[OP01-2-01]

STRUCTURAL INSIGHTS INTO GDP-MEDIATED REGULATION OF A BACTERIAL ACYL-COA THIOESTERASE

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Thioesterases catalyze the cleavage of thioester bonds within a diverse range of activated fatty acids and acyl-CoA substrates. They are expressed ubiquitously in both prokaryotes and eukaryotes, and are subdivided into 25 thioesterase families based on their catalytic active site, protein oligomerization, and substrate specificity. Whilst many of these enzyme families are well characterized in terms of function and substrate specificity, regulation is understood poorly across most thioesterase families. Here, we characterize a TE6 thioesterase from Neisseria meningitidis. Structural analysis based on X-ray crystallographic diffraction data to 2.0 Å revealed each protein monomeric unit harbors a hotdog fold, and that the enzyme forms a hexamer, arranged as a "trimer-of-dimers". Assessment of activity against a range of acyl-CoA substrates revealed greatest activity against acetyl-CoA, and structure-guided mutagenesis of putative active site residues identified Asn24 and Asp39 as essential for activity. Our structural analysis revealed the presence of six GDP nucleotides bound to the enzyme in close proximity to novel disulfide bond interactions which covalently link thioesterase domains in a double hotdog dimer. Structure-guided mutagenesis of residues within the GDP binding pocket identified Arg93 as playing a key role in the nucleotide interaction, and that GDP is required for activity. All mutations were confirmed to be specific and not the result of structural perturbations by X-ray crystallography. Overall, this is the first report of a bacterial thioesterase regulated by GDP, and covalent linkage of thioesterase domains through a disulfide bond, revealing structural similarities with ADP regulation in the human ACOT12 thioesterase.

Keyword: thioesterase, ACOT, GDP, enzyme kinetics, enzyme regulation

[OP01-2-02]

N-TERMINAL DOMAIN OF HELICOVERPA ARMIGERA ULTRASPIRACLE MODULATES INTERACTION WITH SPECIFIC DNA SEQUENCE

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Ultraspiracle (Usp) is an insect nuclear receptor (NR) homologous to human retinoid X receptor (RXR). Likewise other nuclear receptors, Usp possesses typical domain structure. It consists of two globular domains: a DNA-binding domain (DBD) and a ligand binding domain (LBD), beside them, Usp also contains an intrinsically disordered N-terminal domain (NTD) and an unstructured hinge between globular DBD and LBD. In contrast to widely characterized DBD and LBD not much is known about physiological function and exact structure of NTD. Publications concerning NRs rarely concentrate on NTD, thus we decided to investigate if presence of this disordered fragment influences Usp molecular properties. To reach this goal we expressed and purified full length Helicoverpa armigera Usp (HaUsp) and HaUsp deprived of NTD (HaUsp_ΔNTD). As an initial step in silico analyses were performed to determine disordered propensities of HaUsp. Two different approaches with PONDR and DynaMine were used. PONDR estimates disorder tendency of selected amino acid sequence and DynaMine determines its flexibility or rigidity. Both methods indicated that the highest disorder tendency exhibits NTD while DBD and LBD are in fact highly ordered. Subsequently, HaUsp and HaUsp_ΔNTD were expressed fused with His-tag (6xHis) at N termini and purified using two step procedure, consisting of metal affinity chromatography and size exclusion chromatography. As a result homogenous samples of each protein were obtained. Circular dichroism spectrum of HaUsp was apparently characteristic for ordered proteins, however its deconvolution with CDPro package indicated presence of 25% of disordered regions, what demonstrated that HaUsp is partially disordered. Both proteins were then analyzed by electrophoretic mobility shift assay, using two different DNA sequences – ecdysone response element of Drosophila melanogaster heat shock protein gene (hsp27_EcRE) and ecdysone response element of H. armigera HR3 protein gene (HR3_EcRE). As a positive control D. melanogaster Usp (DmUsp) was used. Comparing to the DmUsp, interaction of HaUsp and HaUsp_ΔNTD with hsp27_EcRE was weaker. The differences in the strength of the interactions may arise from the differences in the hinge region sequences and their lengths resulting in different LBD and DBD positioning in relation to each other. Greater mobility of DmUsp domains could

facilitate DNA interaction. In turn, comparing of HaUsp and HaUsp_ΔNTD interactions with the same DNA probe demonstrated significant role of NTD. Interestingly, full length HaUsp was characterized with weaker DNA binding, therefore we suggest that possibly NTD modulates interaction of NRs with specific DNA sequence. It is very likely that some coactivators could be necessary for stronger binding. Furthermore, we suppose that NTDs can regulate NRs - DNA interaction by weakening its NRs binding in the absence of additional factors. Obtained results are good background for further analyses of potential functions of poorly known NTDs and can help to determine theirs influence on the NRs signaling transmission.

Keyword: Nuclear receptor, Ultraspiracle, Intrinsically disordered protein

[OP01-2-03]

SPECIFIC EPH RECEPTOR-CYTOPLASMIC EFFECTOR SIGNALING MEDIATED BY SAM-SAM DOMAIN INTERACTIONS

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The Eph receptor tyrosine kinase (RTK) family is the largest subfamily of RTKs playing critical roles in many developmental processes such as tissue patterning, neurogenesis and neuronal circuit formation, angiogenesis, bone formation, etc. How the 14 members of Eph proteins, via their highly similar cytoplasmic domains, can transmit very diverse and sometimes even opposite cellular signals upon engaging ephrin ligands remains to be a major unresolved question. Here we systematically investigated the bindings of each SAM domain of Eph receptors to the SAM domains from SHIP2 and Odin, and uncover a highly specific SAM-SAM interaction-mediated cytoplasmic Eph-effector binding pattern. Comparative X-ray crystallographic studies of several pairs of SAM-SAM hetero-dimer complexes, together with biochemical and cell biology experiments, not only revealed the exquisite specificity code governing Eph/effector interactions but also allowed us to identify SAMD5 as a new cytoplasmic binding partner of Eph receptors. Finally, these Eph/effector SAM heterodimer structures can explain numerous Eph SAM domains mutations identified in patients suffering from cancers and other diseases.

Keyword: Eph receptor, Ephrin-Eph signal, SHIP2, Odin, SAM domain

[OP01-2-04]

DNA-BINDING AND TRANSCRIPTION ACTIVITY OF P53 AND P73 PROTEINS

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It has been shown that human tumor suppressor protein p53 plays critical roles in various important biological processes including regulation of cell cycle, apoptosis, senescence and metabolism. P53 protein is also commonly mutated in human cancer. Functions of p53 are achieved by protein-protein interactions, but particularly by binding to DNA. As a transcription factor, p53 is well known to bind consensus target sequences in linear DNA. Recent findings demonstrate that p53 binds with higher affinity to target sequences forming cruciform DNA structure. Besides its binding to cruciforms, p53 interacts with quadruplex DNA, triplex DNA, DNA loops, bulged DNA and hemicatenane DNA. On the other hand, it has been demonstrated that restoring the wild-type conformation and DNA-binding activity of mutant p53 is insufficient for restoration of transcriptional activity. Therefore, we have analyzed DNA binding and transactivation activity of p53 and p73 proteins in detail. Our results show that both p53 and p73 proteins bind to p53 target sites according to its potential to form cruciform structure and that transactivation in vivo correlated with relative propensity of the target site to form cruciforms than to its predicted in vitro DNA binding affinity. DNA structural features could therefore be an important determinant of p53 transactivation function. These recent data provide important insights into the complexity of the p53 protein family pathways and the functional consequences of its activation in normal and tumor cells. This work was supported by The Czech Science Foundation (18-15548S) and by the SYMBIT project reg. no. CZ.02.1.01/0.0/0.0/15_003/0000477 financed from the ERDF. References: [1] V. Brázda, J. Čechová, M. Battistin, J. Coufal, E.B. Jagelská, I. Raimondi, A. Inga, The structure formed by inverted repeats in p53 response elements determines the transactivation activity of p53 protein, Biochem. Biophys. Res. Commun. 483 (2017) 516–521. [2] V. Brázda, J. Coufal, Recognition of local DNA structures by p53 protein, Int. J. Mol. Sci. 18 (2017) 375. [3] E.B. Jagelská, H. Pivoňková, M. Fojta, V. Brázda, The potential of the cruciform structure formation as an important factor influencing p53 sequence-specific binding to natural DNA targets, Biochem. Biophys. Res. Commun. 391 (2010) 1409–1414. [4] V. Brazda, P. Muller, K. Brozkova, B. Vojtesek, Restoring wild-type conformation and DNA-binding activity of mutant p53 is insufficient for restoration of transcriptional activity, Biochem. Biophys. Res. Commun. 351 (2006) 499–506.

Keyword: p53, DNA, p53, Protein DNA binding, Cruciform

[OP01-2-05]

DETERMINATION OF THE SOLUTION STRUCTURE OF A POTENTIALLY INFLAMMATION-INHIBITING PEPTIDE (VIPER) DERIVED FROM A POXVIRAL IMMUNE EVASION PROTEIN (A46)

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Toll-like receptors (TLRs) play a role in viral detection leading to cytokine and IFN induction, and as such, they are targeted by viruses for immune evasion. The poxviral protein A46 has been identified to inhibit TLR signalling by interacting with TIR domain-containing proteins of the receptor complex to collectively inhibit all TLR adaptor proteins that positively regulate transcription-factor activation [1]. One 11 aa peptide (KYSFKLILAEY) termed VIPER (Viral Inhibitory Peptide of TLR4) was reported to retain the inhibitory properties of full-length A46 against TLR4 signalling. A 9R homo-polymer delivery sequence at the C-terminus provided delivery of the peptide into cells. 9R-VIPER showed its efficacy in a TLR4-dependent flu-induced lung damage model [2]. In this study, structural comparisons are presented between 9R-VIPER, which is active in preventing TLR4-dependent cytokine induction in cell culture, and a mutant that exhibited loss of function (9R-VIPER L6AE10A), through solution NMR spectroscopy. Assignments of the resonances were accomplished 1H-15N HSQC, 1H-13C HSQC, TOCSY and NOESY at Agilent 800 MHz NMR. Chemical shift assignment and Nuclear Overhauser Effects (NOE) results were used to probe peptide conformation and generate 3D structures. Finally, the 3D structure of the VIPER region in the vaccinia virus A46 protein (residues 88-98) (PDB ID code: 4M0S) [3] is shown and subsequently superimposed with the VIPER and VIPER mutant peptide structure ensembles. We find that despite a relatively minor sequence difference, the loss of hydrophobicity, negative electrostatic interactions as well as structure changes result in subtle but potentially significant differences in the region of the peptide proposed to interface with TLR4. References [1] Stack J, Bowie AG, "Poxviral protein A46 antagonizes Toll-like receptor 4 signaling by targeting BB loop motifs in Toll-IL-1 receptor adaptor proteins to disrupt receptor:adaptor interactions" J Biol Chem 287: 22672-82, (2012) [2] Shirey KA, Lai W, Patel MC, Pletneva LM, Pang C, Kurt-Jones E, Lipsky M, Roger T, Calandra T, Tracey KJ, Al-Abed Y, Bowie AG, Fasano A, Dinarello CA, Gusovsky F, Blanco JC, Vogel SN, "Novel strategies for targeting innate immune responses to influenza" Mucosal Immunol (2016) [3] Kim, Y., Lee, H., Heo, L., Seok, C. & Choe, J. Structure of vaccinia virus A46, an inhibitor of TLR4 signaling pathway, shows the conformation of VIPER motif. Protein Sci 23, 906–914 (2014).

Keyword: VIPER, Solution NMR, Toll-like receptor 4 (TLR4), Poxviral immune evasion protein A46,

Protein/peptide structural study

[OP01-2-06]

HYDROPHOBIC NANOPARTICLES AS BIO-MIMETIC CHAPERONES

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Selective unfolding of native proteins may occur on the hydrophobic surface of nanoparticles, due to hydrophobic interactions among patches of hydrophobic residues in the protein core and the non polar surface of the nanoparticles. We reasoned that these same interactions may be helpful in dissociating hydrophobic bonds in the insoluble protein aggregates often formed as inclusion bodies when overexpressing proteins in heterologous systems. These aggregation events are hard to control, and are limiting the yield in native and functional proteins, in particular when proper folding requires uptake of cofactors. We report here on results obtained by stabilizing the unfolded form of the protein on the surface of geometrically suitable polystyrene nanoparticles, and by promoting subsequent refolding by supplying appropriate "structure-promoting" compounds. In this frame, we tested organic and inorganic cofactors, as appropriate to the specific protein under investigation, as well as substrates and their analogues in the case of enzymes. Under conditions where formation of a native structure is favored (e.g., in the presence of stabilizing agents), refolding of the adsorbed protein around the chemicals used as "structural primers" to promote refolding was proven to lead to formation of a proper hydrophobic core at the protein interior. As anticipated, burial of the exposed hydrophobic sites uopn refolding led to the release of the folded protein from the nanoparticle surface. Results will be discussed in terms of their possible practical relevance, and in terms of the specific conditions required for refolding of individual protein classes.

Keyword: Protein folding, cofactor insertion, chaperones, nanoparticles, nanobiology

[OP01-2-07]

THE STRUCTURAL AND FUNCTIONAL STUDY OF STPR FROM SILKWORM BOMBYX MORI.

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Despite over 3300 protein–DNA complex structures have been reported in the past decades, there remain some unknown recognition patterns between protein and target DNA. The silkgland-specific transcription factor FMBP-1 from the silkworm Bombyx mori contains a unique DNA-binding domain of four tandem STPRs, namely the score and three amino acid peptide repeats. Here we report three structures of this STPR domain (termed BmSTPR) in complex with DNA of various lengths. In the presence of target DNA, BmSTPR adopts a zig-zag structure of three or four tandem -helices that run along the major groove of DNA. Structural analyses combined with binding assays indicate BmSTPR prefers the AT-rich sequences, with each -helix covering a DNA sequence of 4 bp. The successive AT-rich DNAs adopt a wider major groove, which is in complementary in shape and size to the tandem -helices of BmSTPR. Substitutions of DNA sequences and affinity comparison further prove that BmSTPR recognizes the major groove mainly via shape readout. Multiple-sequence alignment suggests this unique DNA-binding pattern should be highly conserved for the STPR domain containing proteins which are widespread in animals. Together, our findings provide structural insights into the specific interactions between a novel DNA-binding protein and a unique deformed B-DNA.

Keyword: silkworm Bombyx mori, STPR domain, unique DNA-binding pattern

[OP01-2-08]

LIPASE IMMOBILIZATION ON POLYPYRROLE-METHYL ANTHRANILATE FUNCTIONALIZED "WORM-LIKE" TITANIUM DIOXIDE NANOCOMPOSITE AS PROMISING NANOBIOCATALYST: CHARACTERIZATION AND MOLECULAR DOCKING INSIGHT

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Enzymes perform an essential role in catalysing extensive reactions holds vast reputation on an industrial scale as well as health sector. Yet, their instability upon repetitive use, as well as their activity inhibition by different solvent remains a cumbersome task of concern. In this study, we report that the activity and stability of enzymes can be enhanced by their immobilization on a novel synthesized polypyrrole–methyl anthranilate-titanium oxide nanocomposite containing amine groups for enzymatic flavour synthesis. Enzymatic flavour synthesis has an upper edge over the traditional chemical route for flavour synthesis in terms of health and cost effectiveness. We demonstrated this by successfully immobilizing lipase from *Rhizopus oryzae* on a Ppy–MA/TiO_2 NC by physical adsorption and a glutaraldehyde-activated covalent coupling procedure. The immobilized lipase successfully synthesised flavour ester in solvent free media and n-hexane having 25.5% and 85.5% ester yields respectively. The catalytic efficiencies of the free and immobilized preparations were determined for the hydrolysis of p-nitrophenyl palmitate. The covalently immobilized lipase displayed a significantly higher activity yield (effectiveness factor of 0.97) in comparison with the adsorbed counterpart (effectiveness factor of 0.86). The binding of lipase to the Ppy–MA/TiO_2 NC was confirmed by transmission electron microscopy, Fourier transform infrared spectroscopy and scanning electron microscopy. TGA and DTA were performed to investigate the thermal stability of the synthesised biocatalysts. The storage stability, solvent tolerance, and reusability of the resulting nanobiocatalyst and the effect of pH and temperature on its catalytic activities were also investigated. The prepared nanobiocatalysts displayed remarkably improved activity in terms of solvent tolerance (activity recovery of 150% and 125% in acetone and isopropanol, respectively) in comparison with its free counterpart. We envisage that the covalent binding method played a profound role in enhancing the properties of the enzyme immobilized on the Ppy–MA/ TiO_2 NC. Molecular docking results confirmed the successful binding of the enzyme-nanocomposite macromolecular complex.

[OP01-2-09]

RESPIRATORY CYTOCHROME C – A MITOCHONDRIAL VISITOR TO THE NUCLEUS REGULATING CHROMATIN DYNAMICS AND DNA DAMAGE RESPONSE IN HUMANS AND PLANTS

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Genome integrity is constantly battered by genotoxic agents. These can induce DNA damage that leads to cell death if not properly repaired. Most studies on the DNA repair process have focused on yeast and mammals, in which histone chaperones have been revealed as key regulators for DNA to be accessible to repair machinery. However, knowledge of their exact role in DNA damage response is far from complete, in particular in plants. Our recent studies reveal that the closely related histone chaperones human SET/TAF-Iβ and plant NRP1 are similarly involved in nucleosome assembly following DNA break in humans and plants, respectively [1,2], and that both histone chaperones interact with cytochrome c (Cytc) in the cell nucleus upon DNA damage. We have used Nuclear Magnetic Resonance (NMR), Isothermal Titration Calorimetry (ITC), Surface Plasmon Resonance (SPR) and Molecular Docking (MD) to provide a structural insight into the complex formed by Cytc with each histone chaperone. Cytc competitively hinders the binding of SET/TAF-Iβ and NRP1 to core histones, thus locking their histone binding domains and inhibiting their nucleosome assembly activities. These results indicate that the underlying molecular mechanism of nucleosome disassembly/reassembly needed for DNA repair is highly conserved throughout evolution [1,2]. We have also recently reported that a phosphomimetic mutant of Cytc exhibits enhanced dynamics, which could be responsible for the observed differences in Cytc functionality in oxidative stress and cell death. Thus, phosphorylation of Cytc becomes a target for further development of robust therapeutic approaches [3]. Over the past decade, evidence has actually emerged suggesting a broader role for Cytc in programmed cell death. Based on the above finding on the ability of Cytc to inhibit the nucleosome assembly activity of histone chaperones, we have hypothesized a dual concentration-dependent function for nuclear Cytc in response to DNA damage. We propose that low levels of highly cytotoxic DNA lesions – such as double-strand breaks – induce nuclear translocation of Cytc, leading to the attenuation of nucleosome assembly and, thereby, increasing the time available for DNA repair. If DNA damage

persists or is exacerbated, the nuclear Cytc concentration would exceed a given threshold, causing the heme protein to block DNA remodeling altogether [4]. [1] González-Arzola, K. et al. (2015) Proc. Natl. Acad. Sci. USA 112: 9908–9913 [2] González-Arzola, K. et al. (2017) Nucleic Acids Res. 45: 2150–2165 [3] Moreno-Beltrán, B. et al. (2017) Proc. Natl. Acad. Sci. USA Plus 114: E3041–E3050 [4] Díaz-Moreno, I. et al. (2018) FEBS Lett. 592: 172–178

Keyword: Cytochrome c, DNA damage repair, Histone chaperones, Programmed cell death, Protein phosphorylation

[OP01-2-10]

CRYSTAL STRUCTURE OF THE V(D)J RECOMBINASE RAG1-RAG2

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V(D)J recombination in the vertebrate immune system generates a highly diverse population of immunoglobulins and T-cell receptors by combinatorial joining of segments of coding DNA. The RAG1–RAG2 protein complex initiates this site-specific recombination by cutting DNA at specific sites flanking the coding segments. Here we report the crystal structure of the mouse RAG1–RAG2 complex at 3.2A˚ resolution. The 230-kilodalton RAG1–RAG2 heterotetramer is 'Y-shaped', with the amino-terminal domains of the two RAG1 chains forming an intertwined stalk. Each RAG1–RAG2 heterodimer composes one arm of the 'Y', with the active site in the middle andRAG2 at its tip. TheRAG1–RAG2 structure rationalizes more than 60 mutations identified in immunodeficient patients, as well as a large body of genetic and biochemical data. The architectural similarity between RAG1 and the hairpin-forming transposases Hermes and Tn5 suggests the evolutionary conservation of these DNA rearrangements.

Keyword: V(D)J recombination, RAG1-RAG2 complex, Crystal structure

[OP01-2-11]

ENLIGHTENING MACROMOLECULAR STRUCTURE-FUNCTION RELATIONSHIP WITH PROTEOPEDIA

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Students and scientists are now able to access images of biomacromolecules both in journal and on the web. However, rather than just relying on text and 2D images to try to understand the function of biomacromolecular structures, it is more effective to be able to interact with a 3D model. To this effect, one can use a collaborative website called Proteopedia1,2 which is a free resource that links written information & 3D molecular models. This wiki web site, http://proteopedia.org, interactively displays structures of proteins, other biomacromolecules and supramolecular complexes. These displays are surrounded by descriptive text containing hyperlinks that change the appearance of the adjacent 3D structure to reflect the concepts discussed in the text. This makes the complex structural information readily accessible and comprehensible, even to non-structural biologists. By authoring content in Proteopedia, one can easily create descriptions linked to the 3D structure, e.g., see a page on the ribosome structure/function, http://proteopedia.org/w/Ribosome. Pages can be viewed on PCs, MACs & LINUX computers and even on iPads via the molecular viewer JSmol3, e.g., a page on HIV-1 protease, http://proteopedia.org/w/HIV-1_protease. It is an invaluable tool for teaching and getting students excited about structural biology4. Content is being added by Proteopedia's >3,750 users, in 60 different countries, in a dozen different languages, including Russian, Chinese, Arabic, and Hebrew: http://proteopedia.org/w/1eve_(Arabic) & http://proteopedia.org/w/1eve_ (Hebrew). A number of journals and book publishers are using Proteopedia to complement their printed and web papers via Proteopedia's "Interactive 3D Complements" (I3DCs) - see, e.g., http://proteopedia.org/w/Journal:Molecular_Cell:1. Recently Acta Crystallographica has begun to use Proteopedia directly in its online papers to aid in visualization of the 3D structures, see, e.g. the Proteopedia Molecular Tour at the bottom of the page http://scripts.iucr.org/cgi-bin/paper?S0907444911047251. Scientists and students are invited to request a Proteopedia user account, at no cost, in order to edit existing pages and to create new ones, see: http://proteopedia.org/w/Special:RequestAccount. References: 1 Hodis, E., Prilusky, J., Martz, E., Silman, I., Moult, J. & Sussman, J. L. Proteopedia - a scientific 'wiki' bridging the rift between 3D

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Keyword: Protein Structure/Function, 3D Visualization, Education, Wiki, PDB

[OP01-2-12]

MOLECULAR BASIS FOR CYTOCHROME C-REGULATED MECHANISM OF CELL SURVIVAL UPON ISCHEMIC INJURY

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One post-translational modification known to regulate proteins is phosphorylation, which is modulated by kinases and phosphatases, affecting for instance the functionality of proteins in redox signaling. This is particularly important in mitochondria, which are the main source of reactive oxygen/nitrogen species (ROS/RNS) in the cell. The two major targets of oxidative phosphorylation (OxPhos) that control signaling are cytochrome c (Cc) and cytochrome c oxidase (COX). Cc can undergo phosphorylated in vivo at several residues. This modification can alter how Cc binds to its physiological partners, in either the mitochondria or cytosol, but these effects are highly dependent on which residue is modified1,2. Tyr97 phosphorylation has been correlated with several pathologies, including ischemia. Indeed, it has been proposed that Tyr97 of Cc is targeted for phosphorylation during the insulin-induced neuroprotection response following an ischemic injury. Here we characterize a phosphomimetic, phosphatase-resistant Cc mutant, which was recombinantly generated by site-specific incorporation of the non-canonical p-carboxymethyl-L-phenylalanine aminoacid at position 97 using an orthogonal amber suppressor tRNA/aminoacyltRNA synthetase (aaRS) pair (Y97pCMF Cc)3. The mutant Cc was not significantly affected in its overall folding and heme environment but had a decreased thermal stability. We found that this post-translational modification of Cc affected the OxPhos process. The electron donation rate to COX by Y97pCMF was higher than wild-type Cc in respiratory supercomplexes. This agrees with a decrease in ROS production of more than 15-fold with respect to the wild-type protein. Altogether, it is suggested that the COX-driven oxidation rate of Cc is controlled by Tyr 97-phosphorylation to maintain low levels of apoptotic-inducing ROS/RNS4. This finding could potentially have therapeutic applications in acute diseases, such as brain ischemia. 1Guerra-Castellano A, et al. Biochim. Biophys. Acta - Bioenerg. 1857, 387–395 (2016). 2Guerra-Castellano A, et al. Proc. Natl. Acad. Sci. U.S.A 114, 3041–3050 (2017). 3Guerra-Castellano A, et al. Chem. Eur. J. 21, 15004-15012 (2015). 4Guerra-Castellano et al. Under revision. Keywords: cytochrome c, phosphorylation, respiratory supercomplexes, caspase activity.

[OP04-4-01]

NOVEL SMALL MOLECULE INHIBITOR OF KPNB1 WITH ANTI-CANCER ACTIVITY

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Karyopherin beta 1 (KpnB1) is a major nuclear transport receptor that mediates the import of cellular cargoes into the nucleus. Recently it has been shown that KpnB1 is highly expressed in several malignant cancers such as ovarian, cervical, neck, and lung cancers. However, not much is known about its role as a therapeutic target for cancer. This project is based on the hypothesis that KpnB1 is necessary for the growth and survival of cancer cells and that a rational drug design approach used to identify small molecule inhibitors against KpnB1 may generate effective anti-cancer treatment for cancers of different tissue origin. The aim of this study is to characterize a novel small molecule inhibitor (C60) of KpnB1 by investigating its effect on the biology of cancer cells, nuclear import inhibitory function as well as its effect on tumour growth in vivo. Subjects and Methods: The effect of C60 on cancer cell biology was investigated in cervical and oesophageal cancer cells by monitoring its effect on proliferation, colony formation, cell cycle progression, migration and invasion. The cell killing effect of C60 was monitored by annexin V assay and PARP cleavage. The nuclear import inhibitory effect of C60 was determined via investigating its effect on the subcellular localisation of KpnB1 and its known cargoes such as NFAT, NFκB and AP-1. The in vivo toxic side effect of C60 has been investigated in nude mice and its effect on tumour growth will be investigated. Results: In this study, we show that C60 inhibited cancer proliferation and colony formation, induced a G1/S cell cycle block and induced apoptosis as shown by PARP cleavage and annexin V assay. Non-cancer epithelial cells and Fibroblasts were mostly unaffected by C60 treatment at concentrations that inhibited cancer cells. In addition, C60 showed a significant inhibitory effect on cancer cell migration and invasion. Furthermore, C60 interfered with the subcellular localisation of KpnB1 as well as that of known KpnB1 cargoes. Toxicology study performed showed that mice tolerated the different doses of C60. Discussion / Conclusion: Taken together, these results show that inhibition of KpnB1 using C60 induces cancer cell death by apoptosis as well as altering KpnB1 subcellular localisation and that of KpnB1 cargo proteins. This study suggests that the nuclear transport protein KpnB1 has potential as an anti-cancer target.

Keyword: Anti-cancer, KpnB1, Small molecule, Inhibitor, Nuclear import

[OP04-4-02]

CHARACTERIZATION OF A549 LUNG CANCER CELL METABOLITE AND GENE EXPRESSION PROFILES UPON TREATMENT OF MOMORDICA CHARANTIA METHANOLIC EXTRACT

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Background: Cancer remains to be one of the leading causes of death worldwide. Lung cancer is one of the most fatal among the different types of neoplasms and the treatment options are limited especially in advanced stages. A significant number of studies suggest that M. charantia possesses anticancer properties but the exact mechanisms as to how it prevents tumor growth progression have yet to be described. Objectives: In this study, we aimed to describe the changes in the metabolite profile of A549 cells after treatment with M. charantia methanolic extract. We also aimed to identify key genes involved in the twelve cancer pathways and to determine the change in their expression levels in A549 cells after treatment with M. charantia methanolic extract. Finally, we aimed to utilize gene expression and metabolite abundance data to create an impact scoring system that determines which pathways are notably altered following treatment with the plant extract. Method: A subculture of A549 cells was subjected to treatment with M. charantia methanolic extract. The gene expression profile of the A549 cells were determined by comparing the treatment group with a control group using quantitative PCR. The metabolite profile was determined using mass spectrometry matched against a selection of cancer-associated metabolites. The data from the gene expression analysis and metabolite profile analysis were used to construct an impact scoring system and the highest scoring pathways were identified. Results and Conclusion: It was determined through quantitative PCR that genes with considerable expression fold changes were VEGF, FGF2, ATM, BRG1, PAI1, and DDB2. Mass spectrometry revealed metabolites of considerable interest, namely oleamide, sphingosine, palmitic acid, trigonelline, creatine, L-carnitine, traumatic acid, and glycerophospho-N-oleoyl ethanolamine. The impact factor scoring analysis determined the top three pathways that were greatly affected upon treatment with the plant extract, which were telomere and telomerase, angiogenesis and hypoxia signaling.

[OP04-4-03]

CHROMOSOME MISSEGREGATION DRIVES STRUCTURAL ABERRATIONS OF CHROMOSOMES IN CANCER.

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The features of tumor include numerical and structural abnormalities of chromosomes. Aneuploidy can occur when chromosomes missegregate during mitosis. However, the possibility is largely ignored that structural constraints imposed on missegregated chromosomes could elicit structural aberrations of affected chromosomes. To investigate structural variations of chromosomes driven by chromosome missegregation, we analyzed the structure of chromosomes in tumors from mice which favor chromosome missegregation and overlook the resulting chromosomal damage, thus K243R/+;p53-/- mice. Our previous research on mitotic checkpoint protein BubR1 revealed that the mouse model (K243R/+) harboring acetylation deficient allele of the protein succumbs to spontaneous tumorigenesis, which emphasizes a causative role of chromosome missegregation during mitosis in tumor development. In this study, we found that fragile telomeres were frequent in both K243R/+ and K243R/+;p53-/- MEFs compared to wild-type or p53-/- mutant MEFs. Also whole arm translocations were found in several tumor cells from K243R/+;p53-/- mice, suggesting that the telomere and centromere become unstable when the mitotic fidelity is abrogated.

Keyword: Chromosome instability, Aneuploidy, Whole arm translocation, Fragile Telomere, BubR1

[OP04-4-04]

TONEBP PROMOTES HEPATOCELLULAR CARCINOGENESIS, RECURRENCE, AND METASTASIS

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- Introduction: Hepatocellular carcinoma (HCC) is a common cancer with high rate of recurrence and mortality. Diverse etiological agents and wide heterogeneity in individual tumors impede effective and personalized treatment. Tonicity-responsive enhancer-binding protein (TonEBP) is a transcriptional cofactor for the expression of pro-inflammatory genes. Although inflammation is intimately associated with the pathogenesis of HCC, the role of TonEBP is unknown. We aimed to identify function of TonEBP in HCC. - Methods: Tumors with surrounding hepatic tissues were obtained from 296 patients with HCC who received completion resection. TonEBP expression was analyzed by qRT-PCR and immunohistochemical analyses of tissue microarrays. Mice with TonEBP haplo-deficiency, and hepatocyte- and myeloid-specific TonEBP deletion were used along with HCC and hepatocyte cell lines. - Results: TonEBP expression is higher in tumors than in adjacent non-tumor tissues in 92.6% of 296 patients with HCC regardless of etiology associated and DEN-induced mouse HCC. Hepatic induction of TonEBP is mediated by a fall in the miR-223 abundance. The TonEBP expression in tumors and adjacent non-tumor tissues predicts recurrence, metastasis, and death in multivariate analyses. Univariate analysis of two layers of patients showed that higher TonEBP expression was significantly associated with bigger tumor, advanced tumor grade, and vascular invasion. TonEBP promotes HCC initiation and growth via oxidative stress and inflammation in various mouse models of HCC. The association between TonEBP and inflammation was confirmed from analysis of the RNA-seq dataset from TCGA. TonEBP-dependent stimulation of tumor growth was dependent on COX-2. TonEBP drives the expression of COX-2 by stimulating the promoter in association with transcription factor YY1 and histone acetyltransferase p300. TonEBP is required for the recruitment of both YY1 and p300 to the promoter in situ. The interaction between TonEBP and YY1 is mediated by RHD and spacer domain. TonEBP deficiency

resulted in reduced COX-2 expression leading to reduced production of prostaglandin E2 in various animal models of HCC and acute liver injury. In mouse models of HCC and acute liver injury, three common sites of TonEBP action in response to diverse etiological agents leading to tumorigenesis and tumor progression were found: cell injury and inflammation, induction by oxidative stress, and stimulation of the COX-2 promoter - Conclusions: TonEBP is a key component of the common pathway in tumorigenesis and tumor progression of HCC in response to diverse etiological insults. TonEBP is involved in multiple steps along the pathway rendering it an attractive therapeutic target as well as a prognostic biomarker.

Keyword: TonEBP, HCC, Poor prognosis, COX-2

[OP04-4-05]

SPATIAL-FLUXOMICS ENABLES QUANTITATIVE CHARACTERIZATION OF CANCER METABOLISM AT SUBCELLULAR LEVEL

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The inability to inspect metabolic activities within distinct subcellular compartments has been a major barrier to our understanding of eukaryotic cell metabolism. Here, we describe a spatial-fluxomics approach for inferring metabolic fluxes in mitochondria and cytosol under physiological cellular conditions. This is achieved by performing isotope tracing in intact cells, followed by rapid subcellular fractionation and LC-MS based metabolomics analysis, and computational deconvolution and metabolic modeling. We applied our method to re-examine one of the emerging metabolic hallmarks of cancer cells – reductive glutamine metabolism which mediates fatty acid biosynthesis under hypoxia and in cells with defective mitochondria. Analyzing isocitrate dehydrogenase (IDH) activity in mitochondria and cytosol (as well as IDH isozymes within mitochondria), we reveal that reductive glutamine metabolism is, in fact, the major producer of cytosolic citrate (rather than glucose oxidation) to support fatty acid biosynthesis also in standard normoxic condition. Applied to investigate metabolic flux reprogramming in cells with defective mitochondria, due to succinate dehydrogenase (SDH) deficiency, the spatial-fluxomics approach revealed a reversal of citrate synthase flux for supporting amino acid and nucleotide biosynthesis. We expect this spatial-fluxomics approach to be a highly useful tool for studying the metabolic interplay between mitochondria and cytosol and elucidating the role of metabolic dysfunction in human disease.

Keyword: cancer metabolism, metabolomics, mitochondria, hypoxia, succinate dehydrogenase

[OP04-4-06]

DELINIATING OXYLIPIN SIGNALING NETWORK IN TRIPLE NEGATIVE BREAST CANCER THROUGH MULTI-OMICS ANALYSES

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Purpose: Fatty acid-derived bioactive lipid mediators (LM), collectively known as oxylipins, act as signaling molecules bridging two hallmarks of cancer: altered metabolism and inflammatory microenvironment. Both hallmarks are important for hormone independent signaling in triple negative breast cancer (TNBC), a breast cancer subtype which does not express targetable hormone receptors and is associated with rapid onset of metastasis and recurrence, and lower survival rate. Our preliminary results suggest that TNBC utilize LMs to fuel important processes in cancer progression differently compared with hormone-positive breast cancer subtypes. This study aims to uncover the role of oxylipin metabolism to provide a novel avenue for TNBC diagnosis or therapy. Method: A quantitative LC-MS/MS metabolomics approach was used to analyze and compare arachidonic acid and linoleic acid-derived oxylipin metabolites in TNBC cell lines and patient-derived triple negative tumor tissues and other breast cancer subtypes and normal mammary epithelial cells and tissues. In parallel, comparative proteomics, multivariate statistics and bioinformatics tools and information from publicly available datasets, i.e., TCGA and METABRIC were utilized to identify metabolite-protein-gene network relationships in TNBC. Results: Our data indicate that elevated concentrations of epoxyeicosatrienoic acids (EETs) and overexpression of their biosynthetic enzymes, CYP450 epoxygenases, are correlated with TNBC metastasis phenotype. Gene and protein network analyses suggest that EETs act as crucial nodes linking metastasis and bioactive lipid metabolism pathways in TNBC tissues. Specific fatty acid binding protein isoforms were identified as important players in EET signaling and metastatic transformation in tumor tissues and may thus have potential as targets for TNBC therapy. Conclusions: : Combination of metabolomics, proteomics and data mining of publicly available transcriptomics data is effective to support hypothesis- and discovery-driven research for sub-classification and target identification of TNBC tumors. We were able to establish a multi-omics, multi-source platform to validate the direct relationship between oxylipin mediator signaling,

metastasis and survival in TNBC patients. Inhibition of identified oxylipin metabolism and transport pathways may have potential for development of personalized treatment for TNBC patients.

Keyword: triple negative breast cancer, oxylipins, metastasis, integrative omics

[OP04-4-07]

REGULATION OF TIGHT JUNCTION PROTEINS BY METASTASTASIS SUPPRESSOR PROTEIN NM23/NDPK IN BREAST CANCER CELLS

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NME1, a metastasis suppressor gene of Nm23, is known to have NDP kinase (NDPK) enzymatic activity. Nm23 plays key roles to inhibit metastasis in multiple stages of metastatic processes. We screened the small molecule compounds as NDPK (Nm23) activator (NMac) from natural product libraries, because NDPK activator possessed anti-metastatic activity. Our novel NMac NAA001 suppresses the invasion of MDA-MB231, triple negative breast cancer cell line by inhibiting Rac1 activation via Nm23-H1. To investigate how NAA001 regulates the cell invasion, we examined the protein profile changes of MDA-MB-231 cells in response to NAA001 treatment combining the protein separation with 2D-PAGE and identified the differentially expressed proteins with nanoUPLC-ESI-q-TOF tandem MS analysis. We identified 16 spots as differentially expressed or modified proteins by NAA001 including keratin 8, heat shock protein 27 (Hsp27), and LIM and SH3 domain protein 1 (LASP1). Of these, LASP1 phosphorylation identified by nanoUPLC-ESI-LC-MS/MS were significantly altered in response to NAA001 treatment. Since LASP1 is known to interact with proteins of tight junction, we examined the LASP1 phosphorylation changes and ZO-2 in Nm23-H1 knock down cells. The results demonstrate that Nm23-H1 inhibits the cell invasion by regulating tight junction via phosphorylation of LASP1 and ZO-2 interaction of phosphoLASP1.
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[OP04-4-08]

REGULATION OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA EXPRESSION IN HYPOXIC TUMOR

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Peroxisome Proliferator Activated Receptor Gamma (PPARγ) is involved in diverse physiological functions including metabolism, inflammation, and differentiation. The ligand-mediated receptor plays as a tumor suppressor in many cancers and clinically sensitizes insulin effect in type II diabetes with its ligand thiazolidinedione treatment. Hypoxia is an important tumor microenvironment contributing to tumor metastasis and therapeutic resistance in cancer. The goal of this study is to investigate PPARγ function in hypoxic area of tumor. To this end, we first established a set of conditions to mimic hypoxic microenvironment including chemical mimetic agents (e.g. cobalt chloride, deferoxamine) and hypoxic culture system for cancer cells. Here, along with transcriptional repression of PPARγ mRNA, we found decreased stability of PPARγ protein under hypoxic condition in lung adenocarcinoma and cervical cancer cells. Interestingly, the decreased stability of the PPARγ protein turns out to be not due to HIF1α-DEC1 axis as a previous report, suggesting alternative pathway to regulate PPARγ protein stability. We wondered if the hypoxia-induced acidic microenvironment would be involved in the PPARγ regulation process, since chronic hypoxic condition increases lactic acid production resulting in acidic microenvironment. Further biochemical experiments using lactic acid revealed that both endogenous and exogenous PPARγ proteins are strongly destabilized upon lactic acid treatment. Moreover, hypoxia synergizes PPARγ instability with lactic acid treatment. Understanding the molecular mechanism for the regulation of hypoxic PPARγ protein stability would provide an important biological insight of PPARγ physiology into inflammatory response, adipocyte differentiation, and tumor suppressive function in particular against the core hypoxic tumor.

Keyword: PPARgamma, Cancer metabolism, Hypoxic tumor, Tumor Microenvironment, NDRG3

[OP04-4-09]

STUDY OF THE EFFECT OF OXIDATIVE STRESS ON BREAST CANCER MULTICELLULAR SPHEROIDS

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Breast cancer is the most diagnosed cancer among women worldwide. Several risk factors including genetically and environmentally are identified as the cause of breast cancer. Investigation on cellular mechanisms related to the important risk factors is essential for an efficient diagnosis and treatment. For a cancer model, an in vitro multicellular tumor spheroid (MCTS) has been accepted to closely recapitulate in vivo cancer condition. Cells in MCTS are deprived of oxygen, nutrients, and extracellular signals causing a production of oxidative stress and reactive oxygen species (ROS) which would not be observed from a conventional cancer monolayer. Here, the aim of this study was to investigate and compare the effects of oxidative stress on the breast MCTS and monolayer. Gene expression involved in the cellular oxidative stress, cell viability, degree of apoptosis, and cell migration were evaluated in this study. In the experiments, the oxidative stress was induced by treating the breast cancer cells (MCF-7) with 30 μM hydrogen peroxide (H2O2) for either 2 or 24 hours. The gene expression of antioxidant enzymes including glutathione peroxidase1 (GPx1), superoxide dismutase 2 (SOD2), catalase (CAT), and glutathione synthetase (GSS) was evaluated by qPCR. The relative fold changes of GPx1, involving in detoxifying H2O2, was upregulated in both MCS and monolayers approximately by 5- and 13-fold, respectively. Interestingly, CAT was downregulated approximately by 6-fold in the monolayer but no significant change was observed in MCTS. Also, when treated with H2O2, the MCTS showed an increase in cell viability, a decrease in late apoptosis and cell death compared with the monolayer. Nevertheless, the cell migration was not affected by the oxidative stress in the MCF-7 monolayer. In addition, the migration of MCTS is being investigated. Altogether, the results preliminary demonstrated that the MCTS and monolayer responded differently to the oxidative stress. Moreover, the culture of cells in MCTS contributed to lower H2O2 sensitivity and more resistance to apoptosis in the oxidative stress condition. In conclusion, this study emphasized on an importance of using MCTS as in vitro model for the study of oxidative stress in breast cancer. Moreover, this knowledge would be beneficial for further investigation.

Keyword: Breast cancer, Multicellular spheroids, Oxidative stress, Reactive oxygen species

[OP04-4-10]

ONCOGENIC ZINC FINGER PROTEIN ZNF322A PROMOTES LUNG CANCER STEMNESS THROUGH TRANSCRIPTIONALLY SUPPRESSING C-MYC EXPRESSION

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ZNF322A, which encodes a C2H2 zinc finger transcription factor, is an oncogene in lung cancer. However, the transcription mechanisms of ZNF322A in lung tumorigenesis especially cancer stemness remain elusive. To characterize the transcription network of ZNF322A in lung cancer, we revealed consensus DNA binding motifs of ZNF322A using cyclic amplification and selection of targets (CASTing) assay and chromatin immunoprecipitation-sequencing (ChIP-seq) analysis. RNA sequencing (RNA-seq) was also performed. Putative transcriptional targets of ZNF322A were identified by integrating ChIP-seq and RNA-seq datasets. Many of the ZNF322A transcriptional target genes were involved in cancer-related processes, such as transcription, cell cycle regulation and migration. Notably, ZNF322A downstream genes showed a significant enrichment in developmental processes, implicating that ZNF322A may participate in cancer stemness and maintenance. Indeed, in vitro sphere formation assay and in vivo tumor initiation assay confirmed that overexpression of ZNF322A promoted lung cancer stemness. In addition, mRNA expression of stemness-related genes such as Oct4, Nanog and Sox2 were increased in ZNF322A overexpressed tumors. Importantly, ZNF322A significantly targeted to MYC gene locus, which encodes for another reprogramming transcription factor. However, c-Myc expression was negatively regulated by ZNF322A through ZNF322A conserved DNA binding motif 2 verified by immunoblotting, qRT-PCR and luciferase promoter activity assays. Since c-Myc downregulation is shown as the key determinant of oxidative phosphorylation dependency in pancreatic cancer stem cells, we therefore performed ATP production assay and XF24 Extracellular Flux Analyzer, and examined the expression of metabolic genes. Our results confirmed that ZNF322A-mediated c-Myc suppression shifted metabolism phenotypes to oxidative phosphorylation. Clinically, c-Myc mRNA expression negatively correlated with ZNF322A protein expression in lung cancer patients. In addition, ZNF322AHigh/c-MycLow expression profile in tumor specimen was revealed as an independent factor for poor outcome of lung cancer patients. Our study provides first evidence that ZNF322A promotes lung cancer stemness by upregulation of Oct4, Nanog and Sox2 expression and downregulation of c-Myc expression. ZNF322A acts as transcription suppressor of c-Myc to maintain stemness by shifting stem-like cancer cells to oxidative phosphorylation metabolism.

Keyword: Cancer stemness, transcription regulation, zinc finger transcription factor, ZNF322A, c-Myc

[OP04-4-11]

FRAILTY IN 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE: THE INFLUENCE OF AGE AND PROTEASOME ACTIVATION

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The increase in frailty is associated with the progression of Alzheimer's disease (AD), one of the most common age-related neurodegenerative disorders worldwide. AD is characterized by the accumulation of amyloid plaques composed of aggregated amyloid-beta (Abeta) peptides, mainly Abeta 42. In the same time, dysfunction of the ubiquitin-proteasome system responsible for the removal of proteins has been observed during aging and in age-related disorders like AD. A proteasome activator, 18 alpha-glycyrrhetinic acid (18-alpha GA), has been shown to enhance levels of proteasome activities and to decrease Abeta deposits in model organisms. We examined the effect of 18-alpha GA supplementation on APP processing and generation of Abeta 42 in the 5xFAD transgenic AD mouse model. This is an early-onset AD mouse model characterized by the deposition of plaques in the cortex and hippocampus as early as at 2 months of age. We exposed both female and male mice to the proteasome activator 18-alpha GA treatment for one month, starting from 2-months of age, when animals already have developed plaques. This is considered an early phase of AD pathology, known to be suitable for therapeutics application. We determined frailty in young (2 months of age) and old (12-13 months of age) 5xFAD mouse, as well in those treated with 18-alpha-GA. Both female and male mice were tested in the open field test, rope test and grip strength test. Body weight was also measured and clinical signs of deterioration in aging were scored. All together these parameters were used to determine frailty in 5xFAD. Obtained results showed a significant increase in frailty in the aged 5xFAD mice. Significant differences were observed between males and females. 18-alpha-GA treatment increased activity of the proteasome in the cortex and hippocampus of 5xFADmice and decreased the number of Abeta plaques. Results indicated potential therapeutic use of 18-alpha-GA.

Keyword: ageing, Alzheimer's disease, frailty, proetasome, 5XFAD mouse

[OP09-3-01]

ORDERED LIM DOMAINS TUNE BINDING KINETICS OF A DISORDERED PARTNER TO REGULATE TRANSCRIPTION COMPLEX FORMATION

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Intrinsically disordered regions (IDRs) are overrepresented among transcription factors, in which they often act as protein-protein interaction motifs. Despite the importance of IDR interactions there is little quantitative information about the role disorder plays in the thermodynamic and kinetic parameters of binding. We have studied the interactions between the LIM domain transcription factors and their disordered partners to understand the role disorder can play in transcription factor complex formation. Transcription factors containing disordered LIM interacting domains (LIDs) interact with the ordered tandem LIM domains (LIM1+2) of LIM-only (LMO) and LIM-homeodomain (LIM-HD) proteins. The resulting complexes regulate gene expression and are important mediators of cell specification, proliferation and differentiation. All LIM-HD/LMO proteins interact with the LID of LIM domain binding protein 1 (LDB1), and overlapping expression of LIM-HD/LMO proteins, LDB1 and other LID co-factors plays an important role in regulating neuronal and brain development. Studying these competitive interactions has previously been difficult due to the aggregation of LIM domains from LMO and LIM-HD proteins in the absence of a binding partner. We have developed FRET-based assays that overcome LIM domain aggregation, which has enabled us to determine the binding affinities of multiple LID:LIM1+2 interactions. The affinities of LMO and LIM-HD proteins for LDB1LID differ by 70-fold. Unusually, the differences in LDB1LID binding affinity are largely determined by the association rate constants, which vary by 2–3-orders of magnitude. These disparate kinetics are not accounted for by differences in electrostatic attractions, rather they reflect differences in binding mechanisms among the related LIM1+2 domains. We have found that LDB1LID can bind single LIM domains with high association rate constants, indicating that the differences in LIM1+2 association occur after the initial encounter. Finally, we have used our equilibrium and kinetic data to model LID:LIM1+2 transcription factor complex as it may occur in motor neuron development, showing that the disparate kinetics facilitate exchange of high affinity binding partners over time. Together, our studies suggest that the inherent flexibility of IDRs can be utilised by ordered domains to generate highly diverse binding kinetics, which may provide a mechanism for temporally regulating transcriptional complex formation and gene regulation during development.

Keyword: Intrinsic disorder, Protein-protein interaction, Transcription, Binding Kinetics

[OP09-3-02]

FUNCTIONAL ROLES OF THE TUMOR SUPPRESSOR GENE FHL2 ON TP53, IL6 AND STAT3

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The four-and-a-half LIM domain protein 2 (FHL2) has been identified as an oncogene or tumor suppressor gene in a tissue / cell specific manner by acting as an adaptor protein to form different protein complexes and involving in various signaling pathways. In hepatocellular carcinoma (HCC), FHL2 was found to suppress tumor cell growth through a TGF- -like signaling pathway. On the other hand, our preliminary results showed that p53, IL6 and STAT3 are potentially related to the function of FHL2. In this study, the functional relationship between FHL2 and the p53 / IL6 / STAT3 signaling axis in liver cells and HCC was examined. To evaluate the significance of our findings in liver cancer patients, mRNA was extracted from 47 pairs of HCC patients' tissues and adjacent normal liver tissues, followed by reverse transcription and real-time PCR. The relative expression levels of FHL2 and STAT3 were then normalized with the housekeeping gene β-actin. We found that the positive correlation between FHL2 and STAT3 expression was statistically significant (P=0.037). We then performed immunohistochemistry (IHC) staining on 20 pairs of HCC and adjacent normal liver tissues using specific antibodies against FHL2, TP53, IL6, and STAT3. Results showed that the levels of FHL2, IL6 and STAT3 proteins were significantly lower in liver cancer tissues when compared with their adjacent normal controls. Moreover, protein levels of both FHL2 and TP53 positively correlated with that of IL6 in HCC tissues. To dissect the functional relationship between FHL2 and TP53 / IL6 / STAT3, we knockdown FHL2 in WRL68 liver cells by siRNA and found that it did not affect the STAT3 protein level but increased the amount of pSTAT3 protein. In addition, the downregulation of FHL2 could upregulate the expression of the p53 gene as well as increase the amount of TP53 protein. Consistent with the IHC staining results, the knockdown of FHL2 decreased the intracellular IL6 protein level by about 30%. Interestingly, the same treatment significantly increased the mRNA level of IL6 by more than four folds. To explore the underlying reason for this strange phenomenon, we quantified the secreted IL6 protein by enzyme-linked immunosorbent assay and found that the knockdown of FHL2 increased the secreted IL6 protein in WRL68 cells by about 50%. In summary, FHL2, IL6 and STAT3 were downregulated in liver cancer. Furthermore, the downregulation of FHL2 could modulate STAT3, p53 and IL6 in liver cells. The exact functional significance of their correlation deserves future investigation.

Keyword: FHL2, IL6, STAT3, TP53, Liver cancer

[OP09-3-03]

TWO COMPLETE REFERENCE HAPLOTYPES FROM A SINGLE INDIVIDUAL

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Reference genome projects have historically selected inbred individuals to minimize heterozygosity and simplify assembly. We challenge this dogma and present a new approach designed specifically for heterozygous genomes. Here, we present "Trio binning", which uses short reads from the parental genomes to partition long reads from an offspring into haplotype-specific sets prior to assembly. Each haplotype is then assembled independently, resulting in a complete diploid reconstruction. On two benchmark human trios, this method achieved high accuracy and recovered complex structural variants missed by alternative approaches, including the highly polymorphic MHC region. To demonstrate its effectiveness on a heterozygous genome, we applied this method to several model organisms including an F1 cross between two cattle subspecies. As a result, we completely assembled both parental haplotypes with NG50 haplotig sizes >20 Mbp and 99.998% accuracy, surpassing the quality of current cattle reference genomes. The newly constructed haplotypes reveal the complex genomic differences between the two cattle subspecies, with a large absence of GBP2 in one haplotype and copy number variations in GBP6 genes relative to the other haplotype. These haplotype representative assemblies will serve as new reference genomes for future resequencing projects, providing opportunities to better understand haplotype variation and biological impact.

Keyword: haplotype, genome assembly, reference genome, heterozygosity, structural variation

[OP09-3-04]

PATHOLOGICAL HYDROGEN PEROXIDE TRIGGERS THE FIBRILLIZATION OF WILD-TYPE SOD1 VIA SULFENIC ACID MODIFICATION OF CYS-111

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Amyotrophic lateral sclerosis (ALS) involves the abnormal posttranslational modifications and fibrillization of copper, zinc superoxide dismutase (SOD1) and TDP-43. However, how SOD1-catalyzed reaction product hydrogen peroxide affects amyloid formation of SOD1 and TDP-43 remains elusory. 90% of ALS cases are sporadic and the remaining cases are familial ALS. In this paper, we demonstrate that hydrogen peroxide at pathological concentrations triggers the fibrillization of wild-type SOD1 both in vitro and in SH-SY5Y cells. Using an anti-dimedone antibody that detects sulfenic acid modification of proteins, we found that Cys-111 in wild-type SOD1 is oxidized to C-SOH by pathological concentration of hydrogen peroxide, followed by the formation of sulfenic acid modified SOD1 oligomers. Furthermore, we show that such SOD1 oligomers propagate in a prion-like manner, and not only drive wild-type SOD1 to form fibrils in the cytoplasm but also induce cytoplasm mislocalization and the subsequent fibrillization of wild-type TDP-43, thereby inducing apoptosis of living cells. Thus, we propose that hydrogen peroxide at pathological concentrations triggers the fibrillization of wild-type SOD1 and subsequently induces SOD1 toxicity and TDP-43 toxicity in neuronal cells via sulfenic acid modification of Cys-111 in SOD1. Our Western blot and ELISA data demonstrate that sulfenic acid modified wild-type SOD1 level in cerebrospinal fluid of 15 sporadic ALS patients is significantly increased compared with 6 age-matched control patients. These findings can explain how hydrogen peroxide at pathologic concentrations regulates the misfolding and toxicity of SOD1 and TDP-43 associated with ALS, and suggest that sulfenic acid modification of wild-type SOD1 should play pivotal roles in the pathogenesis of sporadic ALS.

Keyword: Protein aggregation, Amyotrophic lateral sclerosis, Hydrogen peroxide, SOD1 toxicity, TDP-43

[OP09-3-05]

THE FUNCTION OF THE GOLGI RIBBON STRUCTURE- AN ENDURING MYSTERY UNFOLDS!

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The Golgi apparatus in vertebrate cells consists of individual Golgi stacks laterally fused together in a continuous ribbon structure. The ribbon structure per se is not required to mediate the classical functions of this organelle and the relevance of the "ribbon" structure has been a mystery since first identified ultrastructurally in the 1950s. Recent advances are now recognising a role for the Golgi apparatus in a range of cellular processes, and our current studies are investigating whether the ribbon structure of the Golgi may contribute to the regulation of additional cellular processes. We have developed a cell system to explore the function of the Golgi ribbon by exploiting a finding that the membrane tether of the trans-Golgi network, GCC88, regulates the balance between Golgi mini-stacks and the Golgi ribbon in a variety of cell types 1. We have shown that the regulation of Golgi morphology by GCC88 is an actin dependent process and have identified GCC88 interactive partners responsible for connecting GCC88 to actin filaments. Loss of Golgi ribbons in stable HeLa cells overexpressing GCC88 resulted in a dramatic increase in LC3-II-positive autophagosomes and compromised mechanistic target of rapamycin (mTOR) signalling, whereas RNAi depletion of GCC88 restored a Golgi ribbon and reduced autophagy. In parental cells mTOR was detected on both the Golgi and lysosomes. There was a dramatic reduction in Golgi associated mTOR and phosphorylated active mTOR in HeLa cells lacking a Golgi ribbon compared with parental cells. We demonstrate a strict temporal sequence of fragmentation of Golgi ribbon, loss of Golgi mTOR followed by increased autophagy. Golgi ribbon fragmentation has been reported in various neurodegenerative diseases and we have demonstrated the potential relevance of our findings in neuronal cells using a model of neurodegeneration. Overall, our results have uncovered a mechanism by which the Golgi ribbon negatively regulates autophagy by modulating mTOR signalling. 1. Gosavi P, Houghton FJ, McMillan PJ, Hanssen E, Gleeson PA. (2018). J Cell Sci. 31(3) (in press) doi: 10.1242/jcs.211987

Keyword: Golgi complex, autophagy, signalling, actin cytoskeleton, mTOR

[OP09-3-06]

REWIRING ABERRANT CANCER SIGNALING TO THERAPEUTIC EFFECTOR RELEASE WITH A SYNTHETIC TWO-COMPONENT SYSTEM

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Many cancers are driven by constitutively active signaling networks that promote cell growth, proliferation, or survival. Pharmacological approaches that attempt to block aberrant signaling often suffer from toxic effects on normal cells or are thwarted by the emergence of drug-resistant mutations in target proteins. Here, we present a novel approach termed rewiring of aberrant signaling to effector release (RASER), where oncogenic signaling, instead of being targeted for inhibition, is co-opted to trigger therapeutic responses. Specifically, we developed a synthetic two-component system that integrates ErbB activity to control effector release. By adopting a modular architecture and constructing a complete mathematical model of the system, we could efficiently optimize key system parameters. The resulting ErbB-RASER system responds specifically to constitutively active ErbB, is more specific for constitutively active ErbB than native kinase pathways, and can be programmed to induce a variety of outputs including apoptosis and CRISPR/Cas9 activation of endogenous genes. As the RASER approach allows the customization of sensory modules to specific oncogenic signals, it may be a generalizable method for genetically encoded cancer detection and treatment.

Keyword: Synthetic biology, Signal pathway modeling, Gene therapy, Cancer therapy, Bioengineering

[OP09-3-07]

TELOMERE ATTRITION, ANALYSES OF MNS16A TANDEM REPEATS AND HTERT PROMOTER GENE IN BANGLADESHI POPULATION WITH TYPE 2 DIABETES

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Objectives: Telomeres are protective cap on the ends of DNA of non-coding tandem repeats of TTAGGG. Human telomerase reverse transcriptase (hTERT) is a catalytic subunit of telomerase that maintains the structure of telomeres to protect chromosome from shortening. Further, the variable number of tandem repeats MNS16A in hTERT gene facilitates extension of telomeres by regulating telomerase. Type 2 diabetes (T2D) affects multi-organ and alters telomerase activity and thus, affects telomere length. We aimed to evaluate the relative telomere length (RTL) and risk association of rs2853669 and variants of MNS16A with T2D in Bangladeshi population. Methods: A total of 381 T2D and 245 healthy unrelated Bangladeshi individuals were included in this study. ΔΔCt values facilitated measurement of telomere length using primers for target gene (Telg and Telc) and primers for reference gene albumin (Albu and Albd). Distribution of genotypes for rs2853669 polymorphism was determined using TaqMan® probes followed by allelic discrimination plots. Genetic analysis of MNS16A tandem repeats in hTERT gene was performed using allele specific polymerase chain reaction. Results: The mean RTL was found to be significantly shorter (6.53 ± 3.63) in T2D patients compared to that of healthy individuals (7.60 ± 4.18) ($p < 0.008$). When values were adjusted according to their age and gender, the mean values of RTL did not vary significantly between the groups. Three genotypes (TT, TC, CC) at a respective frequency of 27.01%, 49.84%, 23.15% and 32.21%, 51.05%, 16.74% respectively in T2D and healthy individuals with respect to rs2853669. Both mutant allele C and genotype TC were associated with risk of T2D (OR = 1.26, 95%CI = 0.99, $\chi 2 = 3.68$ and $p = 0.05$ and OR = 1.65, 95%CI = 1.006, $\chi 2 = 3.96$, $p = 0.04$). However, though RTL values were shorter in different genotypes of T2D patients (with regard to rs2853669) compared to their healthy counterparts, the mean differences were not statistically significant. Correlation studies showed that RTLs measured in these two groups of participants were inversely related with their respective ages establishing the involvement of telomere lengths in aging process. With regard to MNS16A polymorphism, DNA bands having 243 or 272 bp and 302 or 333 bp were grouped into short and long alleles, respectively. Both

short alleles, 243 bp and 272 bp were found to be significantly associated (OR: 1.36, p=0.05 and 0.46, p=0.017, respectively) with T2D, when the long alleles (commonly found) were considered as reference. Seven genotypes (302/302, 302/243, 302/272, 243/243, 243/272, 302/333, 272/272) were identified in the present study. When 302/302 genotype (the commonest one) was considered as reference group, the heterozygous genotypes (243/302 and 272/302) were significantly associated with the risk of T2D (OR: 1.47, p= 0.05 and OR: 0.22, p < 0.001), respectively. Conclusion: This study is the first of its kind on Bangladeshi population demonstrates that type 2 diabetic patients have significantly shorter mean RTL irrespective of any rs2853669 genotypes.

Keyword: Relative Telomere Length, Type 2 diabetes, MNS16A, hTERT, Bangladeshi population

[OP09-3-08]

ROLE OF EXOSOME-LIKE VESICLES IN INFLAMMATION-INDUCED INHIBITION OF MYOGENESIS

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Inflammation in insulin-sensitive organs, including adipose tissue, skeletal muscle and liver plays key roles in the development of chronic metabolic diseases. White adipose tissue is not the only organ that develops macrophage infiltration in obesity, but skeletal muscle is also an important organ regulating whole body metabolism and exposed to inflammation in obesity. Skeletal muscle is the largest organ of the body in lean individuals, and skeletal muscle cells dynamically respond to physiological and/or pathological stimuli including inflammation, leading to either muscle atrophy or hypertrophy. The complicated differentiation processes of cells in skeletal muscle against inflammation that induce muscle atrophy are not fully elucidated. Given that skeletal muscle is a secretory organ, we evaluated the effects of inflammation on myogenic signals and myokine expression, and the roles of inflammatory exosomes released by myotubes in myogenic differentiation. Inflammation was induced by treatment of fully differentiated C2C12 myotubes with a cytokine mixture of TNF-α and INF-γ. Exosome-like vesicles (ELVs) were isolated from conditioned media of control or inflamed myotubes and incubated with myoblasts. The expression of molecular switches that contribute to myogenic differentiation, including several kinases, their downstream targets, and myokines, were evaluated using immunoblot analysis in inflamed myotubes and in myoblasts treated with ELVs. Inflammation activated molecular mechanisms contributing to muscle atrophy, including AMPK, p-38 MAPK and JNK, while inhibiting Akt-mediated myogenic signals. Treatment of differentiated C2C12 with a cytokine mixture increased the expression of MAFbx, concurrent with decrease of FOXO3a phosphorylation, while inflammation downregulated the expression of myogenic markers, MyoD and myogenin. In addition, inflammation induced myostatin expression with suppression of a myostatin-counteracting myokine, decorin. Well-characterized ELVs released from inflamed myotubes slightly induced myoblast inflammation and inhibited myogenic mechanisms (p-Akt/p-FOXO3a, p-P70S6K and decorin) while stimulating atrophic signals (MAFbx, p-JNK and p-raptor). Furthermore, the expression of myostatin and decorin in inflammatory ELVs were higher and lower than those in control ELVs, respectively. Inflammation of skeletal muscle has been implicated as a cause of the

metabolic dysfunctioin and muscle atrophy that occur as a result of aging and chronic diseases. In this study, we demonstrated that inflammation induced by treatment of cytokine mixture turned on the molecular switches of muscle atrophy, but suppressed myogenic signaling. More importantly, ELVs from myotubes with inflammation is likely to contribute to the suppression of myogenic signaling pathways. Further characterization of the molecular components of ELVs is warranted for the elucidation of the mechanisms underlying inflammation-induced muscle atrophy.

[OP09-3-09]

UBIQUITIN-LIKE PROTEIN MNSFβ IS DEAGGREGATED AND REGULATES CELL PROLIFERATION

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MNSFβ, a ubiquitin-like protein, covalently binds to various target proteins including proapoptotic Bcl-G. During the course of isolation of E1-like enzyme, we found a novel partner for MNSFβ. This protein was identified as the heat shock 70-kDa protein 8 (HSPA8). Molecular chaperone HSPA8 might promote the folding of the highly aggregable MNSFβ, leading to stabilization of MNSFβ. Indeed, knockdown of HSPA8 significantly reduced the expression of MNSFβ in macrophage cell line Raw264.7 cells. Conversely, overexpression of HSPA8 strongly enhanced the expression levels of MNSFβ. We next examined the effect of HSPA8 on MNSFβ deaggregation by using ProteoStat®, a protein agglutination detection reagent. Although MNSFβ formed insoluble aggregation in vitro, HSPA8 significantly inhibited the aggregation of MNSFβ. Because HSPA8 has been reported to migrate from cytoplasm to nucleus during stress, we investigated the intracellular localization of MNSFβ. MNSFβ-GFP fusion protein localizes in the nucleus in unstimulated Hela cells, yet located to the cytoplasm by actinomycin D, a transcription inhibitor. MNSFβ siRNA increased the level of p53 expression in Raw264.7 cells under nutrient starvation conditions. Collectively, MNSFβ regulates cell proliferation through nucleolus stress response.

[OP09-3-10]

ESTABLISHMENT OF A NEW ESCHERICHIA COLI STRAIN CONSTITUTIVELY EXPRESSING LAMBDA PHOSPHATASE, AND APPLICATION TO THE PREPARATION OF HIGHLY ACTIVE CASEIN KINASE 1

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The Escherichia coli expression system is very useful for large-scale preparation of recombinant proteins. However, protein kinases (PKs) are often autophosphorylated in E. coli cells. Because such "artificial" autophosphorylation may cause unexpected effects on biochemical properties of the PKs, using these kinase preparations may hamper accurate enzymatic characterization of them. Casein kinase 1 (CK1) is a widely expressed Ser/Thr kinase involved in various cellular processes (e.g., circadian rhythm and apoptosis). Since CK1 undergoes autoinactivation via autophosphorylation when it is expressed in E. coli cells, it is important to prevent the undesirable autophosphorylation of the recombinant CK1 in E. coli. To circumvent these problems, we established an expression system using E. coli strain BL21(DE3)pλPP in which lambda protein phosphatase (λPPase) is constitutively expressed. The expressed kinase was purified by affinity chromatography to remove λPPase together with other cellular proteins. First, we examined whether BL21(DE3)pλPP was effective for preparing various PKs as unphosphorylated forms. When Ca2+/calmodulin-dependent protein kinase I , dual specificity tyrosine phosphorylation-regulated kinase 1A, and CoPK02 (CaMK homolog in Coprinopsis cinerea) were expressed by conventional BL21(DE3) or BL21(DE3)pLysE, they migrated as smear bands on SDS-PAGE and were detected by anti-phosphothreonine or anti-phosphotyrosine antibodies. However, when they were expressed by BL21(DE3)pλPP newly established in this study, they were migrated as a clear single band that could not be detected by these antibodies at all. These data indicated that unphosphorylated forms of these PKs were readily prepared by the expression/purification system using E. coli strain BL21(DE3)pλPP. Next, we applied this system to the preparation of three CK1 isoforms, α, δ, and ε, which are known to be important in various cellular processes and have been well studied regarding the role of autophosphorylation. All the CK1 isoforms were readily prepared by the simple protocol as highly active forms, because they were almost completely dephosphorylated by the coexpressed λPPase in E. coli. Moreover, the C-terminally truncated forms, CK1δ(ΔC) and

CK1ε(ΔC), which are used as constitutively active forms of CK1, were prepared as more active forms than those prepared by the conventional BL21(DE3) cells. These results suggest that the use of CK1s and their truncated forms obtained from BL21(DE3)pλPP is suitable for the evaluation of the bona fide properties of CK1s in in vitro analyses. We also compared the kinase activity of CK1δ and CK1δ(ΔC) prepared using BL21(DE3)pλPP with the activity of those prepared by a conventional method in which the produced CK1s were dephosphorylated by λPPase in vitro. These experiments demonstrated that the former had higher specific activity than the latter, indicating that the simple method using BL21(DE3)pλPP is useful for preparation of highly active, unphosphorylated CK1s. In conclusion, the present findings clearly showed that the protein expression/purification method using BL21(DE3)pλPP is a simple and convenient method for preparing unphosphorylated recombinant CK1s with remarkably high specific activity. We believe that BL21(DE3)pλPP will be a generally applicable tool for the expression/purification of "naked" (unphosphorylated) PKs.

[OP09-3-11]

MECHANISTIC STUDIES OF CULLIN-RING E3 LIGASES

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The fate of thousands of protein substrates modified by ubiquitin (UB) depends on cascades of E1 activating, E2 conjugating, and E3 ligating enzymes. Specificity is mainly achieved by different types of E3 enzymes, RING, RBR, and HECT, and its mode of ubiquitin transfer from the E2 to its substrates. The dogma for RING E3 ligases – including the largest subfamily of cullin-RING E3s (CRLs) - was that UB ligation to substrates was exlusively catalyzed by an E2 enzyme. However, our lab recently discovered that many cellular CRLs containing the RING subunit RBX1 activate an RBR type E3, ARIH1, to efficiently mediate substrate ubiquitylation. In this study we address the function of a CRL containing RBX2-CUL5, and its interaction with ARIH2, another type of RBR E3 ligase. We show that ARIH2 is activated to efficiently modify a CUL5 substrate Creatine Kinase B (CKB). Overall, our data define ARIH2 as a component of the human RBX2-CUL5 system, demonstrate that ARIH2 can efficiently and specifically mediate ubiquitylation of a CRL substrate, and provides more evidence that two distinctive E3s can reciprocally control each other for simultaneous and joint regulation of substrate ubiquitylation.

Keyword: ubiquitin, E3 ligase, RING, RBR

[OP10-3-01]

EFFECT OF EDIBLE BIRD'S NEST EXTRACT ON CYTOKINES PRODUCTION IN HUMAN IMMUNITY

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Introduction: For centuries, edible bird's nest (EBN) has been regarded as an immune-enhancing food in traditional Chinese Medicine. However, the potential therapeutic values and the mechanism of actions of EBN in immunity remain largely unknown. Furthermore, there is no clinical evidence to substantially support those claims.

Objective: This study aims to evaluate whether aqueous EBN extract (EBNE) enhances cytokine production in healthy subjects using an in vitro MIMIC model.

Methods: Four healthy subjects were recruited and 60 ml of full blood were collected from them. An in vitro model, named Modular Immune in vitro Construct (MIMIC) was used to simulate the human immune system. In this model, monocytes transendothelial migration through and reverse an endothelial layer was observed under the treatment of EBNE. The innate and adaptive immune responses were evaluated using Peripheral Tissue Equivalent (PTE) and Lymphoid Tissue Equivalent (LTE) modules, respectively, where monocytes were differentiated into dendritic cells with the presence of lipopolysaccharide (LPS), followed by co-culture of the differentiated cells with T and B cells to permit cytokines production. Finally, the cytokines profile was identified by the semi-quantitative human cytokine array G2000.

Results: From the results obtained, 46.21% of monocytes successfully transmigrated reversely and differentiated into dendritic cells, as shown by microscopy and flow cytometry analysis. Differentiated cells displayed dendritic-like morphology through microscopic observation and expression of CD83 and CD86. A wide range of elevated cytokines was observed in EBNE-treated co-cultured immune cells when compared to non-EBNE-treated co-cultured immune cells. Elevation of cytokines included brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor (GDNF) and insulin-like growth factor 1

(IGF-1). These findings suggest that EBNE could support neuronal survival, as well as promote the growth and differentiation of neurons and synapses in the brain through immunity enhancement. EBNE was also found to induce the production of interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10) and interleukin-13 (IL-13). These interleukins are important mediators of immune signaling, which enhance immune cells proliferation and antibodies production during infection.

Conclusion: This study demonstrated for the first time the effects of EBNE on the production of cytokines in MIMIC model. Pro-neurogenic cytokines and antiinflammatory cytokines were found to be elevated in EBNE treated immune cells. With these, it provides a new understanding of the effect of edible bird's nest in improving human immunity, and hence it is crucial to further elucidate the mechanisms of actions of EBN in the future.

Keyword: edible bird's nests extract (EBNE), Modular Immune in vitro Construct (MIMIC), cytokines, pro-neurogenic, anti-inflammatory

[OP10-3-02]

RATIONAL DESIGN OF CYCLIC PROHIBITIN PEPTIDE MIMETICS COULD REDUCE THE EXPANSION OF ADIPOCYTES WITH POTENTIAL FOR TARGETING OBESITY

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Obesity is a major health problem worldwide and the strongest risk factor for diabetes, the fifth leading risk for global deaths. Available anti-obesity therapies have several limitations and inferior results when compared with infectious diseases. The aim of this study is to develop a new generation of drug to treat obesity based on cyclic peptide scaffolds that have increased effectiveness and reduced side effects. Here we report the discovery of a series of cyclic peptide-based therapeutics that target prohibitins with great potential for reducing body weight and diabetic complications. We evaluate a series of multi-functional anti-obesity peptide by combining an epitope that facilitates homing to the surface of white adipose vasculature and an epitope that could assist delivery to adipocytes within a cyclic cell penetrating framework, MCoTI-II (Momordica cochinchinensis trypsin inhibitor-II). This study provides an insight into the use of cyclic disulfide-rich frameworks for developing multi-functional anti-obesity peptide therapeutics with improved potency and low toxicity. We demonstrate that this novel approach has resulted in a potent multi-functional cyclic analogue that could regulate the adipogenesis process by reducing overcrowded adipocytes expansion. This is the first report suggesting the MCoTI-II framework is a promising framework for developing multi-functional anti-obesity peptide and highlights its potential for the treatment of metabolic disorders for treating obesity.

Keyword: cyclic peptide, stable, non-toxic, adipocytes, obesity

[OP10-3-03]

METABOLIC ALTERATIONS IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by impaired cognition and memory loss. Even though AD is primarily a brain disorder, recent evidence has emerged to suggest that AD may be a metabolic neurodegenerative disease, due to the prominent mitochondria failure, oxidative stress and metabolic derangement observed in AD. Here, we aim to investigate the role of metabolic alterations in AD pathology. Transgenic Caenorhabditis elegans (C. elegans) with pan-neuronal expression of human amyloid-beta (Ab) was employed to study the sequence of events underlying metabolic alterations in AD. We observed widespread mitochondrial failure in the form of low ATP levels, reduced respiratory capacity and electron transfer chain complexes dysfunction in the Ab-expressing nematodes. Metabolomics analyses revealed that amino acids, acylcarnitines and krebs cycle intermediates involved in energy production are found at significantly lower levels in Ab-expressing nematodes compared to the controls. Lipidomics analyses further demonstrated a reduction in polyunsaturated fatty acids in Ab-expressing nematodes compared to the controls, which may have be preferentially oxidized by reactive oxygen species (ROS). We also observed profound ROS-mediated damages in the Ab-expressing nematodes, including higher levels of mitochondrial protein carbonyls, suggestive of the occurrence of oxidative stress in AD. These observations are in line with AD being a metabolic neurodegenerative disease, and motivate treatments targeting these pathways for AD.

Keyword: mitochondria failure, oxidative stress, lipidomics, alzheimer disease, metabolic neurodegenerative disease

[OP10-3-04]

CHRONIC EXPOSURE OF ARSENIC SUPPRESSES ADIPOGENESIS, MITOCHONDRIAL RESPIRATION AND THERMOGENESIS IN BROWN ADIPOSE TISSUE

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Arsenic is an element that occurs naturally in the environment. Humans are likely to be exposed to higher amount of arsenic through arsenic contaminated drinking water and consuming foods including fruits and vegetables, and the arsenic can accumulate in the human body at high concentrations. Previous research has shown that arsenic induces oxidative stress linked to metabolic diseases such as obesity and diabetes. In mice, arsenic exposure during embryonic development is linked to an early puberty and adult obesity. Recent findings of brown adipocytes, capable of dissipating energy as heat, in adult humans have promised new hope for obesity treatment and prevention. Therefore, understanding the pathophysiological role of brown adipocytes and uncoupling protein 1 (UCP1) regulation in thermogenesis can provide effective strategies against obesity. Here, we investigated the effects of arsenic on differentiation, mitochondrial respiration and thermogenesis in immortalized murine brown adipocytes. We observed that arsenic significantly reduced brown adipocyte differentiation in dose dependent manner. Arsenic suppressed mitochondrial respiration and biogenesis, leading to attenuated brown adipocytes specific thermogenesis functions including UCP1 in immortalized murine brown adipocytes. In mice studies, we confirmed the heavy arsenic accumulation in brown adipose tissue (BAT) and the suppression of gene expression levels of mitochondria specific markers after the oral administration of arsenic into mice at dose of 10 mg/kg/day for 9 days. Arsenic significantly suppressed the BAT thermogenesis marker UCP1 when exposed to cold temperature for 24 hr at the last day of oral gavage. These results reveal, for the first time, the underlying mechanism of how arsenic aggravates metabolic diseases such as obesity by suppressing brown adipocyte differentiation, mitochondria function and thermogenesis, which can be utilized for development of arsenic-specific inhibitor drugs protecting essential physiological functions of BAT in humans.

Keyword: Brown adipose tissue, Arsenic, differentiation, mitochondria, thermogenesis

[OP10-3-05]

THERAPEUTIC STRATEGY TARGETING B-CATENIN AND RAS FOR SUPPRESSING METASTASIS OF COLORECTAL CANCER

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APC (80-90%) and K-Ras (40-50%) mutations frequently occur in human colorectal cancer (CRC) and these mutations cooperatively promote tumorigenesis in CRC, especially in the development of metastasis. In addition, both β-catenin and Ras levels are highly increased in CRC, especially in metastatic CRC (mCRC). Therefore, targeting both the Wnt/β-catenin and Ras pathways could be an ideal therapeutic approach for treating mCRC patients. In this study, we identified and characterized small molecules that destabilize both β-catenin and Ras via targeting the Wnt/β-catenin pathway, these compounds effectively inhibited cellular events which occur in CRC such as abnormal proliferation, evading apoptosis, and especially epithelial-mesenchymal transition, an initial process of metastasis in vitro and in vivo studies using APCMin/+/K-RasG12DLA2 mice. A small molecular approach degrading both b-catenin and Ras via inhibition of the Wnt/β-catenin signaling would be an ideal strategy for suppressing the development of mCRC at an early stage of tumorigenesis.

Keyword: CRC tumorigenesis, APC mutation, K-Ras mutation, Tumor budding, EMT

[OP10-3-06]

NOVEL THERAPEUTIC STRATEGIES TARGETING THE UNIQUE PROPERTIES OF NEURONS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)/MOTOR NEURON DISEASE (MND)

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ALS/MND is a rapidly progressive, fatal neurodegenerative disease characterised by the death of upper and lower motor neurons. There is no effective treatment for ALS/MND, hence there is an urgent need to discover new therapeutics that can prevent motor neuron death. This is most likely to happen by targeting early changes in the disease. One common and early pathology shared amongst the diverse forms of ALS/MND is disruption of protein trafficking within neurons and protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus is the precursor of almost all trafficking pathways in the cell. ALS-associated mutant forms of Superoxide dismutase (SOD1), Tar-DNA binding protein-43 (TDP-43) and Fused in Sarcoma (FUS) inhibit protein transport between the ER and Golgi apparatus in neuronal cells and lead to ER stress. Therefore, restoring ER to Golgi transport may be an effective therapeutic target. Rab GTPases play a pivotal role in mediating intracellular membrane trafficking events and Rab1 specifically regulates many aspects of ER–Golgi transport. We previously demonstrated that Rab1 can rescue the inhibition of ER–Golgi transport in cells overexpressing SOD1, TDP-43 and FUS. In this study, we investigated the effect of Rab1 in ALS/MND zebrafish and cellular models. Mutant SOD1 (A4V mutation) expression in zebrafish induces motor axonopathy and impairment of motor function, however Rab1 can rescue these defects. Our second aim was to identify whether novel Rab1-mimetic compounds are protective in ALS/MND. We have also demonstrated that these compounds are protective in these zebrafish models and also in neuronal cells expressing mutant SOD1, TDP-43 or FUS. These findings therefore imply that restoring Rab1-mediated ER–Golgi transport is a novel therapeutic target in ALS/MND and this may be effective in multiple forms of ALS/MND.

Keyword: Amyotrophic Lateral Sclerosis, Motor Neuron Disease, Therapy, protein transport

[OP10-3-07]

CERIUM IV OXIDE NANOPARTICLES REDUCE PLATELET AGGREGATION THROUGH SUPPRESSION OF CELLULAR ROS PRODUCTION AND VEGF SECRETION

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Background. Thrombotic disease is extremely harmful to human health, and early detection and treatment can improve the prognosis and reduce mortality. Platelet overactivation, resulting in excessive reactive oxygen species (ROS) production, hypercoagulation and growth factor secretion, plays a crucial role in thrombotic events and abnormal angiogenesis in cancer patients. Cerium (IV) oxide nanoparticles (CONPs) exhibit various regulatory effects on molecular processes and cellular functioning, however their effects on the platelet activity remain unexplored. The aim of our research was to study the effects of CONPs on the platelet aggregation activity, ROS production intensity and vascular endothelial growth factor (VEGF) secretion. Methods. Citrate-stabilized CONPs (2-4 nm) were designed, synthesized and used for the experiment. Native washed platelets were isolated used stage-by-stage centrifugation from a blood of three normal drug-free healthy volunteers. Platelets were incubated with CONPs (0.5 or 5 μM) before activation by ADP or thrombin (5 μM and 1 NIH/ml respectively). Aggregometry was monitored during the first three hours after blood sampling by Solar AT-02 light transmitting aggregometer. Intracellular ROS generation was evaluated with the use of DCF fluorescence. VEGF levels in platelet releasates were assessed by WB. Results. Incubation with CONPs 0.5 μM reduced both thrombin- and ADP-stimulated platelet aggregation approximately by 23%, while using of CONPs 5 μM inhibited aggregating effects of agonists by 53% on average. Such an antagonist-induced "mitigated" reduction of platelet aggregation activity suggests a low cytotoxicity of the used cerium oxide nanoparticles, compared with oxides of other elements. CONP-induced suppression of agonist-stimulated platelet aggregation was correlated with 27% inhibition of intraplatelet ROS production, which are known to be the secondary messengers of platelet signaling pathways. Further, CONPs 0.5 μM decreased the level of 40 kDa VEGF secretion by thrombin- and ADP-stimulated platelets by 52 and 27%, respectively, while CONPs 5 μM diminished VEGF release by 53% and 42%, respectively. Besides, a similar decline of 20 kDa VEGF level in platelet releasates (in compare with 40 kDa VEGF secretion) under the action of both concentrations of CONPs was observed.

Conclusion. Overall, CONPs are shown for the first time to possess anti-platelet activities, based on their ability to reduce ROS formation, resulting in platelet secretion and aggregation inhibition. Thus, our results consider that CONPs may be proposed for the treatment of various pathologies associated with hypercoagulation and angiogenesis disturbances.

Keyword: Cerium Oxide Nanoparticules, Platalets, Aggregation, ROS, VEGF

[OP10-3-08]

α-GLUCOSIDASE INHIBITORY ACTIVITY OF ROASTED COFFEA EXCELSA BEAN

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Alpha-glucosidase hydrolyzes linear and branched oligosaccharides to release glucose resulting to postprandial hyperglycemia (PPHG). Prolonged PPHG is one risk factor for the development of chronic macrovascular diseases. Reducing the rate of carbohydrate digestion by inhibition of α-glucosidase can aid in maintaining blood glucose homeostasis. Coffee, a commonly consumed beverage, has demonstrated significant reduction on risk of type 2 DM through carbohydrate digestion enzyme inhibition. The present study determined the α-glucosidase inhibitory activity of roasted Coffea excelsa bean. Methanol extract of C. excelsa inhibited

Keyword: Alpha-glucosidase, Coffea excelsa, uncompetitive inhibition, total phenolic content, total flavonoid content

[OP10-3-09]

CAMBI, AN ORANGE CALCIUM-MODULATED BIOLUMINESCENT INDICATOR FOR NON-INVASIVE ACTIVITY IMAGING

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Genetically-encoded calcium indicators are useful for detecting calcium influx into electrically excitable cells such as neurons and myocytes, but fluorescence-based indicators are not compatible with non-invasive imaging in larger animals. Here, we describe Orange CaMBI, a new bioluminescent calcium indicator based on the bright orange-emitting luminescent protein Antares. Orange CaMBI features larger calcium responsivity and greater emission of orange-red photons than previously engineered bioluminescent calcium indicators. The combination of high emission and high responsivity in Orange CaMBI enables imaging of calcium transients in single cardiomyocytes and single neurons in vitro by bioluminescent microscopy. With its peak orange-red emission, Orange CaMBI is able to visualize, for the first time, spontaneous calcium influx in muscles in living mice using non-invasive whole-animal imaging. Orange CaMBI is a promising reporter for non-invasive calcium imaging in living mice, and may be especially useful for non-invasive imaging of activity in genetically defined neuronal populations in the brain.

Keyword: Fluorescent proteins, bioluminescence, BRET, calcium, neurons

[OP11-1-01]

BIOINFORMATICS AND SYSTEMS BIOLOGY IN AGING RESEARCH

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Abstract In an "aging society," health duration allowance is widely important. As in 2017, talks in this sequence of conventions in indian¨ nde established that mature is an speciously very composite method, where computational work is furthermost useful for attainment insights and to find interventions that counter aging and prevent or counteract aging-related diseases. The specific topics of this year's meeting entitled, "Symposium on Systems Biology and Bioinformatics in Ageing Research," were primarily related to "Aging and Cancer" and also had a aim on work supported by the Indian Ministry of Education and Research (IMER). The subsequent conference in the sequence, scheduled for October 20–21, 2017, will aim on the use of ontologies for computational investigation into aging, cancer and stem cells. Promoting awareness validation is also at the essential of the set of suggested exploit matters concluding this explosion.

Keyword: Aging, Systems Biology, Bioinformatics, Counteract Aging, ontologies

[OP11-1-02]

EFFECTS OF LONG-TERM HYPERGLYCEMIC-INDUCED CULTURE ON PROLIFERATION AND DIFFERENTIATION OF BONE-DERIVED MESENCHYMAL STEM CELLS

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Prolongation of hyperglycaemic levels in diabetes act as an independent risk factor for short- and long-term deterioration in the progression of microvascular damage in the affected organs. Mesenchymal stem cells (MSCs) have been isolated from various adult tissues and organs and demonstrate the ability to differentiate into multiple mesodermal cell types; such as osteoblasts, adipocytes, chondrocytes, hepatocytes, and neuronal cells. In spite of recent advance in using MSCs as key components in cell-based therapy, treating degenerative disorders, and accelerating repair of tissue injury; studies providing an exhaustive model of glucose-induced toxicity in 2D culture are still lacking. The actual mechanism by which high glucose levels associated with alteration of MSC behaviour in both in vivo and in vitro systems are still unclear. Precisely, the most strategic way to improve MSC-based therapies for skeletal repair with diabetes complications must first embrace a deeper insight into the influence of glucose on MSCs, even prior to their clinical administration. Therefore, this study aims to gain further understanding of bone progenitor cells proliferation and survival, in response to hyperglycemia. It will further elucidate if the high glucose environment significantly promotes alterations in cellular behaviour, which relates to the cells differentiation potential. MSCs were freshly isolated from rats bone marrow and compact bone, and further expanded in culture medium containing αMEM with 20% heat-inactivated FBS, 1% antibiotic-antimycotic and 100μm L-ascorbic acid 2-phosphate with supplementation of each 5.5mM and 25.0mM glucose concentration. Cells were incubated at 37°C, 5% CO2, at the density of 10,000 cells/cm2. MSCs taken from 50 population doublings (PD) of each cell group were further selected for osteogenic and adipogenic induction. Gene expression profiles of MSCs exposed in-vitro to hyperglycemic conditioned medium from either bone marrow cells or compact bone cells were compared. Alizarin Red S staining and immunocytochemistry using LipidTOX were also conducted to assess the differentiation potential of MSCs into osteogenic and adipogenic lineages. Long-term hyperglycemic culture, followed by induction in

25.0mM osteogenic medium seems to suggest that high glucose environment has a profound effect on increasing the expression of osteogenic markers. The qPCR analysis demonstrated that high glucose adipogenic medium significantly induced an overall increase in expression of adipogenic markers, FABP4, C/EBPα and LPL, with adiponectin exhibit the most prominent increase in gene expression within a high glucose-induced environment. In fact, the long-term hyperglycemic-induced cell culture protocol has significantly increased adipogenesis gene expression when exposed to the adipogenic medium. Overall, the information gained from this study assist in a better understanding of cellular and biological functions of MSCs within the hyperglycemic-induced environment, for potential application in disease models and regenerative therapies. Further understanding on the effects of glucose on MSCs does not only assist in identifying and clarifying the pathophysiology of hyperglycaemia but more importantly, it provides an alternative approach in minimising the impact that glucose has on the metabolism and the therapeutic potential of MSC populations.

Keyword: stem cells, hyperglycemia, adipogenic, osteogenic, proliferation

[OP11-1-03]

THE CISPLATIN RESISTANT MECHANISM THROUGH SIRT1 DOWNREGULATION IN NON-SMALL CELL LUNG CANCER

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Lung cancer is the leading cause of cancer-related mortality. Cisplatin is the firstline chemotherapeutic agent to treat NSCLC(non-small cell lung cancer) as well as various malignant tumors. The efficacy limitation of chemotherapy is the acquired resistance. Chemoresistance to cisplatin is affected by genetic or epigenetic changes during prolonged cisplatin treatment. However, the mechanisms promoting or enabling drug resistance is unknown. The cisplatin resistance cells of NSCLC exhibit an mesenchymal like cell character. And we identify that N-cadherin, vimentin of mesenchymal marker increased and E-cadherin of epithelial markers decreased in A549/DDP(cisplatin resistant cell). And our results showed that depletion of SIRT1 contributes to inhibit apoptosis signal, caspase3, Parp, Bax. This result suggested SIRT1 expression may be necessary to inhibit EMT and anti-apoptosis. In the recent study, low SIRT1 expression levels were associated with poor prognostic lung cancer patients. When 1week cisplatin treated at chemoresistant A549, SIRT1, NOX4, Cleaved Parp are downregulated and cell cycle factor, p53, p21, Bcl-2, pro-survival factor AKT are upregulated. In addition to, cisplatin treatment induced G1 arrest at chemoresistant A549. And we investigated about p53 ubiquitination inhibition or synthesis induced by SIRT1 and SIRT1 downregulation mechanism at cisplatin treated cisplatin-resistant A549. Protein and mRNA levels were investigated using western blot, RT-PCR. The cisplatin cytotoxicity was also evaluated using MTT assay in A549 and A549/DDP cells. And proliferation of A549 and A549/DDP cells were measured by ECIS (Electric Cell-substrate Impedance Sensing). And cell cycle distribution of A549 and A549/DDP cells were analysed using FACS in response to cisplatin treatment for 24hours and 1week. In conclusion, we assumed that SIRT may induce the pro-apoptotic effect by deacetylating transcription factor in A549 and SIRT1 downregulation induced cell cycle arrest in A549/DDP. Further studies are needed to validate NOX signals. Those results indicate that SIRT1 and NOX4 has great potential with drug resistant cancer treatment. And represent a novel therapeutic target to inhibit drug resistance.

Keyword: cisplatin, SIRT1, resistance, NSCLC, A549

[OP11-1-04]

CERULOPLASMIN REGULATION OF OXIDATIVE STATUS AND APOPTOSIS IN HUMAN LEUKOCYTES

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Polymorphonuclear leukocytes (PMNLs, neutrophils) play a major role in the initiation and resolution of the inflammatory response. Human ceruloplasmin (CP, ferro:O2-oxidoreductase) is abounding in plasma glycosylated multi-copper ferroxidase that is synthesized primarily in the liver. Its physiological role is not limited to copper deposition and transport. Being the positive acute-phase protein, ceruloplasmin plays a significant role in the development of immune responses. Previously we reported that CP inhibits 5-lipoxygenase and a number of serine proteases of leukocyte origin related to inflammatory and septic processes. This work is a comparative study of the in vitro effects of ceruloplasmin and its derivatives, both physiologic and synthetic, on neutrophil oxidative status and lifespan. Holo-ceruloplasmin, its demetallized (apo-) and partially proteolyzed forms, and synthetic free peptides RPYLKVFNPR (883–892) and RRPYLKVFNPRR (882–893) were investigated. CP was isolated from heparin-stabilized plasma of healthy donors. Proteolyzed CP (CPprot) was obtained after limited proteolysis with human thrombin. Apo-CP was prepared by treating the holo enzyme with copper-chelating agents. It was shown that in contrast to other CP derivatives investigated, the intact enzyme and the product of its partial hydrolysis have a pronounced effect on PMNL oxidative status. Our results indicate, that being effective superoxide radical scavenger, ceruloplasmin induces an immediate sharp increase in other intracellular oxidants, at least in experimental conditions. The ability of CP to exhibit both anti-oxidant and pro-oxidant properties persists after its partial proteolysis. Indeed, we observed a significant increase in the fluorescence emission of the DCF oxidation product when CP or CPprot were added to PMNLs. At the same time, the synthetic peptides weakly inhibited the generation of intracellular oxidants. Contrary to the unidirectional effect on PMNL oxidant status, intact and partially proteolyzed CP acted differently on delayed apoptosis. The inhibition of apoptosis we observed in the presence of CP occurred whether PMNLs were exposed to TNF-α or not. The mode of apoptosis regulation by CPprot, as well as other products of enzyme hydrolysis, switches from inhibition to activation in the presence of TNF-α. The mechanisms of superoxide elimination by CP are not completely clear, but could be linked to the oxidative activity of copper atoms, even

it was shown by us that inorganic copper, when added to the cells in relevant concentrations, did not exert the same influence on PMNL oxidative or apoptotic status. TNF-α plays a critical role in modulating acute and chronic inflammation, largely due to its ability to stimulate myeloperoxidase (MPO) synthesis and secretion. CP and its derivatives differ in their ability to inhibit MPO peroxidase activity. It was found that partial proteolysis of CP dramatically reduces the inhibitory function of MPO on CP without disturbing its ferroxidase activity. CPprot cannot inhibit the peroxidase activity of MPO, but retains ferroxidase activity. Hereby we presented a potential mechanism associated with the change of oxidative status and activation of apoptosis in human neutrophils by CP and its derivatives.

Keyword: neutrophil, ceruloplasmin, apoptosis, oxidative status, inflammation

[OP11-1-05]

THE ROLE OF PDCD5 IN ENDOTHELIAL HOMEOSTASIS

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The endothelium has a crucial role in vascular homeostasis. The role of programmed cell death 5 (PDCD5) in the endothelium has not been revealed. We evaluated the role of PDCD5 in the endothelium and atherosclerosis progression. We conducted a study using a partial carotid ligation model of endothelial-specific PDCD5 knockout (cKO) mice. Also, we examined signal transduction for PDCD5 in human umbilical vein endothelial cells (HUVECs). Then, we investigated correlations between serum PDCD5 and various markers of atherosclerosis in samples taken from the Cardiovascular and Metabolic Disease Etiology Research Center - HIgh Risk Cohort (CMERC-HI) Trial. PDCD5cKO mice showed significantly reduced vascular remodeling compared with wild-type mice after partial carotid ligation. Wild-type PDCD5 competitively inhibited interaction between HDAC3 and AKT, but PDCD5L6R, an HDAC3 binding-deficient mutant, did not. SiRNA knockdown of PDCD5 accelerated AKT and eNOS phosphorylation and nitric oxide (NO) production in HUVECs. In ex vivo mouse aortic ring angiogenesis assays, PDCD5cKO mice had significantly increased numbers of microvessel segments compared with wild-type mice, and microvessel sprouting was not inhibited by a monoclonal antibody against vascular endothelial growth factor. Finally, we found that serum PDCD5 levels reflect endothelial NO production, and are correlated with diabetes mellitus, high density lipoprotein cholesterol, and coronary calcium in human participants. Endogenous PDCD5 negatively regulates the HDAC3-AKT signaling and NO production in endothelium. Serum PDCD5 not only reflects atherosclerosis progression, but itself leads to endothelial dysfunction. Therefore, we conclude that PDCD5 is associated with endothelial dysfunction and may be a novel therapeutic target in atherosclerosis.

Keyword: Atherosclerosis, Endothelium, PDCD5

[OP11-1-06]

HISTONE ARGININE METHYLATION AND TRANSCRIPTIONALLY ACTIVE CHROMATIN

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Chicken has long been recognized as a model system to study the organization and function of a vertebrate genome. Its genome is almost three times smaller than the human genome, but has about the same number of genes, with 60% of them having a single human ortholog. Moreover, there are long blocks of conserved synteny between the chicken and human genomes. In terms of chromosomal organization of genes, the human genome is closer to the chicken than to rodents. Additionally, following 310 million years of separate evolution, conserved noncoding sequences are likely to highlight functional elements in both chicken and human genomes. We recently reported the genome-wide profiles of chromatin signatures (H3K4me3, H3K27ac, salt-soluble domains) in relation to expression levels in chicken polychromatic erythrocytes (PMID: 27226810). In the current study, we will show the genomic distribution of the histone modifying enzymes protein arginine methyltransferases 1 and 5 (PRMT1, PRMT5) and their products H4R3me2a (asymmetric) and H3R2me2s (symmetric), respectively. Further we mapped the open nucleosome-depleted regions using FAIRE-sequencing. We will present our novel findings which show the genomic distribution of the PRMT enzymes and their substrates in relation to other active histone marks and the organization of the transcriptionally active gene domains in the chicken polychromatic erythrocyte. This work was supported by funds from NSERC and a Canada Research Chair to J Davie.

Keyword: epigenetics, histone arginine methylation, gene expression, chicken, erythrocytes

[OP11-1-07]

DETERMINATION OF MITOCHONDRIAL DNA VARIANTS C5178A AND G10398A IN BANGLADESHI TYPE 2 DIABETIC INDIVIDUALS

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Type 2 diabetes (T2D), a metabolic disorder characterized by hyperglycemia due to insulin resistance or insufficient secretion of insulin by the pancreatic beta cells, is the major health concern all over the world. Mitochondrion is involved in energy metabolism by playing a pivotal role in glucose metabolism and insulin secretion. The mitochondrial DNA (mtDNA) G10398A variation (Ala→Thr) within the NADH dehydrogenase (ND3) subunit of complex I of the electron transport chain and C5178A within NADH dehydrogenase subunit (ND1-237, Leu→Met), have emerged as variations of clinical significance in disorders like T2D. This study aims to explore the relationship of mtDNA C5178A and G10398A variations with T2D in Bangladeshi population. A total of 249 unrelated Bangladeshi populations (127 T2D and 122 healthy controls) were enrolled in this study. Specific DNA sequences within mitochondria were amplified by PCR followed by digestion at the polymorphic sites using AluI and DdeI restriction enzymes. It was found that 66.93% T2D and 59.02% healthy individuals carried the major allele 'G' at 10398 position of the mtGenome; while the minor allele 'A' was present in 33.07% T2D and 40.98% healthy individuals. G10398A polymorphism had no association with the T2D when total participants were considered. However, in male study participants, G10398A polymorphism showed significant protective effect against T2D (OR = 0.30, 95% CI: 0.13-0.67, p

Keyword: Mitochondia, Variant, T2D, SNP, As