream genes such as ATG5 and ATG16L1 die ~14 dpf. This is consistent to what we have observed in mice. We have also revealed a novel function of ATG genes in zebrafish, which is conserved in mice. These ATG-deficient zebrafish models are useful resources that can be used for comprehensive analysis of ATG genes in physiological and pathological settings in vivo. This work was supported by JSPS KAKENHI Grants-in-Aid for Scientific Research on Innovative Areas (Grant Number 25111005) and JST ERATO (Grant Numbers JPMJER1702).

Keyword: Autophagy, zebrafish

[SY08-5-2]

SELECTIVE AUTOPHAGY REGULATES LIPID-OXIDATION

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Past one decade, a growing body of evidence shed light on the importance of selective autophagy in removal of soluble proteins, proteins aggregates, damaged mitochondria, and invasive bacteria. Actually, dysfunctions of selective autophagy have been directly linked to human pathogenic conditions such as metabolic disorders, neurodegenerative diseases and cancer. However, the metabolic regulations through selective autophagy are still largely unknown. Here, we show that a deficiency in selective autophagy is associated with suppression of lipid oxidation via a transcriptional regulatory mechanism. The production of acetyl CoA and ketone bodies upon fasting was significantly impaired by loss of Atg7, which arose from transcriptional down-regulation of genes that encode enzymes involved in β-oxidation. Such down-regulation occurred due to suppression of transactivation of PPARα, a master regulator of lipid metabolism. Mechanistically, NCoR1, a nuclear receptor co-repressor 1, which interacts with PPARα and suppresses its transactivation, bound to GABARAP in a LIR-dependent manner and was degraded by autophagy. Thus, deletion of Atg7 caused marked accumulation of NCoR1 and subsequently suppressed PPARα-activity. These results demonstrate that loss of autophagy causes altered lipid metabolism through selective autophagy, possibly predisposing the organism to develop metabolic diseases.

Keyword: Autophagy, PPARα, NCoR1, Lipid-oxidation

[SY08-5-3]

MECHANISM OF AUTOPHAGOSOME FORMATION INFERRED FROM THE ANALYSIS OF THE MOLECULAR FUNCTION OF ATG2

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Autophagy is a membrane traffic pathway highly conserved from yeast to humans. In this pathway, a flattened membrane vesicle called the isolation membrane appears, expands, and closes to form a double-membrane vesicle called the autophagosome that sequesters various intracellular constituents. Subsequently, the autophagosome is fused with the lysosome or vacuole for degradation of the contents. In the budding yeast Saccharomyces cerevisiae, 19 autophagy-related (Atg) proteins important for autophagosome formation have been identified so far. These proteins assemble to organize the pre-autophagosomal structure (PAS), and then cooperatively act to form and expand the isolation membrane to complete the autophagosome. Atg2 forms a complex with the phosphatidylinositol 3-phosphate (PI3P)-binding protein Atg18. This complex localizes to the PAS in a manner dependent on PI3P, which is produced by the autophagy-specific PI3-kinase complex. A previous study showed that the isolation membrane is not formed in cells lacking Atg2 or Atg18, although other Atg proteins accumulate at the PAS in these cells. It was also shown that the Atg2-Atg18 complex localizes to a few sites on the opening edge of the isolation membrane, and these sites are in the vicinity of the endoplasmic reticulum (ER). These results indicate that the Atg2-Atg18 complex functions at a contact between the PAS and the ER to initiate the formation of the isolation membrane. However, the molecular function of Atg2 remains to be elucidated, and thus how the Atg2-Atg18 complex is involved in this process is still unknown. Here, we report the identification and characterization of two membrane-binding domains of Atg2, which are responsible for autophagosome formation. Our in vivo and in vitro analyses suggested that the membrane binding of Atg2 in its C-terminal amphipathic helix is required for Atg18 binding to PI3P, and thus for the targeting of the Atg2-Atg18 complex to the PAS. On the other hand, the N-terminal membrane-binding domain of Atg2 was not involved in the PAS targeting of the Atg2-Atg18 complex, but responsible for the formation of the isolation membrane. Moreover, our data suggested the association of this N-terminal region of Atg2 with the ER. Based on these results, we propose that Atg2, with the two distal membrane-binding domains, links the PAS to the ER to initiate the formation and expansion of the isolation membrane in autophagosome formation.

Keyword: autophagy, autophagosome formation, yeast, Atg protein

[SY08-5-4]

MOLECULAR MECHANISMS OF INITIAL STEPS OF AUTOPHAGY

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Autophagy is an intracellular degradation system that utilizes autophagosomes to deliver cytoplasmic materials to lysosomes for degradation. Upon nutrient starvation in budding yeast, an intrinsically disordered protein Atg13 is dephosphorylated, which enhances its affinity with both Atg1 and Atg17 to form the Atg1 complex. The Atg1 complex functions as a core of the autophagosome formation site, termed the pre-autophagosomal structure (PAS) in yeast, from which autophagosomes are believed to be generated. We performed structural studies on the Atg1 complex and unveiled not only the specific intra-complex interactions constructing the Atg1 complex, but also the inter-complex interactions that leads to the higher-order assembly of the Atg1 complexes. Moreover, biochemical and proteomic analyses revealed how phosphorylation/dephosphorylation of specific Ser residues in the intrinsically disordered region of Atg13 regulates these intra- and inter-complex interactions. We are now reconstituting initial steps of autophagy in vitro, which would be a useful tool to study autophagy initiation in a molecular level and to develop compounds that specifically regulate autophagy.

Keyword: autophagy, Atg1 complex, Atg13, intrinsically disordered, phosphorylation

[SY08-5-5]

A NOVEL APPROACH TO DETECT THE BINDING PROPERTY OF LC3/GABARAP FAMILY WITH ITS TARGET LIRS IN LIVE CELLS

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Macroautophagy involves the intracellular bulky degradation pathway in lysosomes. Each LC3/GABARAP family protein is involved in autophagic process via specific interaction with LC3/GABARAP-interacting protein which has LC3-interacting region (LIR) motifs. Although several consensus sequences of the LIR motif are known, their specific binding properties and it functional significance on autophagy remain elusive. Here, we developed a novel method to detect the binding property of LC3/GABARAP family with its target LIR motifs in live cells. To do this, we generated and expressed mRFP-tagged LIR with 3x nuclear localization signal (3xNLS) and GFP-tagged each LC3/GABARAP(G→A) mutant which is not localized to autophagosome. First of all, we found that the ratio of the fluorescent intensity between nuclear and cytosol (N/C ratio) of all six GFPLC3/GABARAP(G→A) mutant in cells expressing LIR(TP)-mRFP-3xNLS (LIR motif from TP53inp2) was significantly higher than that of cells expressing mRFP-3xNLS, whereas the N/C ratio of a LIR binding-deficient mutant was comparable to that of mRFP-3xNLS expressing cells in all GFP-LC3/GABARAP(G→A) co-expressing cells. There results were consistent with the GST-pulldown assay, further validating the binding property with each LC3/GABARAP-proteins by our novel method. These results support our main idea that LIR-motif containing proteins with 3xNLS could sequester cytosolic LC3/GABARAP proteins into nucleus depending on LIR binding property. We are now investigating the binding property of LC3/GABARAP family with the known or putative LIR motifs using our novel method. In this presentation, I will summarize binding specificity of each LIR motif and present a novel binding protein with LC3/GABARAP family and their functional roles. Collectively, these results suggest its possible application on autophagy research to characterize the role of novel LIR-motif containing proteins.

Keyword: autophagy, selective autophagy, LC3, GABARAP, nuclear localization signal

[SY09-1-1]

BREAST CANCER METASTASIS- BIOLOGICAL IMPLICATIONS AND THERAPEUTIC OPPORTUNITIES

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Breast cancer is a paradigm for two personalized therapies in use- endocrine therapy for hormone receptor positive (ER/PR) patients, and Herceptin for HER2+ patients. However, patients with triple negative breast cancers (TNBC, ER-PR-Her2-) are refractory to these therapies and represent a formidable clinical challenge, as it presents distinct molecular subtypes, follows a notoriously aggressive clinical course compared to most breast cancers, and are impervious to currently available targeted therapies. Surgical resection and standard chemotherapy are the only therapeutic options for women with metastatic TNBC. However, these treatments fail, giving rise to metastatic relapse within 5 years of a diagnosis. Therefore, there is an unmet medical need for targeted therapies for effective treatment of these high-risk breast cancer patients. To develop "mechanism based" effective anti-metastatic therapies, we have delineated specific biological mechanisms underlying key steps in the metastatic cascade, which has unmasked potential vulnerabilities that we wish to exploit for clinical translation. We will describe mechanism-based therapeutic approaches that exploit unique cancer cell autonomous and non-autonomous vulnerabilities in the metastatic cascade related to invasion, dissemination, metastatic outgrowth and therapeutic resistance. To capture the full translational potential of our approaches, a variety of preclinical metastatic breast cancer models, including a collection of PDX models (representing major TNBC molecular subtypes) will be used. Preclinical data from this study provides unique translational opportunities and may lead to the design of clinical trials for TNBC and other high-risk breast cancer patients.

Keyword: BREAST CANCER, THERAPY, EPIGENETICS, MICROENVIRONMENT,

[SY09-1-2]

PATHOGENETIC ROLE OF UBE2O IN CANCER

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UBE2O is localized in the 17q25 locus, which is known to be amplified in human cancers, but its role in tumorigenesis remains undefined. Here we show that Ube2o deletion in MMTV-PyVT or TRAMP mice profoundly impairs tumor initiation, growth and metastasis, while switching off the metabolic reprogramming of tumor cells. Mechanistically, UBE2O specifically targets AMPKα2 for ubiquitination and degradation, and thereby promotes activation of the mTOR-HIF1α pathway. Notably, inactivation of AMPKα2, but not AMPKα1, abrogates the tumor attenuation caused by UBE2O-loss, while treatment with rapamycin or inhibition of HIF1α ablates UBE2O-dependent tumor biology. Finally, pharmacological blockade of UBE2O inhibits tumorigenesis through the restoration of AMPKα2, suggesting the UBE2O-AMPKα2 axis as a potential cancer therapeutic target.

Keyword: UBE2O, Breast cancer, E2 enzyme, AMPK, metastasis

[SY09-1-3]

A MICRORNA:GENE:PSEUDOGENE NETWORK REGULATES TUMORIGENESIS IN PROSTATE CANCER

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Pseudogenes, non-coding homologs of protein-coding genes, were once considered non-functional evolutional relics. Recent studies have shown that pseudogene transcripts can regulate their parental transcripts by sequestering shared microRNAs, thus acting as competing endogenous RNAs (ceRNAs). In this study, we utilize an unbiased screen to identify the ferritin heavy chain 1 (FTH1) transcript and multiple FTH1 pseudogenes as targets of several oncogenic miRNAs in prostate cancer. We characterize the critical role of this FTH1 gene:pseudogene:microRNA network in regulating tumorigenesis in prostate cancer, and show that impairing microRNA binding and subsequent ceRNA crosstalk results in complete phenotype rescue. Our results also demonstrate that pseudogenes are able to regulate intracellular iron levels, which are critical for multiple physiological and pathophysiological processes. In summary, we describe a novel and extensive gene:pseudogene ceRNA network comprising multiple microRNAs and multiple pseudogenes derived from a single parental gene, which regulates iron storage and tumorigenesis in prostate cancer.

[SY09-2-1]

DISCOVERING NOVEL CANCER GENETIC VULNERABILITIES WITH CRISPR SCREENS

Traver Hart[1]

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The ability to do saturating knockout screens on cancer cell lines is transforming cancer genetics and functional genomics. We are now able to comprehensively define which genes are essential for proliferation in a cell line, and to differentiate those which are consistent across genetic backgrounds from those required only in a particular lineage or the presence of an oncogenic driver. Both sets of genes harbor potential therapeutic targets.

This talk will cover lessons learned from the integrated analysis of 400 whole-genome CRISPR knockout screens in cancer cell lines. Key topics include the importance of gold standards for quality control, the unique ability of network approaches to discover novel phenotypes which may represent clinically actionable tumor subtypes, and how the fundamental relationships between our concepts of synthetic lethality, context-dependent essentiality, and genetic interaction might be used to predict cancer-relevant synthetic lethal interactions.

Keyword: CANCER, GENETIC INTERACTION, CRISPR, NETWORK BIOLOGY

[SY09-2-2]

NETWORK-AUGMENTED ANALYSIS OF DISEASE GENOMICS DATA

Insuk Lee[1]

[1]Yonsei University, Korea

Recent genomic revolution opened new avenues to understanding human disease. However, it also revealed complex nature of human disease. For example, currently more than several hundred genes are believed to be associated to human cancer. Genome-wide association study (GWAS) suggests hundreds of disease-related genes, but together explaining only 10-20% of total disease inheritance at most. Because of this overwhelming complexity of disease-causing pathway, modern disease genetics needs to be more systematic and predictive. However, the network organization of disease systems also provide big opportunity to investigate the genetic organization of complex diseases through the molecular networks. Our research group has developed co-functional gene networks for many organisms including human (HumanNet) and various network-guided methods to identify novel disease genes and modules. In this talk, I will present our recent work in network-based augmenting and interpreting cancer somatic mutation data (MUFFINN), GWAS data for complex diseases (GWAB), and gene set enrichment analysis for disease transcriptome data (NGSEA).

[SY09-2-3]

SYSTEMIC FUNCTIONAL ANALYSIS OF MEDICINAL COMPOUNDS WITH A VIRTUAL HUMAN SYSTEM CODA

Doheon Lee[1]

[1]KAIST, Korea

Physiological functions of medicinal compounds have been uncovered in the limited context of their intended purposes or empirical serendipity. Formidable complexity of systemic human physiology often give rises of unintended effects of medicinal compounds during the drug development processes or even after the drug approvals. Though the beneficial unintended effects could lead opportunities of repositioning drugs, the harmful effects might put critical hurdles against successful drug development. We have been developing a virtual human system, CODA, which can explore functional effects of medicinal compounds in the systemic level. CODA integrates three types of physiological knowledge from public structured databases, literature, and in-house experiments into a unified format of physiological interactions. Ten public databases including KEGG, GO, and CTD have been transformed; around 25 million PUBMED abstracts have been text-mined; and more than 3,000 in-house novel findings have been incorporated. We have also developed two types of analysis on the CODA knowledge repository. Given medicinal compounds of interest, CODA can identify possible phenotypic effects in the systemic level. When medicinal compounds and their observed functional effects are given, CODA can enumerate possible effect paths encompassing molecular, functional, and disease level interactions. We have been testing CODA by applying it to various tasks including drug repositioning, drug-drug interactions, and side effect prediction with known benchmark datasets. Though we are enriching CODA with more knowledge sources and more sophisticated analysis techniques, the current version is already providing unique analysis capabilities and one of the most comprehensive information for medical compound analysis.

Keyword: virtual human system, medicinal compound, bioinformatics, systems biology, drug

[SY09-2-4]

GENERAL RULES FOR FUNCTIONAL MICRORNA TARGETING

Daehyun Baek[1]

[1]Seoul National University, Korea

The functional rules for microRNA (miRNA) targeting remain controversial despite their biological importance because only a small fraction of distinct interactions, called site types, have been examined among an astronomical number of site types that can occur between miRNAs and their target mRNAs. To systematically discover functional site types and to evaluate the contradicting rules reported previously, we used large-scale transcriptome data and statistically examined whether each of approximately 2 billion site types is enriched in differentially downregulated mRNAs responding to overexpressed miRNAs. Accordingly, we identified seven non-canonical functional site types, most of which are novel, in addition to four canonical site types, while also removing numerous false positives reported by previous studies. Extensive experimental validation and significantly elevated 3′ UTR sequence conservation indicate that these non-canonical site types may have biologically relevant roles. Our expanded catalog of functional site types suggests that the gene regulatory network controlled by miRNAs may be far more complex than currently understood.

Keyword: microRNA, microRNA targeting, noncanonical site type, target site, posttranscriptional gene expression regulation

[SY09-4-1]

UNDERSTANDING HOW WE GET FAT: DIETARY REGULATION OF ADIPOCYTE STEM CELLS

Matt Rodeheffer[1]

[1]Yale University, USA

We are in the midst of a global obesity epidemic; however, the cause of this recent widespread increase in adiposity remains a matter or debate. Our lab recently identified and characterized the adipocyte cellular lineage in vivo. Directed studies of the regulation of the adipocyte precursors in vivo have shown that within some fat depots adipocyte precursors are transiently activated at the onset of obesity, leading to an increase in the number of adipocytes and adipose mass. Furthermore, we have demonstrated that the mechanism of precursor activation is specific to the obese state. Here we show that high fat diets high in oleic acid, such as lard-based diets, result in adipocyte hyperplasia, while high fat diets low in oleic acid, such as coconut oil, do not stimulate increases in adipocyte number and gain less weight. Oleic acid is also sufficient to activate adipocyte precursors in vivo and stimulates adipogenesis in vitro. These findings suggest that the recent, drastic changes in types of fats in our diets have directly contributed to increased obesity rates.

[SY09-4-2]

MICROENVIRONMENTAL REGULATION OF IN VIVO ADIPOGENESIS

Yun-Hee Lee[1]

[1]Yonsei University, Korea

Adipogenesis continues throughout life and is required for the maintenance of metabolic homeostasis. Beige adipocytes are thermogenic adipocytes that appear in white adipose tissues and have a potential to counteract obesity and metabolic diseases Although progenitors responsible for in vivo beige adipogenesis have been characterized, understanding of molecular identity of the progenitors is limited due to cellular heterogeneity of adipose tissue and unsynchronized natures of in vivo adipogenesis. While multiple intrinsic molecular players in beige adipogenesis have been identified, micro-environmental regulation of progenitors has been crucial for progenitor activation. In this regard, adipose tissue macrophages play a critical role as a niche component of in vivo adipogenesis. Our recent studies demonstrated that progenitor proliferation and differentiation appears to be driven by local signals from M2-polarized macrophages, which clear dead fat cells and by beta3 adrenergic receptors that are expressed in nascent beige adipocytes. We also demonstrated that the mechanisms that lead to beige adipocyte recruitments vary by tissue location and by the nature of adrenergic activation. Mechanistic understanding of in vivo adipogenesis will facilitate identification of novel therapeutic approaches to prevent or reverse metabolic disease.

Keyword: Adipogenesis, Microenvironment, Macrophages, Adipose tissue

[SY09-4-3]

ESSENTIAL ROLES OF PRMT1 IN PANCREATIC β-CELL

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Although the progression of type 2 diabetes initiates with the development of insulin resistance, hyperglycemia does not develop as long as pancreatic -cells compensate for insulin resistance. Type 2 diabetes can be diagnosed when -cells fail to come up with the increased insulin demand due to insulin resistance. Thus, β-cell failure along with insulin resistance is an essential feature of type 2 diabetes. β-Cell death has been thought as a primary cause of β-cell failure. However, given that clear deficiency in glucose stimulated insulin secretion is observed in type 2 diabetes despite the absence of marked differences in β-cell mass, β-cell seems to experience more dynamic changes before β-cell death develops. In fact, recent studies in human and mice indicate that fully differentiated β-cell retains more plasticity than we appreciated before and the loss of β-cell identity results in dedifferentiation of β-cell into alternate endocrine cell fates. Although β-cell dedifferentiation is characterized by the changes in the expression of β-cell specific transcription factors as well as the decrease in insulin production, it remains to be elucidated how β-cell dedifferentiation develops. Here, we propose a new model for β-cell dedifferentiation by knocking out protein arginine N-methyltransferase-1 (PRMT1). PRMT1 is a major type of protein arginine methyltransferase in mammalian cells. Knock out of Prmt1 in developing pancreas (Prmt1 PKO) showed severe defect in development of endocrine pancreas. β-Cell specific Prmt1 knock out (Prmt1 βKO) did not affect mice were grossly normal until they developed glucose intolerance at 12 weeks of age. Glucose stimulated insulin secretion and mitochondrial function were decreased in the islets of Prmt1 βKO mice at 12 weeks of age. Moreover, high-fat diet induced β-cell dedifferentiation in Prmt1 βKO mice. Inducible knock out of Prmt1 in adult β-cell (Prmt1 βiKO) resulted in marked down-regulation of mature β-cell genes and genes involved in mitochondrial function. In conclusion, PRMT1 plays essential roles in maintaining mature β-cell function and identity.

Keyword: PRMT1, beta-cell development, insulin, epigenetic control, diabetes

[SY09-4-4]

CELL SORTING IN PCDH19 EPILEPSY

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At the request of the author, this abstract is not available for sharing.

[SY09-5-1]

SOME IMPORTANT ROLES OF O-GLCNAC MODIFICATION IN CANCER

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O-GlcNAc transferase (OGT) transfers a single GlcNAc to hydroxyl groups of serine and threonine residues on various nuclear and cytoplasmic proteins. in general, increased amount of total O-GlcNAcylation has been found in many cancer cells, and many experimental results imply that this modification might be an important indicator of nutritional status to modulate key physiological pathways that control many cancer cell phenotypes. Many important proteins that involve in cancer progression and metastasis found to be modified with O-GlcNAc. The cellular protein level of OGT is important to regulate cancer cell progression. But the molecular mechanisms regulating the subcellular OGT protein level is unclear. We report that X-linked inhibitor apoptosis protein (XIAP), a well-known caspase inhibitor, acts as an E3 ligase and promotes the proteasome-dependent degradation of OGT in vivo and in vitro. We show that transiently overexpressing XIAP directly poly-ubiquitylated OGT and induced degradation of endogenous OGT levels in an independent of OGT mRNA levels. The HCT116 cells stably overexpressing XIAP show reduced OGT protein levels and decreased growth rate and colony formation compared to the control HCT116 cells. Our study suggests that a novel function of XIAP in the regulation of OGT, which is distinctly different from its well characterized anti-apoptotic properties.

Keyword: O-GlcNAc, cancer, O-GlcNAc transferase, XIAP, metastasis

[SY09-5-2]

PROTEOME-SCALE IDENTIFICATION OF O-GLCNACYLATED PROTEINS USING A FREE RADICAL GENERATING N-TERMINAL CHEMICAL TAGGING PROBE

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The O-linked β-N-acetyl glucosamine (O-GlcNAc) modification, a dynamic post-translational modification (PTM) to serine or threonine residues of nuclear and cytoplasmic proteins, is involved in many different cellular processes including signal transduction and gene regulation. Despite the vital functional roles of protein O-GlcNAcylation in many cellular processes, the area of O-GlcNAc research field has been hampered, mainly due to the lack of techniques for the identification, quantification and site mapping of O-GlcNAc modification in proteins. In this presentation, we will discuss the development of an N-terminal chemical probe to induce a radical-driven peptide fragmentation while retained the O-GlcNAc moiety in the conventional CID mass spectrometry analysis. This free-radical initiating N-terminal chemical labeling allowed us to acquire normal tandem MS/MS patterns without a neutral loss of O-GlcNAc moiety, which is suitable for the precise assignment of O-GlcNAc modification sites. More importantly, we found that the chemical probe also allows a proteome-scale analysis of O-GlcNAcylated proteins as the radical-driven peptide fragmentation for O-GlcNAc-modified peptides is CID-fragmentation dependent. Collectively, combined with the radical generating N-terminal chemical tagging and with the conventional CID mass spectrometry analytical platform, we can confidently identify O-GlcNAc-modified proteins with an accurate determination of O-GlcNAc modification sites. This approach can be used in a variety of applications for O-GlcNAc research, which will provide insights into important functions of protein O-GlcNAcylation in many biological processes.

Keyword: Proteomics, O-GlcNAc, PTM, Protein, Mass Spectrometry

[SY09-5-3]

O-GLCNACYLATION IN B CELL IMMUNITY

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The generation of antigen-specific antibodies and memory B cells is one of the most important immune protections of the host and is the basis for successful vaccination strategies. Whether and how glycans may affect the function of B cells remain largely unknown. Intracellular glycosylation on nuclear and cytoplasmic proteins, O-GlcNAcylation, is catalyzed by O-GlcNAc transferase (OGT) and is removed by O-GlcNAcase (OGA). We showed that protein O-GlcNAcylation accumulates after cross-linking of B cell receptor (BCR) by anti-IgM in B cells and that inhibition of OGA by a specific inhibitor, thiamet G, promotes anti-IgM-mediated activation of B cells. Comparative phosphoproteomics analysis reveled several O-GlcNAc-dependent phosphoproteins in B cell activation. We further created mouse lines in which Ogt is deleted in a B cell-specific manner in early B cell development or in the antigen-experienced B cells. Ogt ablation in B cells alters B cell homeostasis and impairs B cell proliferation upon activation. We identified O-GlcNAcylated proteins by comparative proteomic analysis of wild-type and Ogt-deficient B cells, and found Lyn as one of the O-GlcNAcylated proteins. O-GlcNAcylation of Lyn at serine 19 is crucial for efficient Lyn activation and Syk interaction in BCR-mediated B cell activation. Further, mice lacking Ogt in B cells also showed severe defects in the generation of germinal center and the production of antigen-specific antibody following immunization. These results demonstrate that B cells rely on a monosaccharide to maintain homeostasis, induce BCR signaling and evoke antibody responses.

Keyword: O-GlcNAcylation, B cell, activation, antibody, proteomics

[SY09-5-4]

REGULATION OF ER-GOLGI TRANSPORT THROUGH O-GLCNAC MODIFICATION

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The conventional secretory pathway is indispensable for eukaryotic cells. Newly synthesized membrane and secretory proteins are released from the endoplasmic reticulum (ER) through ER-derived vesicles to their appropriate destination. Vesicle formation is important for steady protein trafficking. O-GlcNAcylation (O-GlcNAc) is a unique protein glycosylation signature, whose dynamic regulation by O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) occurs exclusively for nuclear and cytoplasmic proteins. Because of this locally limited property, the role of O-GlcNAc in the conventional protein secretory pathway is unknown. We report that Sec31A on COPII vesicles, a specific coat protein complex for anterograde trafficking in the ER-Golgi network, is O-GlcNAcylated on S964, which accelerates COPII vesicle formation through control of its binding affinity to ALG-2, a calcium-binding protein. Together, O-GlcNAc on Sec31A regulates conventional secretory vesicle trafficking in the ER-Golgi network. These modifications accelerate COPII vesicle formation and accelerated anterograde transport of vesicle within the ER-Golgi networks.

Keyword: O-GlcNAc, ER-Golgi transport, COPII vesicle, Sec31A, ERES

[SY10-2-1]

GENOMIC DELINEATION OF GLIOMA EVOLUTION

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Individual cancers are comprised of populations of genetically distinct, separated subclones that compete for resources during the disease course, leading to dynamic evolutionary processes. High throughput technologies provide a snapshot of the disease process in space & time. To better understand therapy resistance and apply precision therapeutics, a more refined understanding of glioma evolution is required that can only be provided by multi-dimensional tumor sampling. We have characterized glioma across multiple dimensions in order to delineate evolutionary patterns, to inform optimal sampling for clinical decision making, and to demonstrate that extrachromosomal DNA elements play a critical role in gliomagenesis.

Keyword: Glioma, Sequencing, Computational biology, Cancer

[SY10-2-2]

PDX GENOMICS & INFORMATICS FOR PRECISION TREATMENT OF CANCER

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Clinical cancer genomics towards precision medicine has become an integrative science encompassing multi-layered omics data, animal models, and an advanced bioinformatics analysis. In this talk, we describe our efforts to realize the precision treatment of lung cancer utilizing patient-derived xenograft (PDX) mouse models with extensive analysis of public cancer omics data. We have established 205 PDX models representing 152 lung cancer patients and generated whole exome and transcriptome sequencing data. Compatibility issues between PDX mice and the patient tumors will be addressed based on the deep sequencing data. Concordance of somatic mutations, CNV profiles, and clonal evolution were examined to verify the reliability of PDX mouse models. We also developed a computational pipeline to identify somatic mutations for exome sequencing data of PDX mouse when patient normal or tumor data were missing. Extensive filtering processes were used to remove mouse-originated mutations and germline mutations. Finally, we will briefly describe our effort identify PDX mouse models that could be responsive to checkpoint blockades. A regression model was developed to combine stromal and immunological signature of tumor transcriptome data. Responder mouse models would serve as promising preclinical models to test the efficacy of checkpoint inhibitor drugs in combination with other conventional and targeted therapies.

Keyword: cancer, pdx mouse, precision medicine, bioinformatics

[SY10-2-3]

FANTOM6: FROM DISCOVERY TO FUNCTIONAL UNDERSTANDING OF LONG NON-CODING RNA

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Only 2-3% of our genome is protein coding while the remaining is considered 'junk'. However, we and others revealed that transcription is pervasive in these regions - labeled as long non-coding RNAs (lncRNAs). Recent publications suggest that lncRNA functions are diverse: regulating epigenetic, translation, and transcription processes. Genome-wide association studies (GWAS) and e-QTL analyses strongly suggested biological and pathological significance of these lncRNAs. And our recent strategy to globally map the interaction of lncRNAs to chromatin suggested distinctive cis-/trans-regulation in a cell type specific manner. However, only a small fraction of lncRNA have been studied. To broadly characterize the functional role of lncRNAs, we perturbed 700+ lncRNA in human fibroblasts and iPS cells, and measured: 1) cell growth phenotype, and 2) molecular phenotype by CAGE-seq. We showed that 8-12% of lncRNAs exhibited cell growth phenotype. When focusing on lncRNAs expressed in both cell types, we observed cell-type specific regulation by the same lncRNAs, suggesting the importance of cellular context when characterizing its function. Systematic comparison between cellular and molecular phenotypes revealed strong concordance between the two approaches, which led us to reliably associate new pathways to hundreds of lncRNA that were previously unknown. Overall, about 70% of the knockdowns resulted in a distinct transcriptome change and further integration of the perturbation data with RNA features revealed that polyadenylated (i.e. stable) and antisense transcripts were more likely to be functional. Interestingly, the impact of transcriptome was not significantly correlated with their expression level or conservation, indicating that wide class of lncRNAs exhibit function. The study indicates that long-ncRNA molecules are functionally more diverse and relevant than previously thought and play essential roles in health and disease.

Keyword: lncRNA, FANTOM, functional genomics

[SY10-2-4]

MEDICAL BIG DATA IN THE FIELD

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In many area, big data has driven a number of technological innovations, and now big data is no longer a special word. Unlike earlier rosy expectations, medical big data did not fundamentally change medical care in the field. As far as big data is concerned, there is still a big gap between the bench and the bed. Unlike in other areas, the delay of big data's innovation in medical arena may be due to many reasons including privacy issues, difficulties of proving causality, and other technical issues. Despite these difficulties, the gap between the bench and the bed is gradually being filled, and some of these efforts are introduced here. Patient data stored in many hospitals is probably the most important source of medical big data, but unfortunately these data are scattered among hospitals. Patient data stored in each hospital is often too small to be used in artificial intelligence, so it is necessary to collect the data into one place. Collecting data from multiple hospitals to the one place is often very difficult due to technical reasons, such as the difference in the database structure of each hospital, as well as privacy and conflicting interests among hospitals. Many common data models have been proposed to overcome this difficulty. The Observational Medical Outcomes Partnership (OMOP) Common Data Model (CDM) of Observational Health Data Sciences and Informatics (OHDSI) program is a representative example. OHDSI program, which is a multi-stakeholder, interdisciplinary collaborative to bring out the value of health data through large-scale analytics, has established an international network of researchers and observational health databases. Each hospital converts its electronic medical record to the OMOP CDM structure, stores it on a separate server, and periodically updates it. With this system, the raw data of the hospital is kept within the hospital, and it is free from personal privacy issues and at the same time, it can prevent data being used for other than its intended purpose so that shared data can greatly contribute to the facilitation of research. In many cases, medical claims data are seen as a model for medical big data. Korea has nation-led health insurance for all citizens and it is possible to analyze by using health insurance data for nation-wide aspect of any disease. Korea provides free obligatory health screenings for eligible citizens. Every year, two thirds of the whole adult population of Korea is undergoing health screening. These health screenings data are shared to all eligible researchers through the National Health Insurance Sharing service. So far, several examples of the big data usage in the medical field have been reviewed. It is hoped that technological methods, such as blockchain technology,

to overcome the limitations of medical big data would be developed, so that more research using big data will be implemented and medical innovation will be achieved to contribute toward the promotion of human welfare.

Keyword: big data, predirctive model, epidemiology, statistics, artificial intelligence

[SY10-4-1]

REGULATION OF MUSCLE STEM CELL QUIESCENCE AND ACTIVATION

Thomas Rando[1]

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Many adult stem cells persist in a non-cycling, quiescent state. We previously demonstrated that the quiescent state of adult muscle stem cells (MuSCs) is actively maintained by Notch signaling and a transcriptional regulatory pathway involving the microRNA miR489. Recently, we have explored the dynamics of the quiescent state. Normally, nearly all MuSCs exist in a conventional quiescent state, G0. However, in response to a distant injury, MuSCs enter a distinct quiescent state that we termed GAlert. Cells in GAlert are distinguished from cells in G0 by their increased rate of entry into the cell cycle following stimuli in vivo or explantation ex vivo, increased mitochondrial activity, and a marked induction of mTORC1 signaling. Muscles in which the MuSCs have been induced to enter the GAlert state regenerate much faster than control muscles, suggesting that the alert MuSCs are poised for tissue repair more than G0 cells. Using genetic models, we found that mTORC1 activity is both necessary and sufficient for the transition of MuSCs from G0 into GAlert. Moreover, we show that the systemic signals that mediate the reversible transition of MuSCs from G0 to GAlert are conveyed through the HGF receptor, cMet. We recently showed that the systemic signals responsible of induction of MuSCs into the alert state involve the activation of the circulating protease, hepatocyte growth factor activator (HGFA), which in turn activates local HGF, allowing it to bind to cMet receptors on the MuSC membrane. The GAlert state represents a novel form of cellular memory in which a previous stimulus results in a long-lasting cellular response that allows stem cells to respond more rapidly and effectively to subsequent challenges for tissue homeostasis and repair.

Keyword: stem cell, muscle, quiescence

[SY10-4-2]

MICRORNA EXPRESSION CONTROLS SKELETAL MUSCLE AGING

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Age-associated degeneration of skeletal muscle, called sarcopenia, is a major cause of morbidity, mortality, and overall declines of quality of life in the elderly. Several studies have demonstrated that miRNAs have crucial roles in regulation of skeletal muscle homeostasis including muscle differentiation, regeneration, and metabolism. We found that microRNAs down-regulated in skeletal muscle aging are enriched as a cluster in the Dlk1-Dio3 imprinted genomic region, the largest microRNA cluster found in mammal, located on human chromosome 14 (mouse chromosome 12). Forty pre-microRNAs in this locus were down-regulated in old mouse myoblast. The imprinted Dlk1-Dio3 genomic region, contains the paternally expressed genes Dlk1, Rtl1, and Dio3 and the maternally expressed non-coding RNA genes Meg3 (Gtl2) and Meg8 (Rian) as well as the antisense Rtl1 (anti-Rtl1). It has been known that overexpression of Dlk1 and Rtl1 is strongly correlated to muscle hypertrophy in Callipyge sheep and that MEF2A induces Gtl2-Dio3 microRNA cluster-mediated WNT signaling in skeletal muscle regeneration. Further analysis of microRNA-mRNA interaction network enabled identifying the microRNA-contributed muscle homeostasis and aging. Notably, several miRNAs in the Dlk1-Dio3 cluster promoted an increase in myotube diameter when transfected into C2C12 myotubes. Fifty-three mature miRNAs in the cluster have predicted binding sites on the 3′-UTR of Smad4 transcript encoding a transcription coactivator involved in muscle protein degradation. We also observed that 18 human miRNAs encoded in Dlk1-Dio3 cluster were declined in their expressions with age in human gluteus maximus muscle, consistent with our mouse data. Taken together, our findings support the theory that a group of miRNAs clustered in the Dlk1-Dio3 locus suppress muscle atrophy by tandemly targeting an atrophy-related gene and are coordinately downregulated in aging muscle, providing potential targets for development of therapeutics against muscle aging.

Keyword: microRNA, sarcopenia, SMAD4, skeletal muscle, aging

[SY10-4-3]

ROLE OF CELLULAR AGING IN CARDIO-METABOLIC DISEASE

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Epidemiological studies have shown that age is the dominant risk factor for lifestyle-related diseases. The incidence and the prevalence of diabetes, heart failure, coronary heart disease and hypertension increase with advancing age. However, the molecular mechanisms underlying the increased risk of such diseases that is conferred by aging remain unclear. Cellular senescence is originally described as the finite replicative lifespan of human somatic cells in culture. Cellular senescence is accompanied by a specific set of phenotypic changes in morphology and gene expression including negative regulators of the cell cycle such as p53. Primary cultured cells from patients with premature aging syndromes are known to have a shorter lifespan than cells from age-matched healthy persons. It is also reported that the number of senescent cells increases in various tissues with advancing age. Interestingly, such accumulation of senescent cells in aged animals is attenuated by caloric restriction that regulates the lifespan regulatory system and delays age-associate phenotypes. I therefore hypothesize that cellular senescence in vivo contributes to the pathogenesis of age-associated disease. An important feature shared by several types of senescent cells is persistent up-regulation of inflammatory molecules and accumulating evidence has suggested a critical role of senescence-induced inflammation in metabolic and cardiovascular disease. Here I will present our recent data on the role of cellular senescence in age-related pathologies and will discuss the potential of anti-senescence as a novel therapeutic strategy for age-associated diseases.

Keyword: Aging, Cellular senescence, p53

[SY10-4-4]

PROTEASOME ACTIVATION DELAYS AGING AND PROGRESSION OF AGE-RELATED DISEASES

Efstathios Gonos[1]

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Aging and longevity are two multifactorial biological phenomena whose knowledge at molecular level is still limited. We have studied proteasome function in replicative senescence and cell survival (Mol Aspects Med 35, 1-71; Ageing Res Rev 23, 37-55). We have observed reduced levels of proteasome content and activities in senescent cells due to the down-regulation of the catalytic subunits of the 20S complex (J Biol Chem 278, 28026-28037). In support, partial inhibition of proteasomes in young cells by specific inhibitors induces premature senescence which is p53 dependent (Aging Cell 7, 717-732). Stable over-expression of catalytic subunits or POMP resulted in enhanced proteasome assembly and activities and increased cell survival following treatments with various oxidants. Importantly, the developed "proteasome activated" human fibroblasts cell lines exhibit a delay of senescence by approximately 20% (J Biol Chem 280, 11840-11850; J Biol Chem 284, 30076-30086). Similar proteasome activation in human mesenchymal stem cells not only increases their lifespan, but also enhances stemness significantly (Free Rad Biol Med 103, 226-235). Moreover, additional findings indicate that the recorded proteasome activation by many inducers is Nrf2-dependent (J Biol Chem 285, 8171-8184). Finally, we provide evidence that proteasome activation is an evolutionary conserved mechanism, as it can delay aging in vivo and, importantly, it also confers deceleration of aggregation-related pathologies, such as Alzheimer's or Huntington's diseases (FASEB J 29, 611-622). Given these findings, recent work has identified a proteasome activator that decelerates aging and Alzheimer's disease progression (Antiox Redox Signal 25, 855-869).

Keyword: aging, longevity, proteasome, proteolysis

[SY10-5-1]

GLYCOSYLATION IN MUSCULAR DYSTROPHY

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Muscular dystrophy is a group of genetic disorders characterized by progressive muscle weakness. Dystroglycanopathy, a new classification of muscular dystrophy often associating with abnormalities in the central nervous system, was established in the early 2000s. The common biochemical feature of dystroglycanopathy is abnormal glycosylation of alpha-dystroglycan. Alpha-dystroglycan carries a unique glycan, O-mannosyl glycan, which is required for binding with various ligands including laminin to form stable complexes in the membrane. We reported GlcNAc 1-2Man glycan structure and this linkage is synthesized by POMGnT1 that is identified as causative for muscle-eye-brain disease, one of dystroglycanopathies. Then, we found that the initial Man transfer to the Ser/Thr residues of alpha-dystroglycan is catalyzed by a POMT1/POMT2, and causative for Walker-Warburg syndrome, another type of dystroglycanopathy. In just the last few years, the entire structure of O-mannosyl glycan has been proposed. The tandem ribitol-phosphate and GlcA-Xyl repeat structures are unique, and the former structure is essential for the latter structure formation. The tandem ribitol-phosphate structure was shown to be synthesized through the sequential enzymatic actions of fukutin and FKRP as ribitol-phosphate transferases and LARGE synthesizes multiple GlcA-Xyl repeats. Fukutin, FKRP, and LARGE are causative for different dystroglycanopathies. Finally, we find that fukutin forms a complex with POMGnT1, and the POMGnT1-fukutin complex is important to form a platform that requires further glycosylation of the GlcA-Xyl repeat by LARGE. Collectively, structures and processing of O-mannosyl glycans are highly complicate, and possible regulatory mechanism of O-mannosyl glycans will be discussed.

Keyword: Glycosylation, Muscular dystrophy, O-Mannosyl glycan, Ribitol-phosphate,

[SY10-5-2]

PROTEOGLYCANS IN AXON REGENERATION AND NEURAL PLASTICITY

Kenji Kadomatsu[1], Kazuma Sakamopto[1], Tomoya Ozaki[1] and Yuanhao Gong[1]

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Axons in the central nervous system cannot regenerate after injury. It is due to low intrinsic capacity of regeneration and emerging inhibitory molecules upon injury. Therapeutics for neuronal injuries are not currently available and have long been awaited. Chondroitin sulfate (CS) is one of the strongest inhibitors, while heparan sulfate (HS) promotes axon growth. Both CS and HS are glycosaminoglycans, which are long sulfated glycans with repeating disaccharide units, and thus have similar structures. Therefore, it is a big question why CS and HS show opposite effects on axon growth. We addressed this question to understand molecular mechanisms underlying the axon regeneration inhibition. Through identification of critical structures of CS and HS for axon growth, we found a "chance and necessity" rule of functional domains of CS and HS in their receptor organization. CS monomerizes its receptor PTPR sigma (receptor-type protein tyrosine phosphatase sigma), blocks the autophagy flux, leads to dystrophic endball formation, and consequently suppress axon regeneration. HS reverses this pathway.

Keyword: Axon, Regeneration, Autophagy, Chondroitin sulfate, Heparan sulfate

[SY10-5-3]

TOWARD CURING NGLY1-DEFICIENCY

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The cytoplasmic peptide:N-glycanase (PNGase) is the enzyme widely conserved throughout eukaryotes. This enzyme is involved in the degradation of misfolded/non-functional glycoproteins destined for the degradation process called ERAD (ER-associated degradation). In 2012, a patient harboring mutations of PNGase gene (NGLY1) was first reported. Symptom of these patients includes developmental delay, multifocus epilepsy, involuntary movement and liver dysfunction. From this report, it is clearly suggested that the cytoplasmic PNGase play a pivotal role in normal human development. We analyzed Ngly1-deficient mice and found that they are embryonic lethal in C57BL/6 (B6) background. Surprisingly, the additional deletion of Engase, encoding another cytosolic deglycosylating enzyme called ENGase (endo-β-N-acetylglucosaminidase), resulted in the partial rescue of the lethality of the Ngly1-deficient mice. Additionally, we also found that a change in the genetic background of B6 mice, produced by crossing the mice with an outbred mice strain (ICR) could rescue the embryonic lethality of Ngly1-deficient mice. Viable Ngly1-deficient mice in a B6 and ICR mixed background, however, showed a very severe phenotype reminiscent of the symptoms of NGLY1-deficiency subjects. Again, many of those defects were strongly suppressed by the additional deletion of Engase in the B6 and ICR mixed background. We also showed that in Ngly1-KO cells, ERAD process was compromised. Interestingly, not only delayed degradation but also the deglycosylation of a model substrate was observed in this cell. The unexpected deglycosylation was found to be mediated by ENGase. Surprisingly, the ERAD dysregulation in Ngly1-KO cells were restored by the additional KO of Engase gene. These observations collectively suggest that the ENGase represents one of the potential therapeutic targets for this genetic disorder. In this symposium, we will overview our most recent progress on our NGLY1-research, and also introduce our efforts to develop drugs for NGLY1-deficiency. References 1. Fujihira, H., et al. (2017) PLOS Genetics 13: e1006696. 2. Huang C, et al. (2015) Proc Natl Acad Sci U S A 112:1398-1403

Keyword: NGLY1, N-glycan, catabolism, ENGase

[SY10-5-4]

THE DEVELOPMENT OF GLYCOCONJUGATE VACCINES AGAINST MULTI-DRUG RESISTANT PATHOGENIC BACTERIA

Shih-Hsiung Wu[1]

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Combating drug-resistant A. baumannii with high mortality is a challenge in this post-antibiotic era due to the limitation of available drug choices. Although the treatments of tigecycline, colistin, and sulbactam have favorable clinical outcomes, the low serum level and bacteriostatic nature of tigecycline, nephrotoxicity of colistin and emerging resistance against sulbactam limit their use in critically ill patients. Therefore, it is an urgent need for a novel therapeutic modality. Bacterial surface polysaccharides elicit antibodies during infection and are essential for resistance to complement killing. Capsular polysaccharide (CPS) (K antigen) localized on the outer layer of the bacteria is immunogenic and plays an important role in the pathogenesis. Until now, there are more than 20 A. baumannii CPS structures have been determined. The chemical structures of the capsular polysaccharide from two clinic A. baumannii SK44 and 54149 in Taiwan were determined by the cleavage of specific phage enzymes with the analysis of GC-MS, NMR, and mass spectrometry. Glycoconjugate vaccine comprising the degraded products of bacterial surface polysaccharide is believed to be an alternative to combat bacterial infection. With these two strains as models, we would like to introduce our studies in the preparation of glycoconjugate vaccines by using chemoenzymatic methods.

[SY10-5-5]

INSPIRATION FROM NATURAL PRODUCTS TO DEVELOP SMALL MOLECULES FOR MODULATION OF SUGAR-PROCESSING ENZYMES: POTENTIAL TREATMENT FOR LYSOSOMAL STORAGE DISEASES

Wei-Chieh Cheng[1]

[1]Academia Sinica

A unique molecular library, inspired from naturally occurring polyhydroxylated alkaloids, consisting of all sixteen synthetic ADMDP (1-aminodeoxy-DMDP) stereoisomers has been prepared and evaluated for inhibitory activity against α-Gal A, and ability to impart thermal stabilization of this enzyme. The results of this testing led us to develop a novel pharmacological chaperone for the treatment of Fabry disease. 3-Epimer ADMDP was found to be an effective pharmacological chaperone, able to rescue α-Gal A activity in the lymphoblast of the N215S Fabry patient-derived cell line, without impairment of cellular β-galactosidase activity. When 3-epimer ADMDP was administered with rh-α-Gal A (enzyme replacement therapy) for the treatment of Fabry patient-derived cell lines, improvements in the efficacy of rh-α-Gal A was observed, which suggests this small molecule can also provide clinical benefit of enzyme replacement therapy in Fabry disease. Besides, two polyhydroxylated pyrrolidines with the (3R,4S,5R) configuration pattern underwent rapid substituent diversity by conjugating the primary aminomethyl moiety of each with a variety of carboxylic acids to generate two libraries (2 x 60 members). Our bioevaluation results showed one member with the (2R,3R,4S,5R) configuration pattern and bearing a 5-cyclohexylpentanoyl group as a substituent moiety possessed sufficient chaperoning capability to rescue α-Gal A activity in the lymphocyte of the N215S Fabry patient-derived cell line and other α-Gal A mutants in COS7 cells.

Keyword: lysosomal storage disease, fabry disease, , lysosomal α-galactosidase A, pharmacological chaperone, pyrrolidine-based iminosugar

[SY10-5-6]

GENERATION OF AN AGLYCOSYLATED ANTIBODY-PRODUCING MICE

Yong-Sam Kim[1], Nan-Ee Lee[1], Sun Hee Kim[1] and Jeong Heon Ko[1]

[1]KRIBB, Korea

Aberrant protein glycosylation is frequently observed in various disease states including cancer, thereby making efforts to utilize a specific glycoprotein of a protein as a disease biomarker feasible. The immunoassay using biomarker-specific antibodies is one of the most reliable, robust and convenient methods. Conceptually, the combination of an antibody and a lectin (or glycan-specific antibody) may enable detection of a specific glycoform of a biomarker when applied to the sandwich ELISA platform. However, the glycans in immunoglobulin G interfere with the interactions between an antigen and a lectin, thereby making such immunoassay fundamentally unfeasible. Several efforts have been made to overcome this problem including deglycosylation using PNGase-F, enzymicdigestion of an antibody using pepsin, and chemical modifications of glycans of antibodies. However, such efforts had to be made individually for each biomarker and thus each antibody and, furthermore, rendered unsatisfactory analytical outcomes. To resolve this matter fundamentally, we genome-engineered mice by mutating the N-glycosylation consensus site (asparagine-coding codon) into non-asparagine coding sequences. We found that aglycosylated antibodies were produced in the genome-engineered mice following immunization with antigens. The stability of the aglycosylated antibodies produced from the engineered mice was equivalent to that of conventional antibodies. Moreover, the aglycosylated antibody in combination with a lectin was reliably applicable to the ELISA platform to quantify a specific glycoform with diagnostic validity. The genome-engineed mice can also be used as a host for generation of an aglycosylated antibody to measure a specific glycoform of biomarkers.

Keyword: Genome editing, Aberrant glycosylation, Diagnosis, Alpha-feto protein, hepatocellular carcinoma

[SY11-2-1]

HOST IMMUNITY AND ENERGETICS

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Organisms acquire energy from their environment, which they allocate to growth, reproduction, and somatic maintenance. In energy-rich environments, investment in reproduction and growth is favored, whereas in energy-limiting environments, organisms favor investment in somatic maintenance programs. Since the various maintenance programs compete with each other for energy, it has been postulated that it might result in physiologic trade-offs under energy-limiting conditions. However, the nature of these physiologic trade-offs and their significance remain poorly understood. Here, we will present evidence that competition for energy between immunity and homeothermy results in physiologic trade-offs. These new findings, their mechanisms, and implications will be highlighted at the meeting.

Keyword: Host Immunity, homeothermy, obesity

[SY11-2-2]

TEMPERATURE-DEPENDENT REPROGRAMMING OF BEIGE CELL IDENTITY

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Beige and brown adipocytes generate heat in response to reductions in ambient temperature. When warmed, both beige and brown adipocytes exhibit morphological 'whitening', but it is unknown whether or to what extent this represents a true shift in cellular identity. Using cell type-specific profiling in vivo, we uncover a unique paradigm of temperature-dependent epigenomic plasticity of beige, but not brown, adipocytes, with conversion from a brown to a white chromatin state. Despite this profound shift in cellular identity, warm whitened beige adipocytes retain an epigenomic memory of prior cold exposure defined by an array of poised enhancers that prime thermogenic genes for rapid response during a second bout of cold exposure. We further show that a transcriptional cascade involving glucocorticoid receptor and Zfp423 can drive warm-induced whitening of beige adipocytes. These studies identify the epigenomic and transcriptional bases of an extraordinary example of cellular plasticity in response to environmental signals.

Keyword: adipocytes, cellular identity, epigenomic

[SY11-2-3]

ADIPOSE TISSUE REMODELING IN METABOLIC DISEASES

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The increase in circulating inflammatory factors found in obese subjects and the discovery of inflammatory cell accumulation in adipose tissue (WAT) has opened fascinating researches in obesity and diabetes. While substantial knowledge has been brought by rodent studies, this nevertheless allowed reconsidering certain aspects of human obesity physiopathology and of its links with its complications. White adipose tissue (WAT) contributes significantly to the occurrence and maintenance of low-grade inflammation in obesity. The phenotype and the biology of WAT cellular components are profoundly altered by several major processes as adipose cell hypertrophy and immune cells accumulation which include macrophages, lymphocytes and mast cells. In addition to adipocyte metabolic dysfunction eventually associated with cell hypertrophia (i.e. perturbed lipogenesis and lipolysis capacity), cellular stress including inflammation, oxidative, reticulum endothelial stress and hypoxia are part of the biological alterations which attract and retain inflammatory cells within the WAT and is suspected to promote insulin resistance. Perturbed paracrine dialogs between adipocytes, preadipocytes and inflammatory cells contribute to modify cell biology. However, in human studies, variation in inflammatory cell accumulation is not always associated with modification of insulin sensitivity, and the mediators linking hypertrophied WAT to its obesity downstream complications are still to be identified. Together with inflammatory cell accumulation, the evaluation of transcriptomic interactions characterizing human adipose tissue demonstrated the strong relationship linking inflammatory processes to extra cellular matrix (ECM) remodelling components. Our group showed that interstitial fibrosis accumulates in obese WAT as in many organs affected by low-grade inflammation in chronic diseases (i.e. liver, lung, kidney pathologies). We recently provided insights into the composition in WAT fibrosis showing a different pattern and distinct physiopathological significance in subcutaneous and omental WAT. We also identified progenitor cells contributing to fibrosis accumulation. A major finding is the diminished fat mass loss in patients with high level of scWAT fibrosis. The aim of this lecture will be to discuss up-to-date current knowledge regarding the importance of inflammation and adipose tissue remodelling in human obesity and in its metabolic complications. Selected references 1: Divoux A, Tordjman J, Lacasa D, Veyrie N, Hugol D, Aissat A, Basdevant A, Guerre-Millo M, Poitou C, Zucker JD, Bedossa P, Clément K. Fibrosis in human adipose tissue: composition, distribution and link with lipid metabolism and fat mass loss. Diabetes. 2010 Aug 16. [Epub ahead

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Keyword: obesity, diabetes, inflammation, fibrosis, adipose tissue

[SY11-2-4]

OBESITY-INDUCED LIFESTYLE-RELATED DISEASES: LET'S SEE BOTH THE WOOD AND TREES

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Energy homeostasis is maintained locally through cell-cell interaction and systemically through metabolic organ network. During the course of obesity, free fatty acids, when released from obese adipose tissue through the macrophage-induced adipocyte lipolysis, are overflown to the liver via the portal vein, where they are accumulated as ectopic fat. It is likely that non-alcoholic steatohepatitis (NASH) occurs through the dysregulation of metabolic organ network. Using a recently developed rodent model of human NASH, we have provided evidence for the role of resident macrophages, in addition to recruited macrophages, in the progression from simple steatosis to NASH. Sodium glucose cotransporter 2 (SGLT2) inhibitors, an oral antidiabetic drug, promotes urinary excretion of glucose by blocking its reabsorption in the renal proximal tubules. We have demonstrated that SGLT2 inhibition results in increased accumulation of lipid in the adipose tissue without marked inflammatory changes in obese mice. As a result of "healthy expansion" of the adipose tissue, ectopic fat accumulated in the liver may be redistributed to the adipose tissue, thus preventing or at least delaying the onset of NASH and eventually hepatocellular carcinoma in a rodent model of human NASH. Thus, SGLT2 inhibitors represent the unique class of drugs targeting metabolic organ network. In this talk, I would stress the importance of seeing both the wood and trees to understand the pathogenesis of obesity-induced lifestyle-related diseases.

Keyword: oral antidiabetic, drug, Obesity

[SY11-3-1]

GENE THERAPY APPLICATIONS FOR UPPER MOTOR NEURONS

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Upper motor neurons are unique for their ability to receive, integrate and transmit cortical input towards spinal cord targets for the initiation and modulation of motor movement. They are large pyramidal neurons located in layer V of the motor cortex, receiving a vast amount of information both form long distance and local circuit neurons and sending out a signal to spinal targets. Their degeneration is characteristic of many neurodegenerative diseases in which voluntary movement is impaired, such as primary lateral sclerosis (PLS), hereditary spastic paraplegia (HSP) and amyotrophic lateral sclerosis (ALS). Improving their health has significant consequences for improved motor connectivity and function. We have used AAV-mediated approaches to label them as a distinct neuron population within the complex structure of the brain, and to genetically modify them selectively without affecting other brain or neuron populations in the brain. Our initial experiments took advantage of their projection path into the spinal cord, and utilized retrograde transduction approach to label them via precise injections from the dorsal funiculus of the spinal cord. This approach helped us make the discovery about the health and stability of their apical dendrite and the extent of its degeneration in neurodegenerative diseases. We found that upper motor neurons undergo cellular degeneration much earlier than once thought and that their apical dendrites degenerate and loose spines even prior to symptom onset. This was a remarkable finding, suggesting that cortical degeneration is an early event in ALS. In a different set of experiments we used AAV mediated gene delivery approach directly into the motor cortex, targeting specifically the upper motor neurons with the correct combination of promoter, capsid protein, and gene expression. Our investigations lead to the identification of AAV2-2 as the most potent serotype for upper motor neuron transduction by direct cortex injection. Upon one time injection, among all cells transduced about 70% were upper motor neurons, and the second largest population transduced were the callosal projection neurons. Our approach did not primarily target astrocytes and/or microglia and was more geared towards genetic modulation of upper motor neurons. This is an important step for gene delivery and genetic engineering approaches that are required for building effective treatment strategies for neurodegenerative diseases in which voluntary movement is impaired.

Keyword: CSMN, AAV, Gene Therapy

[SY11-3-2]

INVOSSA: A CELL-MEDIATED GENE THERAPY FOR OSTEOARTHRITIS

Sujeong Kim[1]

[1]Kolon Life Science, Korea

Invossa is a cell mediated gene therapy for osteoarthritis. It is a mixture of primary human chondrocytes (hChonJ cells) and irradiated human chondrocytes modified to express TGF-β1 (hChonJb#7 cells). Clinical trials have shown that Invossa treatment improved pain and function in patients with knee OA. We investigated the mechanism of action of Invossa in a rodent OA model. When OA rats were treated with Invossa, the anti-inflammatory cytokine IL-10 and anti-inflammatory M2 macrophages were highly escalated. Based on the results, we suggest that the anti-inflammatory environment induced by Invossa treatment is responsible for the analgesic effect. The phase III trial was conducted to determine safety and efficacy of Invossa in patients with OA. Participants were randomized into the treatment group and the placebo group. The primary evaluation parameter was IKDC and VAS, and the secondary evaluation parameters were WOMAC score, KOOS, X-ray and MRI. Invossa treatment group showed statistically significant improvement in IKDC and VAS compared to placebo. Invossa gained marketing approval for treatment of knee osteoarthritis from the Ministry of Food and Drug Safety on July 12, 2017.

Keyword: Invossa, gene therapy, osteoarthritis, chondrocyte

[SY11-3-3]

DEVELOPMENT OF A NOVEL AND INNOVATIVE DNA MEDICINE FOR DIABETIC PERIPHERAL NEUROPATHY: SCIENTIFIC BASIS AND CLINICAL RESULTS

Junghun Lee[1]

[1]Viromed, Korea

Diabetic peripheral neuropathy (DPN) is a devastating condition caused by damaged peripheral nerves, whose clinical symptoms vary from numbness to extreme pain. However, current treatment options are limited to transient pain relief and may accompany serious side effects. Hence, there is a high unmet medical need for the development of a disease-modifying drug for DPN patients that can reduce pain levels and help patients recover sensory function as well. One of the strategies in developing novel therapeutics for DPN is to utilize endogenous growth factors involved in the pathogenesis of peripheral neuropathy. We found that hepatocyte growth factor (HGF) was highly increased in damaged peripheral nerves, and that inhibiting c-Met, its receptor, aggravated the damage, which together suggested HGF/c-Met pathway might play a role in nerve regeneration. We focused on three different physiological changes, neuropathic pain, re-myelination and axon outgrowth, and investigated whether the supply of exogenous HGF to injured nerves could improve the disease condition. We strategically selected plasmid DNA because it is simple, safe, and easy to engineer and manufacture. HGF expressing vector (VM202), once injected to skeletal muscle, produced a significant level of HGF protein and had effects on nearby peripheral nerves; it exhibited pain-relieving effects, promoted re-myelination process and enhanced axonal outgrowth. These data strongly suggested that VM202 could facilitate the repair of damaged nerves, consequently leading to modification of the disease condition. Based on these pre-clinical data, we moved on to conduct clinical trials with patients suffering from painful DPN. Data from the multi-center, double-blind, placebo-controlled phase II study suggested that administration of VM202 in DPN patients may have therapeutic effects, which are reduction of pain symptoms and enhancement of sensory function. Taken together, our data collectively suggested that VM202 is a safe and effective drug candidate that can provide a significant amount of benefits to DPN patients, through its disease-modifying mechanisms. Phase III trial is currently underway to confirm both its safety and efficacy.

Keyword: VM202, Neuropathic pain, HGF, PNS

[SY11-3-4]

DEVELOPMENT AND MANUFACTURING OF SAFE AND EFFECTIVE GENE THERAPY VECTORS FOR CLINICAL USE

Scott Loiler[1]

[1]Nationwide Children's Hospital/Ohio State University, USA

The recent successes of gene therapy clinical trials have led to the logarithmic growth of new companies and the development of new therapeutic applications for diseases that had no treatment options just a few years ago. Many disease targets are requiring systemic delivery of high titers of vector which are now reaching the limits of dosing safety in some instances and strain on manufacturing capacity. Adding to the manufacturing strains are the new gene editing technologies that are rapidly progressing to the clinic and promise and problems that they entail during development. The expansion of new treatments has led to the need for increased manufacturing capacity and scalable production process. I will discuss our current manufacturing process that was used to generate the large amounts of vector needed to successfully to treat Spinal Muscular Atrophy patients as well as our transition to a more scalable production process for phase II studies and beyond. I will also discuss some of the critical parameters for testing and new requirements of regulatory authorities to incorporate into new processes to meet current and future demands of a robust chemistry, manufacturing and control (CMC) program.

Keyword: Gene therapy, Adeno-associated virus, AAV, Manufacturing

[SY11-4-2]

MOLECULAR MECHANISM OF HUMAN NUCLEOTIDE EXCISION REPAIR

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DNA repair pathways are essential to counteract the threat of endogenous and exogenous damage to DNA. While the maintenance of genome stability is key to cellular survival and human health, many agents used in antitumor therapy also damage DNA and repair pathways cause resistance to treatment with these agents. This presentation will illustrate our chemical and biological approaches toward understanding these central issues of DNA repair using the human nucleotide excision repair (NER) pathway as an example. We are in particular interested in how protein-protein and protein-DNA interactions in mediating progression through the NER pathway. NER operated through the dynamic assembly and disassembly of the NER proteins at the site of damage. One central factor is XPA, which coordinates damage recognition with the dual incision to excise DNA lesions through interactions with the TFIIH, RPA and ERCC1-XPF protein. The importance of the XPA interaction network on the architecture of NER complexes and coordination of the various steps in NER will be discussed.

Keyword: DNA Repair, Genomic Inegrity, Cancer, Cisplatin, Xeroderma Pigmentosum

[SY11-4-3]

ROLES OF ATAD5 IN PRESERVING GENOMIC STABILITY UNDER REPLICATION STRESS

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[1]Institute for Basic Science, Korea

ATAD5 (ATPase Family, AAA Domain Containing 5) protein is important for maintaining genomic stability and suppressing tumorigenesis through regulating functions of eukaryotic sliding clamp, proliferating cell nuclear antigen (PCNA). Replication stress, which slows or stalls progression of the replication fork, contributes to genomic instability. Despite effects of ATAD5 depletion on various replication-related features suggest the presence of replication stress, the exact roles of ATAD5 in preserving fork stability under replication stress remains mostly unknown. ATAD5 depletion reduced the recruitment of RAD51, which promotes fork regression and fork restart under replication stress, to stalled forks under replication stress. Accordingly, fork regression was reduced by ATAD5 depletion. We found that ATAD5 interacted with RAD51 and their interaction was enhanced under replication stress in an ATR dependent manner. The identity and roles of DNA breaks following fork stalling/regression are controversial. Interestingly, we found that RPA32 S4/S8 phosphorylation, a marker for single strand DNA-associated breaks, following replication stress was reduced by ATAD5 depletion. Replication stress-induced DNA breaks and recruitment of MUS81 endonuclease were also reduced by ATAD5 knockdown. Finally, ATAD5 facilitated restarting of stalled replication forks and stabilized genome under replication stress evidenced by reduced chromosome breakage. Taken together, all data suggest that ATAD5 plays important roles in stabilizing and restarting stalled forks under replication stress.

Keyword: Replication stress, Fork stability, Fork restart, ATAD5, RAD51

[SY11-4-4]

NEW TRAIP BINDING PROTEIN, NTBP, PLAYS AN IMPORTANT ROLE IN DNA DAMAGE RESPONSE PATHWAY

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TRAIP localizes to the DNA damage site. At the DNA damage site, the function of TRAIP is to repair the damage and amplify the DNA damage signal. In this study, we have identified NTBP as a new TRAIP binding protein and demonstrated that TRAIP regulated the NTBP localization to DNA damage sites. Also we demonstrated that foci formation of RAD51 and RPA2 was reduced in NTBP-depleted HeLa cells. Furthermore cell death caused by the DNA damaging agent was increased in NTBP-depleted HeLa cells. So, our data demonstrates that the new TRAIP binding partner, NTBP, controls the localization of TRAIP to the DNA damage site, resulting in an increase in DNA damage response pathways and repair efficiency.

Keyword: DNA damage, TRAIP, NTBP

[SY11-5-1]

GANGLIOSIDE GM3 MOLECULAR SPECIES AS NOVEL ENDOGENOUS LIGAND FOR TLR4

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Ganglioside GM3 has been known to participate in insulin signaling by regulating the association of the insulin receptor in caveolae microdomains (lipid rafts), which is essential for the execution of the complete insulin metabolic signaling in adipocytes. Macrophage-secreted factors including proinflammatory cytokines, TNF-α and IL1-β, in adipose tissues have been known to limit the local adipogenesis and induce insulin resistance, however, the interplay between adipocytes and macrophages upon regulation of GM3 expression is not clear. GM3 was virtually absent in primary adipocytes differentiated from macrophage-depleted mesenteric stromal vesicular cells, which accompanies enhancement of insulin signaling and adipogenesis. We found that the expression of GM3 is governed by soluble factors including steady-state levels of proinflammatory cytokines secreted from resident macrophages. The direct involvement of GM3 in insulin signaling is demonstrated by the fact that embryonic fibroblasts obtained from GM3 synthase (GM3S) deficient mice have increased insulin signaling, when compared to wild type embryonic fibroblasts, which in turn leads to enhanced adipogeneis. In addition, GM3 expression in primary adipocytes is increased under proinflammatory conditions as well as in adipose tissue of diet-induced obese mice. Moreover, GM3S deficient mice fed high fat diets become obese but are resistant to the development of insulin resistance and chronic low-grade inflammatory states. Thus, GM3 functions as a physiological regulatory factor of the balance between homeostatic and pathological states in adipocytes by modulating insulin signaling in lipid rafts. Furthermore, we have identified the significant increases of GM3 molecular species possessing pro-inflammatory actions in human serum with metabolic syndrome. Collectively, we propose a novel inflammation amplification loop triggered by GM3 molecular species.

Keyword: Ganglioside GM3, metabolic disorder, innate immunity, TL4R, inflammation

[SY11-5-2]

East Asian Joint Symposium on Glycoscience Session3

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Lipid glucosylation is a highly-conserved system in living organisms, indicating its critical role in life. The recent remarkable development of the mass spectrometer significantly revealed the enormous complexity of lipids and enabled us to discover novel glycolipid classes especially in CNS tissues, such as glucosylated sterols3) and glucosylated phosphatidic acid (phosphatidylglucoside, PtdGlc)4). These three brain lipids are associated with lipid raft/lipid microdomains, indicating that lipid glucosylation contributes not only to change their original lipid properties but also to serve as new functional molecules in various cellular processes. As a typical example, one of metabolite, lysoPtdGlc functions as a G protein-coupled receptor 55 endogenous agonist5). LysoPtdGlc/GPR55 signaling axis regulates modality-specific sensory axon guidance in the spinal cord. Identification of each glucosylation enzymes is important for understanding the biological significant of lipid glycosylation. Here, I will discuss a novel type of lipid glycosylation mediated by glucocerebrosidase GBA and metabolic crosstalk among different class of lipids.

[SY11-5-3]

ATTRACTIVE AND REPULSIVE FIELDS OF POLYSIALIC ACID SYNTHESIZED BY ST8SIA2 AND ST8SIA4

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Polysialic acid (polySia or PSA) is a unique and functionally important glycotope especially in vertebrate brains. It is involved in several neural functions, such as memory, circadian rhythm, and social behaviors. PolySia is synthesized by two polysialyltransferases, ST8SIA2 and ST8SIA4. Interestingly, altered expression of polySia and ST8SIA2 is reported to have some association with psychiatric disorders such as schizophrenia and bipolar disorder. An underlying mechanism for polySia-involved phenomena is considered to depend on its anti-adhesive effect due to its bulky and hydration properties, which is so-called a repulsive field. However, we have recently introduced an attractive field as a new mechanism for the polySia functions, under which polySia provides a reservoir scaffold for various neurological active molecules (1,2): the brain-derived neurotrophic factor (BDNF) (3-6), catecholamine neurotransmitters (7), and the fibroblast growth factor 2 (FGF2) (6,8). Importantly, binding properties of polySia to BDNF and FGF2 highly depend on the degree of polymerization of polySia (3,8). Recently, we compared the structure and function of polySia chains synthesized by ST8SIA2 and ST8SIA4 in detail and found that these two enzymes synthesized polySia chains with different properties. In addition, we analyzed the coding single nucleotide polymorpholisms (SNPs) of ST8SIA2 gene found in the schizophrenia patients and showed that the SNPs affected the quality and quantity of polySia and its functions (9,10), in which both attractive and repulsive fields are demonstrated to be imporotant. All these results indicate that highly regulated polySia expression is important for normal brain functioning. References: 1) Sato, Kitajima (2013) Front Cell Neurosci 7, 61; 2) Sato, Kitajima (2013) J Biochem 154, 115; 3) Kanato et al. (2008) Glycobiology 18, 1044; 4) Hane et al. (2012) Pure Appl Chem 84, 1895; 5) Sumida et al. (2015) J Biol Chem 290, 13202; 6) Hane et al. (2015) Glycobiology 10, 1112; 7) Isomura et al. (2011) J Biol Chem 286, 21535; 8) Ono et al. (2012) J Biol Chem 287, 3710; 9) Hane et al. (2017) Biochem Biophys Res Commun 478, 1123; 10) Hane et al. (2016) Biochim Biophys Acta 1860, 1739.

Keyword: polysialyltransferase, Schizophrenia, BDNF, sialic acid, NCAM

[SY11-5-4]

IDENTIFICATION OF ST3GAL1 TARGET PROTEINS WHICH FACILITATE TUMOR GROWTH, ANGIOGENESIS AND IMMUNE EVASION

Tan-Chi Fan[1], Huiling Yeo[1], Wen-Der Lin[1], Eric Sheng-Wen Chen[1], Ko-Wa Chen[1], Jung-Tung Hung[1], John Yu[2] and Alice L. Yu[2]

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It has been reported that ST3Gal1 plays an important role in promoting tumor growth but its substrates have remained elusive. Here we identified 3 protein targets and demonstrated how each contributed to tumor promotion via ST3Gal1-mediated sialylation. The first protein substrate is glial cell line-derived neurotrophic factor (GDNF) family receptor alpha 1 (GFRA1). Binding of GDNF to GFRA1 induces trimerization with RET and facilitates subsequent phosphorylation, regulating neuronal cell proliferation and differentiation. GFRA1 and RET are overexpressed in estrogen receptor (ER)-positive breast cancers, and GDNF signaling leads to ER phosphorylation and estrogen-independent transcriptional activation of ER-dependent genes. We found that silencing of ST3Gal1 in breast cancer reduced GDNF-induced RET, AKT and ER phosphorylation, with decreased cell proliferation. ST3Gal1 silencing greatly impaired the interaction between GFRA1 and RET and dampened their downstream signaling. Intriguingly, GDNF can upregulate the transcription of ST3Gal1, forming a positive feedback loop. Mechanistically, we showed the presence of Sp1 binding site on ST3Gal1 promoter by ChIP assay. Furthermore, GDNF induced Sp1 phosphorylation via PI3K/AKT pathway, leading to the upregulation of ST3Gal1. The 2nd target of ST3Gal1 we identified is Vasorin (VASN) which binds to TGF-β1 and inhibits TGFβ1-induced angiogenesis. Conditional medium collected from ST3Gal1-silenced cells significantly reduced tube-formation ability of HUVEC, which was accompanied by downregulation of angiogenesis gene expression, including FGF1, FGF13, MMP, and VEGF-A. Such suppression was abrogated by anti-VASN mAb, suggesting that VASN is important in angiogenesis. Desialylation of VASN by ST3GAL1-silencing or sialidase enhanced its binding affinity to TGF-β1, dampening TGF-β1 signaling and angiogenesis, as indicated by impaired tube formation of HUVECs and reduced activation of Smad2 and Smad3 in MCF7 cells. Since TGF-β1 can transcriptionally activate ST3Gal1, our findings illustrated a feedback regulatory loop in which TGF-β1 up-regulates ST3Gal1 to circumvent the negative impact of VASN binding to TGF-β1. Examination of 104 primary breast cancer samples and their adjacent normal tissues showed that the expression levels of ST3GAL1 ($P < 0.0001$) and TGF-B1 ($P < 0.0001$) were significantly higher in tumor part than normal part, while

the reverse was true for VASN (P < 0.0001). Kaplan Meier survival analysis showed significantly shorter relapse free survival for those with lower expression of VASN (P=0.0226, Hazard Ratio= 2.24, 95% CI:1.14-5.89). Combination of low VASN with high ST3GAL1 yielded an even greater risk of recurrence (P=0.0224, Hazard Ratio= 2.917, 95% CI:1.18-8.97). Another ST3Gal1 target is CD55, which is an important complement regulatory protein, preventing cells from complement mediated cytotoxicity. We showed that O-linked desialylation of CD55 by ST3Gal1 silencing resulted in increased C3 deposition and complement-mediated lysis of MDA-MB-231 cells and enhanced their sensitivity to antibody-dependent cell-mediated cytotoxicity. These data demonstrate that ST3Gal1 mediated O-linked sialylation of CD55 acts like an immune-checkpoint molecule for cancer cells to evade immune attack. Taken together, our findings have provided molecular mechanisms underlying the role of ST3Gal1 in tumor growth, angiogenesis and immune evasion and supported the development inhibitors of ST3Gal1 as a new strategy for cancer therapy.

Keyword: ST3Gal1, GFRA1, Vasorin, angiogenesis, CD55

[SY11-5-5]

O-GLCNAC REGULATES SIGNALING IN EMBRYONIC STEM CELLS

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Mouse embryonic stem (ES) cells are pluripotent stem cells derived from pre-implantation embryos. They maintain the undifferentiated state via several signaling such as LIF/STAT3, BMP/SMAD, and WNT/β-CATENIN, whereas FGF4/ERK1/2 signaling induces ES cell differentiation (2). ERK1/2 is phosphorylated by MEK. Therefore, the MEK-ERK1/2 pathway is inhibited to maintain the undifferentiated state of mouse ES cells. However, the inhibition mechanism of the MEK-ERK1/2 pathway in mouse ES cells is not fully understood. O-linked β-N-acetylglucosaminylation (O-GlcNAcylation) is a post-translational modification characterized by the attachment of a single N-acetylglucosamine (GlcNAc) to the serine (Ser) and threonine (Thr) residues of nuclear or cytoplasmic proteins (2). O-GlcNAc transferase (OGT) transfers GlcNAc to Ser or Thr of core proteins and O-GlcNAc is removed from proteins by O-GlcNAcase (OGA). O-GlcNAcylation competes with phosphorylation as OGT catalyzes the addition of O-GlcNAc at or in proximity to phosphorylation sites. Consequently, O-GlcNAc is believed to regulate signaling pathways by inhibiting the phosphorylation of their cytoplasmic components. Here, we showed that the O-GlcNAc on Thr-410, the phosphorylation site of PKCζ, inhibits PKCζ phosphorylation and accordingly, the FGF4-PKCζ-MEK-ERK1/2 pathway in mouse ES cells (3). The reduction of OGT induces the reduction of O-GlcNAc levels on PKCζ, while FGF4 stimulation enhances PKCζ phosphorylation. Phosphorylated PKCζ then phosphorylates MEK, which in turn phosphorylates ERK1/2. Phosphorylated ERK1/2 induces ES cell differentiation into primitive endoderm (PrE). To maintain the undifferentiated state, O-GlcNAc inhibits the FGF4-PKCζ-MEK-ERK1/2 pathway via the inhibition of PKCζ phosphorylation, thereby inhibiting differentiation into PrE cells. Our results demonstrate a novel mechanism for the maintenance of the undifferentiated state of mouse ES cells via the inhibition of the FGF4-PKCζ-MEK-ERK1/2 pathway by O-GlcNAcylation on PKCζ. (1) Lanner F, Rossant J. (2010) Development 137, 3351–3360. (2) Torres CR, Hart GW (1984) J. Biol. Chem. 259, 3308–3317. (3) Miura T, Kume M, Kawamura T, Yamamoto K, Hamakubo T, Nishihara S (2018) Stem Cell Reports. 10, 272-286.

Keyword: O-GlcNAc, embryonic stem cell, signal, FGF, PKC

[SY11-5-6]

SOLUBLE SIGLEC-14: FUNCTION AND GENERATION MECHANISM

Po-Chun Jimmy Huang[1], Penk-Yeir Low[2], Iren Wang[2], Shang-Te Danny Hsu[2] and Takashi Angata[2]

[1]National Taiwan University, [2]Academia Sinica

Siglecs are a family of receptor-type glycan-recognition proteins that recognize sialylated ligands at the extracellular domain and transduce regulatory signals at the intracellular domain. Most Siglecs are expressed on leukocytes, and participate in fine-tuning of immune responses. Human Siglec-14 is expressed on myeloid cells and recognizes select bacterial pathogens, eliciting pro-inflammatory responses. We have previously demonstrated that Siglec-14 and its inhibitory counterpart Siglec-5 modulate myeloid inflammatory responses toward some bacterial pathogens, influencing clinical outcomes. Although Siglec-14 is a type 1 transmembrane protein, a soluble form of Siglec-14 is also found in human blood. As the functions of many membrane receptors are modulated by soluble counterparts, which are generated by alternative splicing and/or proteolytic cleavage of the membrane-bound form, we are intrigued by the presence of soluble Siglec-14. Thus, we investigated the generation mechanism and the function of soluble Siglec-14. We found that soluble Siglec-14 is derived from an alternatively spliced mRNA that retains intron 5, which contains a termination codon and thus prevents the translation of the exon 6 encoding the transmembrane domain of Siglec-14. The translated segment in the intron 5 encodes a unique C-terminal 7-amino acid extension, which allows specific detection of this isoform by an antibody. We found that a majority of soluble Siglec-14 carries this C-terminal extension, implying that alternative splicing is the primary mechanism that generates soluble Siglec-14. With regard to the function, we found that soluble Siglec-14 suppresses bacteria-elicited pro-inflammatory responses of myeloid cells that express transmembrane Siglec-14. Soluble Siglec-14 attenuates the signaling downstream of toll-like receptor 2 (TLR2), likely by interfering with the interaction between transmembrane Siglec-14 TLR2 on cell surface. We also found that the intron 5 of Siglec-14 pre-mRNA contains a guanosine-rich segment that assumes a tertiary structure called G-quadruplex, which may regulate the efficiency of intron 5 splicing. Based on these findings, we propose that soluble Siglec-14 is a negative regulator for the pro-inflammatory responses triggered by transmembrane Siglec-14.

Keyword: Siglec, sialic acid, myeloid cells, soluble receptor, bacteria

[SY01-3-5]

A NOVEL RED-SHIFTED EXCITATORY CHANNELRHODOPSIN WITH MULTIPLE PROPERTIES ENABLING MARKEDLY IMPROVED INTEGRATION OF CA2+ IMAGING WITH OPTOGENETIC CONTROL.

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A rapidly-evolving goal of modern neuroscience is to combine the power of optogenetic activity-control with the information on specific activity patterns that is accessible via genetically encoded fluorescent activity-sensors. This goal has been challenging to fully achieve, due in part to the overlapping spectra of actuators and sensors. Here, we report a novel excitatory channelrhodopsin (ChR) nicknamed here 'MO20' that was discovered through sequence analysis and biophysical characterization of rhodopsin-like proteins from over 600 microbial organisms. MO20 showed robust excitation by redshifted light in the range of λ ~ 585-650nm, suitable for compatibility with GFP-based activity sensors with minimal optical cross-talk. Peak photocurrent values under orange (λ ~ 585nm) and red (λ ~ 650nm) light were 4.5±0.6 and 1.0±0.16 nA, respectively; these values were significantly higher than for previously characterized red-shifted ChRs under the same illumination conditions when tested in parallel. Speed was also suitable; MO20 exhibited more than 90% spiking fidelity in trains at frequencies up to 40Hz (orange light); and action potentials could be readily induced in neurons with orange light with irradiance values down to 0.005mW/mm2. In a neuronal culture imaging setting with the Ca2+ sensor GCaMP6m, MO20 showed 10-fold faster rise-and-decay Ca2+ signal kinetics and significantly higher fluorescent signal amplitude than the leading red-shifted ChRs. This striking difference likely relates to reduced spectral overlap between opsin and sensor, faster channel kinetics, larger photocurrent, and pH independence. These unprecedented biophysical properties define a tool for more powerful and precise probing of neuronal function via integration activity sensors and actuators.

Keyword: Optogenetics, Neuron excitation, Calcium imaging

[SY02-3-6]

CG101 INHIBITS CANCER CELL PROLIFERATION AND TUMOR GROWTH BY TARGETING THE PYRUVATE KINASE M2 AND PEROXIREDOXIN 1

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It is reported that CG101, isolated from cinnamon, has anti-tumor effects through the modulation of multi-target molecules. Pyruvate kinase M2 (PKM2) and peroxiredoxin 1 (Prdx1) are up-regulated in multiple cancer types and are considered as potential targets for cancer therapy. Here, we report that CG101 binds directly to PKM2 and Prdx1, which acts as a PKM2 activator and Prdx1 inhibitor, respectively. We identified PKM2 and Prdx1 as target molecules of CG101 by affinity chromatography coupled with mass spectrometry and further validated using label-free biochemical methods and biophysical assays. As a PKM2 activator, CG101 increases pyruvate kinase activity by promoting the tetrameric state of PKM2. CG101 suppresses protein kinase activity of PKM2 by decreasing the phosphorylation at Tyr105. Moreover, this leads to a decrease of PKM2-mediated STAT3 phosphorylation at Tyr705 and a down-regulation of target genes, including MEK5, survivin, and cyclin D1. Furthermore, CG101 suppresses tumor growth and the release of tumor extracellular vesicles by dephosphorylating PKM2. As a Prdx1 inhibitor, CG101 inhibits the peroxidase activity and inactivates Prdx1 by increasing the oxidized dimer and phosphorylation at Tyr194. Collectively, our results suggest that CG101 may be a potential anticancer agent targeting PKM2 and Prdx1 in cancer progression. This work was supported by the KRIBB Research Initiative Program, the Bio-Synergy Research Project (NRF-2012M3A9C4048777), and the Bio & Medical Technology Development Program of the National Research Foundation & funded by the Korean government (2015M3A9B5030311 and NRF-2017M3A9A8032417).

Keyword: Natural product, Cancer, Metabolism, Antioxidant, Multi-target

[SY02-5-6]

STRUCTURAL AND FUNCTIONAL INSIGHTS INTO HUMAN GAMMA-SECRETASE

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Alzheimer's disease is one of the most devastating age-related, neurodegenerative diseases worldwide. The four-component intramembrane protease γ-secretase is intricately linked to the development of Alzheimer's disease. By single-particle, cryo-electron microscopy (cryo-EM), we solved the three-dimensional structure of human γ-secretase at atomic resolution. Molecular mechanism for substrate recognition and cleavage was proposed based on the atomic resolution structure of γ-secretase complex. We explored the molecular mechanism of its enzymatic function, especially for the Familial Alzheimer's Disease related mutants, and analyzed 138 reported mutations in PS1 by individually reconstituting the mutant PS1 proteins into γ-secretases and examining their abilities to produce Aβ42 and Aβ40. Structural and functional studies of γ-secretase provide an important framework for the understanding of AD pathogenesis, and molecular basis for drug design targeting human γ-secretase.

Keyword: Alzheimer's disease, Cryo-electron microscopy, γ-Secretase

[SY03-1-6]

N-TERMINAL EUKARYOTIC EXTENTION DOMAIN OF HUMAN TRYPTOPHANYL-TRNA SYNTHETASE ACTIVATES MACROPHAGE VIA TLR SIGNALING

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Tryptophanyl-tRNA synthetase (WRS) is an essential enzyme for protein synthesis as it catalyzes tryptophan ligation to its cognate tRNA. Interestingly, WRS undertakes several non-canonical biological activities beyond its catalytic reactions. While N-terminal truncated form of WRS (mini-WRS) functions as an angiostatic ligand, FL-WRS secreted from monocytes in response to pathogen infection works as an endogenous ligand of TLR4-MD2 for innate immune activation. Here, we show that the N-terminal eukaryotic extension domain, which consists of 154 amino acids in FL-WRS (N154) and is present in humans but not in prokaryotes, activated TLR signaling. N154 significantly induced chemokine and TNF-α secretion, comparable to the effect of FL-WRS in macrophage. FL-WRS and N154, but not mini-WRS, induced the activation of the NF-κB and ERK signaling pathways. Furthermore, blockade of TLR2 and TLR4-MD2 by siRNA abrogated N154-induced MIP-1α and TNF-α production. Finally, the protein-protein docking study proposed the interaction mode of N154 and TLR4-MD2 and mutational analysis further revealed that N-terminal 10 and 152 residues are critical for the macrophage activation. Taken together our data suggested that the N154 of WRS is sufficient to recapitulate the FL-WRS activity.

Keyword: full-length tryptophanyl-tRNA synthetase (FL-WRS), innate immunity, endogenous ligand, TLR4-MD2

[SY04-1-5]

SURFACE FUNCTIONALIZATION DEPENDENT SUBCELLULAR LOCALIZATION OF SUPERPARAMAGNETIC NANOPARTICLE IN PLASMA MEMBRANE AND ENDOSOMES

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In our research, we elaborate the application of thermal decomposition based Fe3O4 superparamagnetic nanoparticle (SPMNP) for subcellular fractionation in cell biology context. Here, we used thermal decomposition based seed mediated growth for the synthesis of 8 nm Fe3O4 core SPMNP. We performed surface functionalization of SPMNP with phospholipids (Pl) and dimercaptosuccinic acid (DMSA). Surprisingly, we observed surface functionalization dependent SPMNP localization in subcellular compartments such as plasma membrane, endosomes and lysosomes. By using SPMNP based subcellular localization with pulse-chase methodology, we could use SPMNP for high pure-high yield organelle (plasma membrane, endosomes and lysosome) fractionation. Previously, several groups have used SPMNP based subcellular fractionation to isolate endosomes and lysosomes with high purity-yield for subcellular omics. For example using Dextran coated SPMNPs, several research groups were able to decipher endosomal trafficking in lysosomal storage disorders. Due to generic nature of SPMNP, our methodology has additional advantage in isolating subcellular compartments such as plasma membrane, endosome and lysosome from any given adherent cells. With additional optimization step, our methodology could be applied for suspension cells. In addition, our methodology does not include any acidic treatment or antibody based pulldown and subcellular compartments are isolated under native physiological conditions. Hence, this methodology would facilitate enzymatic studies, isolating intact membrane protein complexes and structural studies. Thus, SPMNP that are distinctly localized in subcellular compartments can be used as technology for subcellular fractionation that can complement existing tools for cell biology research. As a future perspective, our methodology can be used to isolate subcellular compartments in primary cells and can be extended to in vivo analysis for biochemical and structural biology studies. Keywords: Superparamagnetic nanoparticle (SPMNP), Plasma membrane, Early or Late Endosomes, and Lysosomes. Funded by Envirotransgene® Biosolutions Global.

Keyword: Superparamagnetic nanoparticle (SPMNP), Plasma membrane, Early or Late Endosomes, Lysosomes

[SY04-5-5]

AUTOPHAGY MEDIATES ENHANCEMENT OF PROANGIOGENIC ACTIVITY BY HYPOXIA IN MESENCHYMAL STROMAL/STEM CELLS.

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Mesenchymal stromal/stem cells (MSCs) have been a promising source for cell therapy in angiogenesis related diseases through self renewal, transdifferentiation capacity or paracrine mechanism. To improve the efficacy of MSC cell therapy, various priming methods have been developed. Hypoxic priming has been reported to enhance the therapeutic efficacy of MSCs by increasing the secretion level of growth factors and cytokines. Interestingly, recent studies have reported that MSCs primed with hypoxic condition show a increase of autophagy. Therefore, in this study, we investigated whether proangiogenic activity increased by hypoxic condition is associated with autophagy. First, we confirmed that hypoxia increased migration promoting activity of conditioned medium (CM) from MSCs, with upregulation of hypoxia-inducible factor-1 and increase of LC3-II level. To ask this question using pharmacological agents, WJ-MSCs were pretreated with 3-methyladenine (3MA) or chloroquine (CQ) (autophagy inhibitors) for 48 h and incubated in fresh EBM-2 under hypoxic condition for 24 h to harvest CM. When HUVEC migration was induced by the CM, we observed that pretreatment with 3MA or CQ significantly reduced migration. To further confirm the involvement of autophagy, ATG5 and ATG7, autophagy-related proteins, were knockdowned through siRNA transfection, followed by exposure to hypoxic condition and CM collection. When the CM was tested in HUVEC migration assay, siATG5/7 transfection significantly decreased migration, with decrease of LC3-II level. When the factors increased in MSCs by hypoxic condition was examined using the Human Angiogenesis Array kit, angiogenin and VEGF were markedly increased. Furthermore, we found that knockdown of ATG5/7 abrogated the secretome effect of MSCs increased by hypoxia. Thus, we found that MSCs primed with hypoxic condition show enhanced proangiogenic activity through secretion of mainly angiogenein and VEGF, and that this process is mediated by autophagy. Therefore, it could be suggested that hypoxia-mediated autophagy provides a new therapeutic strategy by contributing to increased paracrine effect of MSCs

Keyword: Hypoxia, Autophagy, Mesenchymal stem cells, Angiogenin, VEGF

[SY05-1-6]

DEPLETION OF SIRT3 LEADS TO THE IMPAIRMENT OF ADIPOGENIC DIFFERENTIATION AND INSULIN RESISTANCE VIA INTERFERING MITOCHONDRIAL FUNCTION OF ADIPOSE-DERIVED HUMAN MESENCHYMAL STEM CELLS

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Upregulation of mitochondrial function and oxidative metabolism is a hallmark in the differentiation of stem cells. However, the mechanism underlying the metabolic reprogramming during the differentiation of human mesenchymal stem cells (hMSCs) is largely unclear. Sirt3 has emerged as a sensor in regulating mitochondrial function and antioxidant defense system in cellular responses to energy demand or environmental stimuli, but its roles in stem cell differentiation has not been extensively studied. In this study, we used adipose-derived hMSCs (ad-hMSCs) to investigate the role of Sirt3 in adipogenic differentiation and in the function of mature adipocytes. We showed that at the early stage of adipogenic differentiation, Sirt3 upregulation is essential for the activation of biogenesis and bioenergetic function of mitochondria. In addition, we found that induction of Forkhead box O 3a (FoxO3a), an upstream factor that regulates MnSOD gene transcription, is involved in the upregulation of antioxidant enzymes at the early stage of adipogenic differentiation. Silencing of Sirt3 by shRNA significantly decreased the protein level of FoxO3a and subsequently downregulated a number of FoxO3a-mediated antioxidant enzymes and increased oxidative stress in ad-hMSCs after adipogenic induction. Remarkably, depletion of Sirt3 compromised the ability of ad-hMSCs to undergo adipogenic differentiation and led to adipocyte dysfunction and insulin resistance. These findings suggest that Sirt3-mediated protein deacetylation plays an important role in the regulation of oxidative metabolism and antioxidant defense in stem cell differentiation, and that Sirt3 deficiency may play a role in insulin resistance.

Keyword: Sirt3, Mitochondria, Stem cells, Differentiation, Insulin insensitivity

[SY05-1-7]

INVESTIGATING NOVEL NAD+ PRECURSORS AND THEIR THERAPEUTIC POTENTIAL AGAINST AGE-ASSOCIATED PHYSIOLOGICAL DECLINE AND METABOLIC DISEASE

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Ageing is one of the major risk factors for a variety of pathophysiological abnormalities and changes, including the development of insulin resistance, obesity, cardiovascular disease, chronic inflammation and cancer. Nicotinamide adenine dinucleotide (NAD+) is an essential cofactor to all living cells and is involved in important processes such as glycolysis and oxidative phosphorylation. More importantly however, it is the rate-limiting factor and key substrate to important enzymes involved in homeostatic regulation in the cell, namely, the sirtuins and poly ADP-ribose polymerases (PARPs). With age and in age-related metabolic diseases NAD+ declines, leading to a decrease in activity of these regulatory enzymes and consequently causing metabolic dysfunction and disease. Our overall aim is to provide potential therapeutic strategies to prevent this metabolic dysfunction and subsequent onset of disease through administration with novel NAD+ precursor molecules. With an abundance of NAD+, we hypothesize an increase in activity of the sirtuin and PARP enzymes which may alleviate the effects of, or even prevent certain metabolic diseases associated with age.

Keyword: NIcotinamide adenine dinucleotide, NAD+, Ageing, NAD+ precursors

[SY05-2-6]

ADHESION MOLECULE TARGETTED IMMUNETHERAPY TOWARDS A PROPHYLAXIS AGAINST VISCERAL LEISHMANIASIS

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Visceral Leishmaniasis is a macrophage associated disorder for the treatment of which antimony based drugs like SAG and SSG were the first choice in the recent past. The clinical value of antimony therapy is now declined against VL because increasing cases of Sodium Antimony Gluconate (SAG) resistance have reached outstanding proportion in Bihar, India. We have also evaluated the effect of combining CD2 with conventional antimonial (sb) therapy in protection in BALB/c mice infected with either drug sensitive or resistant strain of Leishmania donovani with 3 million parasites via-intra-cardiac route. Mice were treated with anti CD2 adjunct SAG sub-cutaneously twice a week for 4 weeks. Assessment for measurement of weight, spleen size, anti-Leishmania antibody titer, T cell and anti-leishmanial macrophage function was carried out day 0, 10, 22 and 34 post treatments. The combination therapy was shown boosting significant proportion of T cells to express CD25 compared to SAG monotherapy. Although, the level of IFN-γ was not statistically different between combination vs monotherapy ($p = 0.298$) but CD2 treatment even alone significantly influenced IFN- γ production than either SAG treatment ($p = 0.045$) or with CD2 adjunct SAG treatment ($p = 0.005$) in Ld-S strain as well as in Ld-R strain. The influence of CD2 adjunct treatment was also documented in anti-leishmanial functions in macrophages. As shown, the super-oxide generation began enhancing very early on day 10 after SAG treatment with CD2 during which SAG action was at minimum. Interestingly, the super-oxide generation ability remained intact in macrophage after treatment with immuno-chemotherapy even in mice infected with Leishmania resistant strain. Unlike SAG treatment, treatment of SAG with CD2 also led to production of nitric oxide and TNF-α, resulting in resulting in most effective clearance of L. donovani from infected macrophages. Our results indicate that CD2, which can boost up a protective Th1 response, might also be beneficial to enable SAG to induce macrophages to produce Leishmanicidal molecules and hence control the infection in clinical situation like Kala-azar. Drug resistance is the major impedance for disease control but the encouraging results obtained after infecting mice with resistant strain of the parasite strongly imply that this drug can be effective even in treating resistant cases of Kala-azar.

Keyword: CD2, Visceral Leishmaniasis, SAG

[SY09-1-4]

CHARACTERIZATION OF ANDROGEN RECEPTOR SIGNALING PATHWAY REGULATED BY ANDROGEN AND PROTEIN KINASE A IN PROSTATE CANCER

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Androgen receptor (AR) plays an important role in prostate cancer development and the activation of the AR signaling cascade is critical for survival and progression. The AR can be activated by the binding with androgen as well as through a variety of protein kinases, including cyclic AMP-dependent protein kinase A (PKA). Inhibition of the AR activity is still one of the effective treatment options for advanced prostate cancer patients and EPI-001, the second generation AR antagonist, has shown the significant inhibitory effect on tumor growth and cancer progression. Here, we investigated the differences in proteomes associated with androgen- and PKA- induced conditions in VCaP prostate cancer cells. In addition, the effect of EPI-001 targeting the N-terminal domain of the AR was assessed to investigate whether the differences in observed protein expression were directly affected by AR-mediated mechanisms. Differential patterns of protein expression in prostate cancer cells treated with EPI-001 followed by DHT or foskolin were quantified and eleven protein spots with significant expression changes among five groups were identified by mass spectrometry analysis. Functional information of identified proteins has revealed that most of proteins were involved in metabolic process and have the molecular function of catalytic activity. There is very little information available concerning the possible involvement of identified proteins in androgen signaling pathway. However, most of proteins were known to be associated with cancer disease and the mRNA and protein expression of selected proteins were validated, which offer new clues as an effector molecules in prostate cancer development. Aberrant protein levels may reflect molecular changes significantly regulated by androgen and/or PKA signaling pathways which may ultimately play a role in prostate cancer progression and provide the possible molecular mechanism of the AR activation in CRPC.

Keyword: Androgen, Androgen receptor, Castration-resistant prostate cancer, Prostate cancer, Proteome