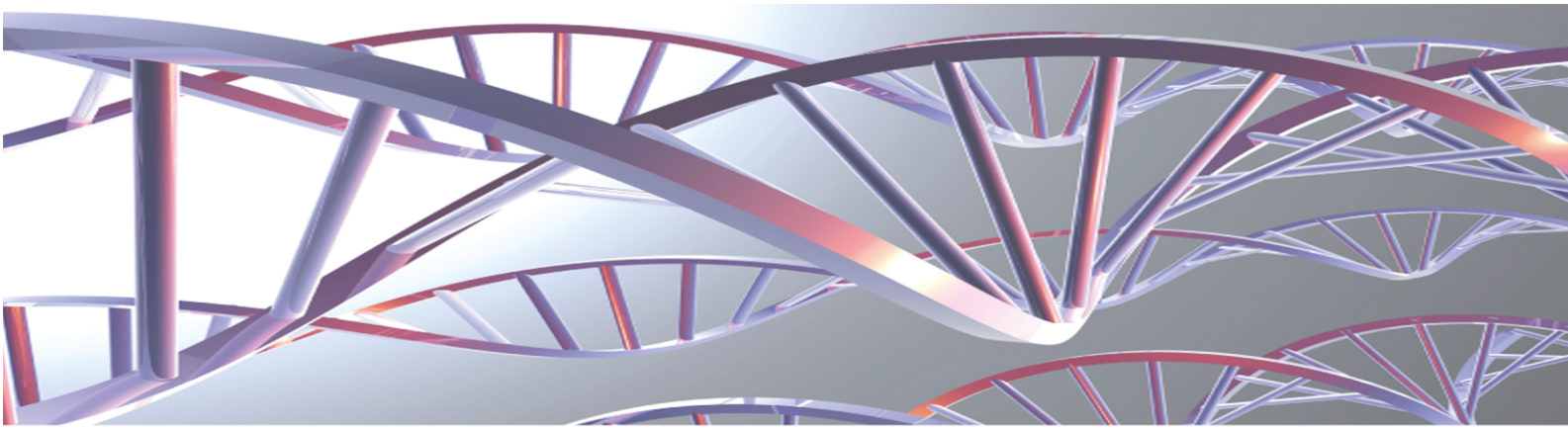

2014 HONG KONG INTER-UNIVERISTY BIOCHEMISTRY POSTGRADUATE SYMPOSIUM

June 14,2014

Jointly organized by:
The University of Hong Kong
The Chinese Univeristy of Hong Kong
The Hong Kong University of Science and Technology

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	Role of nuclear lamins in the regulation of SIRT6 **Ms. Shrestha Ghosh (HKU)**
	Roles of progenitor cells for intervertebral disc regeneration in "healer" mice **Ms. Ying Zhang (HKU)**
	Dust mite and allergy: Multi-omic approach reveals a broad spectrum of allergens **Mr. Yim Aldrin Kay Yuen (CUHK)**
	Use of a tunable resistive pulse sensing system to detect drug response from the mitochondria at organelle level **Mr. Loo Fong Chuen, Jacky (CUHK)**
10:35 – 10:50	**Tea break**
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	Combinations of quasi-independent binding sites underlie diversity of membrane target recognition by ankyrins **Mr. Chao Wang (HKUST)**
	How vacuolar sorting receptor (VSR) protein interact with their cargo protein - Crystal structures of apo and ligand-bound forms of the protease-associated domain from an Arabidopsis VSR **Ms. Luo Fang (CUHK)**
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	Loss of LIM-homeodomain genes, Lhx1 and Lhx5, disrupts dendritic spine morphogenesis of Purkinje cells and causes ataxia in mouse **Ms. Lui Nga Chu (CUHK)**
	The dynamic process of microglial colonization of the developing zebrafish brain **Ms. Tienan Wang (HKUST)**
	Regulation and functions of liprinα1 in activity-dependent synapse development **Ms. Huiqian Huang (HKUST)**
17:35 – 18:00	**Closing remarks and award presentation** Professor Danny Chan (HKU) Dr. Shannon Au (CUHK) Dr. Ho Yi Mak (HKUST)

Keynote sessions

Professor Daniel Finley *Harvard Medical School*

Dr. Finley's laboratory published groundbreaking studies into the intricate workings of proteasome-dependent protein degradation, with a focus on the proteasome itself. The laboratory employed genetic and biochemical approaches to gain insights into the proteasome's 35-plus component structure, the assembly, and the mechanics (both enzymatic and non-enzymatic) of this biological machine, which is critical for cellular homeostasis. These recent studies have capitalized on yeast genetics to facilitate the interrogation of proteasome activities and the cellular mechanisms that integrate the proteasome activities into the cellular metabolic equilibrium. The lab's studies on the deubiquitinating enzyme Ubp6 (the human ortholog is Usp14) uncovered multiple activities for this tightly associated component of the proteasome. Elegant studies demonstrated that Ubp6 is adept at stalling the catabolism of ubiquitinated substrates, thus curbing proteasome activity. This activity does not involve its deubiquitinating enzymatic activity, demonstrating that Ubp6 possesses dual functions that work together to counterbalance protein degradation. The laboratory also found that Ubp6 is important for cementing the Hul5 ubiquitin ligase to the proteasome complex. Other experiments have shown that the architectural integrity of the proteasome, consisting of a regulatory and core particle, is preserved by specific molecules, as well as the process of protein degradation itself. The laboratory has also investigated proteasome activity in cells undergoing a stress response. The lab found that effective regulatory constraints are operable for maintaining proteasome levels and perturbations in ubiquitin levels, which trigger a stress response, lead to an altered composition of proteasomal subunits rather than increases in the number of proteasome complexes, as is observed following proteasome stress.

Professor Jiahuai Han *Xiamen University*

Dr. Jiahuai Han is known for the discovery of the p38 signaling pathway, one of the most important pathways in intracellular signaling transduction. This pathway plays important roles in many biological processes including cell cycle regulation, cell proliferation, cell differentiation and senescence, as well as immune reactions, development and tumorigenesis. Another current focus of Han`s lab is molecular mechanisms of RIP3 dependent necrosis, which is based on a recent finding in this laboratory revealing that RIP3 is a major player in controlling programmed necrosis. The research in Han's Lab will lead to a better understanding of the molecular mechanisms of inflammation, cardiovascular disease and tumorigenesis, and thus provides new ideas for the development of therapeutic intervention for these diseases.

His research interests are the molecular mechanisms in cellular stress responses including inflammation and inflammation related diseases. Specifically interested in:

- The relationship between cellular stress responses and cell differentiation, senescence and death.
- The relationship between cellular stress responses and malignancy.
- The relationship between cellular stress responses and metabolic pathways.
- The functions of cellular stress response pathways in septic shock, atherosclerosis, colitis and other inflammation related disease.

Student oral presentation

Session 1

Role of nuclear lamins in the regulation of SIRT6

Shrestha Ghosh (HKU)
(Supervisor: Dr. Zhongjun Zhou, HKU)

Sirtuins are a family of proteins which have been involved in a multitude of biological functions, especially longevity expansion. SIRT6 is a mammalian sirtuin with emerging functions in a spectrum of cellular events, such as cellular senescence, premature aging, DNA damage response, metabolism, extending lifespan and also tumor suppression. Although SIRT6 has been established to regulate a range of cellular functioning, its upstream regulation has remained elusive till date. Given its crucial roles in so many vital processes, identification of its endogenous activators can serve as effective therapeutics. On the other hand, lamin A is a nuclear lamina protein involved in the maintenance of nuclear structure, gene regulation and several other important functions. A point mutation in LMNA gene (encoding lamin A) results in progerin (the mutant isoform of lamin A containing 50 amino acids deletion at the C-terminus), which causes Hutchinson-Gilford Progeria syndrome (HGPS), a severe early onset premature aging syndrome. In my study, I have identified lamin A as the endogenous activator of SIRT6 towards histone deacetylation and also DNA double-strand break repair. Intriguingly, progerin exhibited impaired activation towards SIRT6 functioning. To understand the biological significance of this observation, we investigated SIRT6 functioning in fibroblasts derived from HGPS patients. Interestingly, HGPS fibroblasts also displayed impairment in SIRT6-mediated DNA damage repair process, thus indicating that impaired SIRT6 functioning is another defective mechanism resulting in progeria.

Dust Mite & Allergy: Multi-omic Approach Reveals a Broad Spectrum of Allergens

Yim Aldrin Kay Yuen (CUHK)
(Supervisor: Professor TF Chan, CUHK)

House dust mite (HDM) is the most pervasive inhalant allergen source worldwide, with over 50% of allergic disease cases being attributed to them. Allergens derived from HDM are associated with sensitization and asthma, and it affects people of all ages. It is estimated by the World Health Organisation that 300 million individuals were affected with asthma in 2011, and approximately 250,000 people die prematurely each year from asthma. It is therefore important to have a comprehensive study on HDM to advance our understanding of HDM allergens. In 1920s and 30s, it was thought that HDM contained only a single allergen; it was until 1969, Voorhost et al. reported that HDM indeed harbored a mixture of allergens. In 1970s, Halmai and Alexander further isolated faecal pellets from the bodies of mites and performed skin-prick tests to demonstrate their allergenicity. With decades of effort, the functions of 23 HDM allergen groups have been revealed, yet the full spectrum of HDM allergenic components remains incomplete. In this study, we have isolated Dermatophagoides farinae (D. farinae) – the dominating HDM in southeastern China, and performed both DNA and RNA sequencing to assemble the draft genome & transcriptome. In addition to genome assembly, 112k genomic sequencing reads were able to map uniquely to a list of microbial species, hence determining the microbiome of D. farinae. Cross-comparison between D. farinae and other mite species such as spider mite & predatory mite also reveals the particularly broad spectrum of allergen genes harbored in D. farinae. It is believed that this work can be used as a future investigative tool for identification and characterization of HDM allergens.

Roles of Progenitor Cells for Intervertebral Disc Regeneration in "Healer" Mice

Ying ZHANG [1,*] Chi XIONG [1] Cheuk Wing CHAN [1] Daisuke SAKAI [2] Danny CHAN [1]
[1]Biochemistry, The University of Hong Kong, Hong Kong, China,
[2]Orthopaedic Surgery, Tokai University School of Medicine, Isehara, Kanagawa, Japan

Introduction: Intervertebral disc (IVD) degeneration is a major cause of back pain that can also lead to sciatica, affecting the quality of life. Current treatments are limited to salvage surgical operations. Biological treatments to relieve symptoms or to restore disc are not available as we know little about the biology of IVD degeneration and its potential to regeneration. While most people will develop disc degeneration with aging, there are individuals who are protected even at the age (older than 50 years) when over 90% of the population would succumb to the problem, suggesting the presence of protective genes. Furthermore, maintenance of progenitor cells within the nucleus pulposus (NP) is thought to play an important role in disc homeostasis. A hypothesis is that genetic factors can confer a protection against disc degeneration via better maintenance of resident progenitor cells. There exist strains of "healer" mice (MRL/MpJ, LG/J) that have better regenerative potentials of cartilage tissues[1,2]. Thus, we propose to address the NP progenitor cell pools in these healer mice in relation to the degeneration and potential repair/regeneration potentials of the disc.

Materials and Methods: Good healer (MRL and LG/J) and poor healer (C57/BL6C, and SM/J) mice were used in this study. Histological comparison of tail disc sections was assessed from 8 to 24 weeks of age. Progenitor cell pools and differentiated NP cells were assessed using immunohistochemistry using specific cell markers, Tie-2 and disialoganglioside (GD2), that were recently identified[3]. Tail looping at 8 weeks of age for a fixed period was used as an environmental perturbation that will induce degeneration. Unlooping the tail after the period of looping can assess healing processes with appropriate controls.

Results: A comparison of MRL and C57 mice showed neither observable histological differences, nor signs of degenerative processes from 8-week to 24-week of age. Following tail looping for 4, 5, 6 and 8 weeks, there were significant distortion of the annulus fibrosus (AF) and NP at the compressed and distended sides; in terms of loss of NP cells, AF tears and ruptures, and cell death in the AF. After the tails are unlooped for 4 weeks, there are restoration of NP and AF structures such as cell number in both MRL and C57 mice. However, superior healing is seen for MRL mice at all time-points studied; especially in TL6/TL7, TL7/TL8 and TL8/TL9 disc levels, in which the disc structure restores better via continuous expansion of NP region, cell repopulation and lamellae orientation recovers in the compressed AF sides with a clear NP AF boundary. In C57 mice, the AF lamellae structure remained disorganized following unlooping. Interestingly, in the absence of tail looping, SM/J tail discs already showed severe degeneration even at 8-week-old, while that of LG/J mice were relatively normal, suggesting an impact on developmental or maturation in SM/J IVDs. Immunohistochemistry analysis of progenitors related marker Tie-2 and GD2 shows different expression pattern from 4 to 24 weeks, in which MRL maintain more Tie-2 negative, GD2 positive cells during aging, indicating a role of this cell pool in maintaining disc homeostasis.

Conclusion: LG/J and MRL/MpJ mice have better IVD structure and maintenance than C57BL/6J and SM/J with aging, indicating genetic variations can significantly influence disc function. MRL/MpJ mice can better maintain a NP and AF boundary than C57BL/6J mice from mechanical loading, suggesting a potential "protective" effect and also MRL/MpJ mice maintain a higher number of Tie-2-/GD2+ cells, suggesting this pool of cells may have better function for disc maintenance. In depth analyses with more time points and molecular markers of IVD cells are needed to gain a better understanding of the "protective" genetic influences in the "healer" mice

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2. Rai, M. F. *et al*. Heritability of articular cartilage regeneration and its association with ear-wound healing in mice. *Arthritis Rheum.* **64,** 2300-2310 (2012)
3. Sakai, D. *et al*. Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. *Nat Commun*. **3,** 1264 (2012)

Use of a Tunable Resistive Pulse Sensing System to Detect Drug Response from the Mitochondria at Organelle Level

Loo Fong Chuen (CUHK)
(Supervisor: Professor Kong Siu Kai, CUHK)

Single organelle analysis from cancer cells provides a better understanding of the drug effects from the isolated organelles for anti-cancer drug development. Since mitochondria play a key role in regulating the life and death of cancer cells, we have developed a nanometer-scale sensing technique using the Tunable Resistive Pulse Sensing (TRPS) system to compare the drug response from single mitochondrion (diameter around 500 nm) isolated from the liver cancer cells with or without multidrug resistance (MDR).

This TRPS technique is based on the detection of blockade events when mitochondria pass through a tunable nano-pore for the change in time duration and current magnitude after drug treatment. In this presentation, the use of the TRPS system for the detection of mitochondrial response upon drug treatment will be highlighted. We first optimized the experimental conditions and verified the system by gold nanoparticles, and then applied the system to detect the drug response from single mitochondrion isolated from cancer cells. We found that more mitochondrial damages include mitochondrial swelling and change in surface charge were observed in the cancer cells with MDR when compared to that without MDR after anti-cancer drug candidate polyphyllin D treatment.

Session II

Csi1p recruits alp7p/TACC to the spindle pole bodies for bipolar spindle formation

Fan Zheng (HKU)
(Supervisor: Dr. Chuanhai Fu, HKU)

Accurate chromosome segregation requires timely bipolar spindle formation during mitosis. The transforming acidic coiled-coil (TACC) family proteins and the ch-TOG family proteins are key players in bipolar spindle formation. They form a complex to stabilize spindle microtubules, mainly dependent of their localization to the centrosome (the spindle pole body/SPB in yeast). The molecular mechanism underlying the targeting of the TACC-ch-TOG complex to the centrosome remains unclear. Here, we show that the fission yeast Schizosacchromyces pombe TACC ortholog alp7p is recruited to the SPB by csi1p. The csi1p interacting region lies within the conserved TACC domain of alp7p while the carboxyl-terminal domain of csi1p is responsible for interacting with alp7p. Compromised interaction between csi1p and alp7p impairs the localization of alp7p to the SPB during mitosis, thus delaying bipolar spindle formation and leading to anaphase B lagging chromosomes. Hence, our study establishes that csi1p serves as a linking molecule tethering spindle stabilizing factors to the SPB for promoting bipolar spindle assembly.

Functions of BCL-2 family in mitotic cell death

Shan Huang and Randy Y.C. Poon
Division of Life Science, The Hong Kong University of Science and Technology

Antimitotic drugs such as Taxanes are some of the most effective anticancer agents. By interfering the function of mitotic apparatus or antagonizing crucial components of the mitotic machinery, these drugs can inhibit mitotic progression in cancer cells and further induce apoptosis after prolonged mitotic arrest. However, abnormal mitotic exit mechanisms including mitotic slippage and multipolar division occurred in antimitotic drug-treated cells and are strongly linked to drug resistance. Therefore, it is important to unravel the question of how mitotic cell death occurs at the molecular level. The BCL-2 (B-cell lymphoma 2) protein family regulates apoptosis by controlling mitochondria outer membrane integrity. In this systematic study, the whole BCL-2 family was screened to identify members that may be involved in antimitotic drug-induced mitotic cell death. The effects of downregulation of individual BCL-2 family members in HeLa and HCT116 cells were evaluated by time-lapse live cell imaging. Through analyzing the kinetics of mitotic cell death, these studies indicated that multiple anti-apoptotic members, including BCL-B, BCL-W, BCL-XL and MCL1, were involved in Paclitaxol-induced mitotic cell death. Overexpression of these proteins partially repressed the mitotic cell death induced by paclitaxel or nocodazole. The expression of BCL-XL, BCL-W and MCL1 were modified during the prolonged mitotic arrest, indicating that the active roles of BCL-2 proteins in mitotic cell death may be carried out by mitotic-specific regulations. Since BCL-2 inhibitors are being evaluated as potential anticancer agents, this study highlighted the potential and molecular basis of synergism between BCL-2 inhibitors and antimitotic drugs.

Combinations of Quasi-independent Binding Sites Underlie Diversity of Membrane Target Recognition by Ankyrins

Wang Chao (HKUST)
(Supervisor: Professor Mingjie Zhang, HKUST)

Ankyrin adaptors together with their spectrin partners coordinate diverse ion channels and cell adhesion molecules within plasma membrane domains and thereby promote physiological activities including fast signaling in the heart and nervous system. Ankyrins specifically bind to numerous membrane targets through their 24 ankyrin repeats (ANK repeats), although the mechanism for the facile and independent evolution of these interactions has not been resolved. Here we report the structures of ANK repeats in complex with an inhibitory segment from the C-terminal regulatory domain and with a sodium channel Nav1.2 peptide, respectively, showing that the extended, extremely conserved inner groove spanning the entire ANK repeat solenoid contains multiple quasi-independent target binding sites capable of accommodating target proteins with very diverse sequences via combinatorial usage of these sites. These structures establish a framework for understanding the evolution of ankyrins' membrane targets and provide mechanistic insights into diseases caused by mutations in ankyrins and/or their binding partners, with implications for other proteins containing extended ANK repeat domains.

How vacuolar sorting receptor (VSR) protein interact with their cargo protein - Crystal structures of apo and ligand-bound forms of the protease-associated domain from an Arabidopsis VSR

Luo Fang (CUHK)
(Supervisor: Professor Kambo Wong, CUHK)

In plant cells, soluble proteins reach vacuoles because they contain vacuolar sorting determinants (VSDs) that are recognized by vacuolar sorting receptor (VSR) proteins. Previous reports suggest that the protease-associated (PA) domain of VSR is involved in sequence-specific recognition of the NPIR motif of cargo-proteins such as aleurain. Here, we present crystal structures of PA domain of AtVSR1 (AtVSR1-PA) at a resolution of 1.6 Å and its complex with aleurain peptide at a resolution of 1.9 Å. The apo form of AtVSR1-PA reveals that its surface conserved residues are clustered on one regionside of the molecule, which forms a ligand-binding surface. The complex structure showed AtVSR1-PA interacts with a novel site of aleurain peptide, not the known NPIR motif. Moreover, the C-terminal swinging movement of PA domain induced upon peptide binding allows central region to relocate near the binding pocket, thus helping to bind cargo-peptide. Mutagenesis study of residues involved in receptor-cargo interaction was performed to test the roles of these residues in interaction and subcellular localization. Taking advantage of our structural and functional study, we proposed a model of molecular mechanism for the interaction between VSR and its cargo.

Session III

Eya1 is essential for branchial arch segmentation and branchial epithelium development through regulating Notch signaling pathway

Haoran Zhang (HKU)
(Supervisor: Professor MH Sham, HKU)

Craniofacial anomalies are common features of Branchio-Oto-Renal (BOR) syndrome patients. Mutations in the Eya1 gene have been found in around half of the BOR patients, but the pathogenic mechanisms mediated by Eya1 in the craniofacial malformations remain unknown. In this study, we use Eya1 mutant mice as a disease model to study the abnormal early branchial arch (BA) development. Eya1-/- mutant embryos have hypoplastic BA2. The formation of branchial cleft was severely affected. Interestingly, Notch signaling was down-regulated in the mutant branchial epithelium. We hypothesize that Eya1 may interact with the Notch signaling pathway to regulate BA development. The aim of this study is to investigate the molecular mechanisms underlying the epithelial cell defects during abnormal BA development in BOR syndrome.

By cell lineage tracing experiments, we identified a group of Sox2 and Sox3-positive branchial epithelial progenitors in normal embryos. Analysis of Eya1-/- mutants suggested that these progenitors failed to contribute to the formation of branchial clefts. To test whether Notch signaling is involved in mediating these progenitors, we overexpressed an activated form of the Notch1 receptor (NICD) in Eya1-/- mutants. We found that over-expression of NICD resulted in ectopic epithelial progenitors, the branchial cleft defects of the Eya1-/- mutants were partially rescued. Furthermore, we showed that Eya1 could physically interact with the NICD protein and stabilize it. Our results indicate that the interaction between Eya1 and NICD is required for the specification and maintenance of epithelial progenitors, thereby controls the formation of branchial clefts and segmentation of branchial arches. In BOR syndrome patients who have mutations in Eya1 gene, some major craniofacial phenotypes, such as pinnae deformities and external auditory canal stenosis may be due to the abnormal development of branchial epithelium and branchial segmentation defects.

Loss of LIM-homeodomain genes, Lhx1 and Lhx5, disrupts dendritic spine morphogenesis of Purkinje cells and causes ataxia in mouse

Lui Nga Chu (CUHK)
(Supervisor: Professor KM Kwan, CUHK)

Abnormal development of Purkinje cells, the only efferent neurons in cerebellar cortex, damages cerebellum function and thus impairs motor coordination and body balance, leading to clinical symptoms like ataxia. Previously, we identified that LIM-homeodomain genes, Lhx1 and Lhx5, can regulate early Purkinje cell differentiation in developing cerebellum. However, their functional roles in postnatal development of differentiated Purkinje cells were poorly understood. Here both Lhx1 and Lhx5 were conditionally inactivated in Purkinje cells in postnatal mouse cerebellum, resulting in mutant mice suffering from motor disability and body imbalance. The control mice with one functional copy of Lhx1 or Lhx5 did not show any defects. Though the general morphology of Purkinje cells in the double Lhx1/5 mutant mice were comparable with the control mice, detailed examination showed that dendritic spine morphogenesis of Purkinje cell dendrites was disrupted in mutant mice. A large portion of dendritic spines of Purkinje cells in mutant mice failed to maturate. The mutant Purkinje cell dendrites had mislocalized F-actin cytoskeleton. In addition, we found that Lhx1 and Lhx5 could transcriptionally activate an actin-bundling protein, espin, indicating that Lhx1 and Lhx5 could govern F-actin cytoskeleton localization through espin. The F-actin mislocalization in Purkinje cell dendrites therefore caused the failure of dendritic spine maturation because F-actin is an important scaffold of dendritic spines. Overall, our findings illustrate that Lhx1 and Lhx5 are functionally redundant and essential for dendritic spine maturation, and thus maintain the proper functioning of differentiated Purkinje cells in mouse cerebellum.

The Dynamic Process of Microglial Colonization of the Developing Zebrafish Brain

Tienan WANG (HKUST)
(Supervisor: Professor Zilong Wen, HKUST)

Microglia are the resident macrophages of the central nervous system (CNS), and they play important roles in neuron development and neuron function. Yet, the migration routes and dynamic behaviors of microglial precursors during their colonization of the CNS remain largely undefined. Here, by utilizing in vivo time-lapse imaging and genetic amenable ability of zebrafish, we show that microglia precursors enter and colonize the optic tecum, where the majority of microglia reside during early zebrafish development, via the bi lateral periphery of the brain or passing through the ventral brain. The colonization process of the optic tectum by microglial precursors is independent of the circulation and involves two distinctive steps, homing and settling, both of which appear to be driven by the neuronal cell death that occurs during normal neurogenesis. Our work provides the cellular basis for the in vivo observation and mechanistic study of microglial colonization of the brain.

Regulation and functions of liprinα1 in activity-dependent synapse development

Huiqian HUANG (HKUST)
(Supervisor: Professor Nancy Ip, HKUST)

The Liprinα family proteins, originally identified as interacting protein of the receptor protein tyrosine phosphatase LAR (LAR-RPTP), are suggested to play an indispensable role in dendrite development and formation of excitatory synapse. And the expression of one of its family members, liprinα1, can be regulated by neural activity. However, the regulation and function of liprinα1 in neural development remains unclear. We found that liprinα1 was highly expressed at the post-synaptic fractions during early postnatal stages, when activity-dependent synapse development actively occurs. Furthermore, liprinα1 was identified as an in vivo substrate of Cdk5, and this phosphorylation was regulated in cultured neurons by neuronal activity. Interestingly, phosphorylation of liprinα1 in the mouse visual cortex was also modulated during eye opening, suggesting that liprinα1 phosphorylation might be involved in activity-dependent synapse development in vivo. Importantly, knockdown of liprinα1 in cultured hippocampal neurons led to defects in dendritic arborization and reduction in spine density. Together, our results reveal a novel mechanism of activity-dependent synapse development that involves phosphorylation of liprinα1.

Poster presentation

Theme 1: Cell biology

Poster Number

1 **Identification of 'erasers' for histone crotonylation mark**

Bao Xiucong (HKU)
(Supervisor: Dr. Xiang Li, HKU)

Posttranslational modifications (PTMs) on histones play crucial roles in a wide range of fundamental cellular processes, such as gene transcription, DNA replication and chromosome segregation. Lysine crotonylation (Kcr) is a newly discovered histone PTM that is specifically enriched at active gene promoters and potential enhancers in mammalian cell genomes. Accumulation of Kcr on sex chromosomes was observed immediately following meiosis of male germinal cells and Kcr specifically marks testis-specific X-linked genes that are postmeiotically expressed. However, the cellular functions of Kcr still remain elusive, which is, in large part, due to a lack of knowledge about enzymes that regulate the addition or removal of this novel PTM. Here, we identified human Sirt3 as the first histone decrotonylase by using a chemical proteomics approach that combines photo-cross-linking strategy and SILAC-based quantitative mass spectrometry. Our biochemical, structural and cellular biology studies revealed that Sirt3 can selectively recognize histone crotonylation 'mark' and catalyze their hydrolysis in vitro and in cell and that the unique pi-pi interaction in Sirt3-H3K4Cr complex contributes its decrotonylation activity. The discovery of Sirt3 as an enzyme that regulates lysine crotonylation not only opens opportunities for examining the physiological significance of this new histone PTM, it may also help to unravel unknown cellular mechanisms controlled by Sirt3.

2 **Structural characterization of a bile acid-specific mouse liver sulfotransferase (mL-STL)**

Chan Yan Chun (CUHK)
(Supervisor: Dr. Lee Sau Tuen, CUHK)

Cytosolic sulfotransferase (SULT) is a superfamily of enzyme that catalyzes the transfer of a sulfuryl group from the cofactor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to various hormones, neurotransmitters, drugs, and xenobiotic to assist detoxification. A novel mouse liver cytosolic sulfotransferase (mL-STL) gene was discovered in our laboratory. In silico functional prediction revealed that mL-STL belongs to SULT2A family, which can sulfonate hydroxysteroids including dehydroepiandrosterone, pregnenolone and bile acids. Interestingly, we found that the mL-STL protein is specific to bile acids only but no other prototype substrates. Historically, bile acid is known to solely facilitate dietary lipid absorption, but recent studies discovered that bile acid can also function as metabolic regulator in maintaining energy homeostasis. Thus it is reasonable to hypothesis that sulfonation of bile acids by mL-STL can be one of the physiological pathways to regulate the bile acid signals which in turn regulate the glucose, lipid and energy homeostasis. As bile acid is essential in energy homeostasis, characterization of mL-STL protein might help develop novel strategies for treating diabetes, obesity and metabolic diseases. Current research is aiming to characterize the structurally specificity of mL-STL with bile acids. To investigate its bile acid specificity, crystal structure of recombinant His-tag mL-STL protein was studied by co-crystalizing protein with cofactor. We obtained an initial X-ray diffraction result with 3.8 Å resolution. To resolve the protein structure to study the substrate binding pocket, further optimization is required with the addition of bile acid in the co-crystallization.

Poster Number

3 **DYRK1A is an RNF169-Interacting Partner involved in DNA Double-Strand Break Signaling and Repair**

Chen Jie (HKU)
(Supervisor: Dr. Michael SY Huen, HKU)

Chromatin ubiquitylation surrounding DNA double-strand breaks (DSBs) orchestrates the recruitment of DNA damage signaling and repair proteins, and is critically important in the protection of genome integrity. Notably, mechanistically how ubiquitylation reactions are confined to domains flanking DSBs has remained elusive.

Recently, we identified the Ring Finger Protein RNF169 as a putative negative regulator of DSB signaling. RNF169 competed for DNA damage-associated ubiquitin conjugates, displaced DNA damage mediator and checkpoint proteins from DSBs, and hastened cell recovery from DNA damage checkpoint arrest.

To further explore the mechanistic bases of negative regulation of DSB signaling, we purified RNF169 protein-complexes and have identified the Down syndrome-related protein DYRK1A as an RNF169-interacting partner. DYRK1A accumulated at DSBs via its binding to RNF169, and is important for cell resistance to genotoxic stress. Pertaining to a broader role in chromatin regulation, we found that increased dosage of DYRK1A effectively dissembled nuclear bodies, including those of splicing speckles and DNA damage foci. Intriguingly, DYRK1A inactivation not only substantially increased spontaneous DNA damage foci, but also led to markedly elevated efficiency of DSB repair. We propose that DYRK1A is endowed with suppressive properties on chromatin functions, and that the RNF169-DYRK1A complex encodes a bipartite molecular module to restrain DSB signaling and repair processes.

4 **How $Cd2^+$ affects TCDD-induced AHR pathway in a zebrafish liver cell line**

Chen Ying Ying (CUHK)
(Supervisor: Dr. King Ming Chan, CUHK)

Trace metal ions, such as cadmium ($Cd2^+$), and trace organics typified by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD), are common co-contaminants in the environment that pose health risks and significant environmental impacts because of their accumulation in food chains and multiple toxic effects. However, the knowledge of their joint toxic effects is not thoroughly investigated. In this study, we evaluated the effects of $Cd2^+$ on the regulation of the cytochrome P450 1A1(CYP1A1) gene induced by TCDD. To determine the effects, zebrafish liver cells (ZFL) were treated with TCDD (3nm or 30 nm) with or without increasing concentrations of $Cd2^+$ (0–30 μm), Alamer-Blue assay, Ethoxyresorufin-O-deethylase (EROD) activities, real-time PCR analysis，western blot analysis and luciferase assay were employed to determine the cytotoxicity, enzyme activity, gene and protein expression levels, as well as transcriptional regulation of CYP1A1. Our results showed that TCDD, at both concentrations, can inhibit $Cd2^+$ induced cell viability and the inhibition effect is especially stronger at higher concentration of TCDD (30 nm). $Cd2^+$, at all concentrations used significantly inhibited TCDD-mediated induction of CYP1A1 activity and aryl hydrocarbon receptor (AHR2), aryl hydrocarbon receptor nuclear translocator 2b (ARNT2B) as well as CYP1A1 mRNA expression levels. Luciferase assay of different deletion mutants of the zebrafish CYP1A1 gene promoters also showed that co-treatment with $Cd2^+$ (0–15 μM) and TCDD (3 nm) produced inhibition in the activity of gene construct harboring distal promoter region (P -2626/-2009) of CYP1A1 and 3XRE reporter gene compared to TCDD (3 nm) treatment alone. In contrast, co-treatment with $Cd2^+$ (30 μM) and TCDD (3 nm) resulted in an increased induction in the proximal promoter (P-2626/+36) region of CYP1A1 induction compared to TCDD (3 nm) treatment alone, indicating the different contributions of CYP1A1 promoter regions on the inhibition effects induced by $Cd2^+$. These data showed that the ability of $Cd2^+$ to alter the capacity of AhR ligands to induce the bioactivating phase I enzymes (CYP1A1) might affect the carcinogenicity and mutagenicity of the AhR ligands and will help us to understand the mechanism of $Cd2^+$ modulating on TCDD-mediated induction of cytochrome P450 1A1.

Poster Number

5 **Ubiquitination and proteosome-dependent degradation of the activated form of human liver-enriched transcription factor CREB-H regulated by protein kinase A**

Yun Cheng, Hei-Man Vincent Tang, Wei-Wei Gao, Jian-Jun Deng, Chi-Ping Chan and Dong-Yan Jin
Department of Biochemistry, The University of Hong Kong

CREB-H is a membrane-bound bZIP transcription factor which is mainly expressed in liver and small intestine. CREB-H plays important roles in the regulation of lipid metabolism, iron metabolism, gluconeogenesis and acute phase response. CREB-H is proteolytically activated by regulated intramembrane proteolysis to generate a C-terminal truncated form known as CREB-HΔTC, which translocates to the nucleus to activate target gene expression. We have previously shown that CREB-HΔTC has a short half-life. In this study we report on ubiquitination and proteasome-mediated degradation of CREB-HΔTC. Proteasome inhibition led to the accumulation of CREB-HΔTC. The degradation of CREB-HΔTC was mediated by lysine 48-linked polyubiquitination of CREB-HΔTC. A DSGXS destruction box was identified in CREB-HΔTC and was also found to be conserved among orthologous proteins from different species. Disruption of this DSGXS destruction box resulted in stabilization of CREB-HΔTC. A potential E3 ubiquitin ligase implicated in CREB-HΔTC degradation was identified and characterized. In addition, CREB-HΔTC was also found to be phosphorylated by protein kinase A, leading to its stabilization. Taken together, our work revealed a new signaling pathway that controls ubiquitination and degradation of CREB-HΔTC. The rapid ubiquitination and degradation of CREB-HΔTC ensures transient and tightly regulated activation of its target genes in liver.

This work is supported by S.K. Yee Medical Research Fund (2011).

6 **The Activation of Transient Receptor Potential Channel Vanilloid 3 (TRPV3) Suppresses Adipogenesis**

Cheung Sin Ying (CUHK)
(Supervisor: Professor Chung Hau Yin, CUHK)

Obesity is a major risk factor for metabolic diseases. Adipocytes in adipose tissues influence obesity, insulin resistance and diabetes mellitus. Therefore, discovery of anti-adipogenic pathways is crucial for the development of clinical therapies against obesity. We identified that the activation of $Ca2^{+}$ permeable channel Transient Receptor Potential Channel Vanilloid 3 (TRPV3) prevented differentiation of 3T3-L1 preadipocytes. The activation of TRPV3 by activators (-)-epicatachin and diphenylboronic anhydride (DPBA) was determined by fluorometric calcium imaging studies and patch clamp electrophysiology. The 3T3-L1 cells were induced to differentiate in the presence of the TRPV3 activators. Adipogenesis in stimulated 3T3-L1 preadipocytes was determined by oil red O-staining of intracellular lipid droplets and quantitative real-time RT-PCR. The activators attenuated adipogenesis at a dose-dependent manner and could be reversed by the TRPV3 inhibitor Diphenyltetrahydrofuran (DPTHF) and TRPV3 siRNA. Our immunoblotting results validated that the activation of TRPV3 attenuated insulin receptor and phosphoinositide 3-Kinase/Akt signaling, which downregulated the expression of CCAAT/enhancer binding protein alpha (C/EBPalpha) and peroxisome proliferator-activated receptor gamma (PPARgamma) in 3T3-L1 cells. TRPV3 also co-immunoprecipitated with insulin receptor substrate-1 (IRS-1), confirming association between TRPV3 and IRS-1 in 3T3-L1 preadipocytes. Compared with wild-type mice, we observed reduction in TRPV3 expression in ob/ob and db/db mice. We conclude that TRPV3 activation suppresses adipogenesis. The TRPV3 channel may regulate adipocyte metabolism.

Poster Number

7 **Regulation of ubiquitination and degradation of CRTC1 transcriptional coactivator by salt-inducible kinase SIK1**

Gao Wei Wei (HKU)
(Supervisor: Professor DY Jin, HKU)

cAMP response element (CRE) binding protein (CREB) is a bZIP transcription factor centrally involved in the regulation of cellular proliferation, survival, energy metabolism and stress response. CREB-regulated transcriptional coactivator (CRTC) is an essential factor which potently activates the transcriptional activity of CREB by promoting the recruitment of TAFII130/135. Currently, several kinases and phosphatases are known to regulate the function of CRTCs. For example, salt-inducible kinases (SIKs) belonging to AMPK protein kinase family inhibit the activity of CRTCs through a phosphorylation-dependent nuclear export mechanism. In this study, we provide the first evidence for destabilization of CRTC1 protein by SIK1. Enforced expression of SIK1 resulted in diminution of the steady-state amount of CRTC1, whereas expression of kinase-defective mutant of SIK1 had no influence on the level of CRTC1, indicating the requirement of the kinase activity of SIK1 for the suppressive effect on CRTC1. Addition of proteasome inhibitor MG132 restored CRTC1 protein level. More importantly, ubiquitinated CRTC1 species were detected in the ubiquitin pull down assay in the presence of kinase-proficient SIK1. Taken together, our data suggest a novel regulatory mechanism for CRTC1 through which SIK1 suppresses the CREB-mediated gene transcription by destabilizing CRTC1 in a phosphorylation-induced, ubiquitination-mediated and proteasome-dependent manner.

8 **Role of ATM Signaling in PALB2-dependent DNA Damage Responses**

Yingying Guo[*1,2,3], Wanjuan Feng[1,2,3], Shirley M.H. Sy [4] & Michael S.Y. Huen[1,2,3]
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Mutations of the PALB2 gene lead to a number of hereditary cancer-predisposing syndromes, including Fanconi anemia and hereditary breast and ovarian cancer syndrome. Originally identified as a core DNA repair factor, emerging evidence now implicates PALB2 in cell cycle checkpoint control, DNA replication, oxidative stress regulation and transcription, highlighting the multi-functionality of the tumor suppressor. Notably, mechanistically how its expanding repertoire of functions are orchestrated remains unexplored.

By halting cell cycle progression, checkpoint machineries allow time for restoring of genome integrity prior to resumption of cell growth and division. However, it is not well understood how cell cycle arrest is coordinated with DNA repair processes, and whether DNA repair proteins signal to restart cell cycle following completion of DNA repair. Intriguingly, recent observations that PALB2 plays a role in cell cycle regulation raise the interesting possibility that the tumor suppressor may encode a protein hub to coordinate DNA repair and cell proliferation. However, exactly how PALB2 function in cell cycle checkpoints may be spatiotemporally coupled to DNA repair is not known.

In exploring how DNA damage signaling coordinates with PALB2-dependent DNA repair, we have identified a panel of putative phosphorylation sites on the PALB2 polypeptide. Employing a combination of biophysical, biochemical and genetic approaches, we found that ionizing radiation (IR) triggered PALB2 phosphorylation at Ser157 and Ser376 in an ataxia telangiectasia mutated (ATM) kinase-dependent manner. Interestingly, our data indicated that PALB2 phosphorylation status at Ser157 and Ser376 neither affected its relocalisation to DNA damage sites nor were they required for DNA repair. Instead, these DNA damage-regulated PALB2 phosphorylation events are temporally coupled to cell recovery from checkpoint arrest. We propose that ATM-mediated PALB2 phosphorylation represents a means to coordinate cell cycle checkpoint regulation and DNA repair processes. Given the potential relationship between PALB2 phosphorylation and tumorigenesis, our study may provide a diagnostic biomarker of cancer predisposition.

Poster Number

9 **Aptamer library resampling**

Kinghorn Andrew (HKU)
(Supervisor: Dr. Julian A Tanner, HKU)

Aptamers are nucleic acid based binding molecules capable of specific, high affinity binding. Aptamers are isolated from a library of random ssDNA or RNA sequences using SELEX, a process involving successive rounds of selection for analyte binding and amplification. Due to insufficient library sequence space coverage and the stochastic nature of SELEX the probability of selecting the fittest aptamer is extremely low. You are however extremely likely to select family members of the fittest aptamer. Herein we describe 'Resample', a computer program coded in Visual Basic which takes an input of an aptamer family motif and outputs a library representing every aptamer permutation of the family motif. By taking sequences isolated using SELEX and using sequence alignment you can deduce with high probability the aptamer family motif of the highest affinity aptamer. This motif can then be used by 'Resample' to combinatorially generate an aptamer library containing many novel high affinity aptamers and most probably the highest affinity aptamer which exists. This library could then be synthesised in solution or onto the surface of a microarray and subsequent selections performed to isolate extremely high affinity aptamers. Increasing aptamer affinity in this way is extremely valuable for reducing the limit of detection of aptamer based diagnostic tests for disease as well as increasing efficacy of aptamer based therapeutics.

10 **Effects of lysogeny on the ecophysiological fitness of E. coli**

Jennifer LAI, Hao ZHANG, Miranda CHIANG (HKUST)
(Supervisor: Dr. Stanley LAU, HKUST)

Escherichia coli is a symbiont of warm-blooded animal. However, E. coli was found in external environment without fecal input, where it was faced a large number of environmental stresses that were absent in the animal host. It is well known that prophages (i.e. DNA of phages residing in the genome of bacterial cells) play a significant role in the genome diversification and niche expansion of pathogenic E. coli strains from one host species to another, but whether prophages can facilitate to E. coli niche expansion from the animal host to the external environment still remains unknown. In our study, a model system composed of an environment E. coli strain, and a pair of pre- and post-lysogenic facal E. coli strain was used to elucidate the effect of lysogeny on the ecophysiological fitness of E. coli. By analyzing the whole genome sequences of the three strains, it is confirmed that the pre- and post-lysogenic strains are isogenic, with a P2-like prophage from the environmental strain is acquired by the post-lysogenic strain. Sediment and seawater microcosm experiments show that the post-lysogenic fecal strain decay significant slower than the pre-lysogenic strain. The metabolic profiles of the pre- and post-lysogenic pair are also appeared to be different. By applying genetic engineering, the P2 prophage acquired by the post-lysogenic fecal strain is knocked out completely and the impact of the prophage deletion to the host survival is undergoing investigation.

Poster Number

11 **Molecular basis of bacteriolysin-induced degradation of SUMO E2 Ubc9**

Li Jie Xin (CUHK)
(Supervisor: Dr. Shannon Au, CUHK)

SUMOylation is a reversible protein post-translational modification indispensable for viability in all eukaryotes, which is a sequential catalytic cascade involves E1, E2 and E3 enzymes. Modulation of SUMOylation has emerged as a strategy exploited by many pathogens during infection. Cholesterol-dependent cytolysin (CDC) is a large family of pore-forming toxins that commonly associated with bacterial pathogenesis. Pore formation on cell membrane is thought to be the major functions of CDCs, while increasing number of studies showed that CDCs can also modulate diverse host post-translational modifications during infection, including SUMOylation. Three CDCs, LLO, PLY and PFO were found to trigger a proteasome-independent degradation of SUMO E2 conjugating enzyme Ubc9, leading to a global decrease of SUMOylation, which partially linked to a yet-to–be identified aspartyl protease. However, the underlying mechanism of the LLO-induced Ubc9 degradation is unknown.
Here we show that recombinant LLO, PLY and two other CDCs, SLO from S. pyogenes and SLY from S. Suis, all possessed inhibitory effect on both Ubc9 stability and SUMOylation when applied to both HeLa cells and THP-1 cells. Results from cell fractionation and immunostaining suggested that the degradation event was mainly occurred within nuclei. By using different Ubc9 mutants defective in covalent or non-covalent SUMO binding, we demonstrated that interaction between SUMO and Ubc9 is not critical for CDC-induced Ubc9 degradation. On the other hand, we found that phosphorylation of Ubc9 was involved in this degradation process. CDC-induced Ubc9 degradation was enhanced when pre-incubating cells with phosphatase inhibitors. Furthermore, degradation of Ubc9S71A mutant was suppressed upon LLO treatments. These results suggested that CDCs share similar strategies during bacterial infection and phosphorylation of Ubc9 may be one of the key steps during CDC-induced degradation.

12 **Mcp1p tracks microtubule plus ends to destabilize microtubules at cell tips**

Li TianPeng (HKU)
(Supervisor: Dr. Chuahai Fu, HKU)

Microtubule plus ends are dynamically regulated by a wide variety of proteins for performing diverse cellular functions. Here, we show that the fission yeast Schizosaccharomyces pombe uncharacterized protein mcp1p is a microtubule plus-end tracking protein which depends on the kinesin-8 klp6p for transporting along microtubules towards microtubule plus ends. In the absence of mcp1p, microtubule catastrophe and rescue frequencies decrease, leading to an increased dwell time of microtubule plus ends at cell tips. Thus, these findings suggest that mcp1p may synergize with klp6p at microtubule plus-ends to destabilize microtubules.

Poster Number

13 **RGS19 up-regulates Nm23-H1/2 expression via stimulating multiple transcriptional factors**

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The Nm23 gene family is comprised of ten genes that are involved in a variety of physiological and pathological processes ranging from cell proliferation, differentiation, apoptosis, cell cycle regulation, tumorigenesis to metastasis. Nm23-H1 and H2 are the most widely studied isoforms due to their ability to suppress metastasis. Loss or reduced expression of Nm23-H1/2 is often associated with aggressive metastatic potential in different types of tumors. Conversely, elevated expression of Nm23-H1/2 has been shown to suppress metastasis. We have recently shown that both Nm23-H1 and H2 are up-regulated by RGS19 (regulator of G-protein signaling 19, a GTPase activating protein for $G\alpha_{i/o}$ and $G\alpha_{q}$ subunits) in multiple cell types. However, the mechanism involved in RGS19-induced transcriptional regulation of Nm23 remains unknown. By means of *in silico* comparison of promoter sequences and luciferase reporter assays, we showed that the transcription factor AP-1 might be involved in the up-regulation of Nm23-H1/2 by RGS19. Both transient and stable expressions of RGS19 in HEK293 cells are able to stimulate the pAP-1-luc reporter. Since Nm23-H1 and H2 do not appear to utilize identical signaling partners, we have identified sixteen amino acids on the exposed surfaces of Nm23-H1 that are different from H2. To examine if these residues are responsible for specific protein-protein interactions between Nm23-H1/2 and diverse binding partners, we have constructed seven mutants with double or triple mutations that may alter the specificity of signaling. These Nm23 mutants will be characterized for their ability to regulate tumorigenesis and metastasis (Supported by HKUST 663110 and RPC11SC07).

14 **Development of an aptamer-based biosensing system for measuring luteinizing hormone pulsatility for infertility treatment**

Liang ShaoLin (HKU)
(Supervisor: Dr. Julian A Tanner, HKU)

Normal fertility in human involves a highly orchestrated signal communication between the hypothalamic-pituitary-gonadal. The pulsatile release of Luteinizing Hormone (LH) from pituitary gland is the key element in this ”concerto” for the simulation of sex steroid hormones synthesis and the production of mature eggs. Specific alterations in LH pulsatile pattern are linked to hypothalamic dysfunction in female patient with anovulatory infertility - by knowing the information of this pattern, clinicians can decide whether the patient needs treatment with pituitary hormones. However, there is currently no clinical feasible test to assess LH pulsatility due to the need of frequent blood sampling in every 10 minutes for 8 hours, which requires dedicated experts to carry out antibody-based immunoassay and causes significant blood loss to the patient. Here we propose a novel diagnostic strategy by using an electrochemical aptamer-based point-of-care (POC) biosensing system to continuously monitor the LH concentration in the patient with infertility. Our LH aptamer is a single-stranded DNA sequence generated via an in vitro selection method which embedded counter-selection steps against FSH and hCG, two structural similar gonadotropins with LH, to avoid cross-reactions. The aptamer can be modified with methylene blue (MB) redox label and coated on the gold surface, which forms a system that can monitor LH-induced structural switching of the aptamer by determining electrochemical currents that are associated with the distance between the redox label and the electrode surface. This system has the potential to be further developed as a POC diagnostic tool for the infertile patient caused by LH defect and assist clinicians to ”personalized” treatment with hormonal therapy.

Poster Number

15 **Gene Expression Noise in Transcriptional Negative Auto-regulation in Mammalian Cell**

Liu Lizhong (HKU)
(Supervisor: Dr. Huang Jiandong, HKU)

Negative auto-regulation (N.A.R) is the simplest and most widely spread type of genetic network motif. Although previous work has demonstrated that N.A.R can reduce gene expression noise, the dynamic relationship between repression strength and its noise reduction effect has not been well documented in Mammalian cell. Here we construct N.A.R cascades using repressor tetR to suppress its own promoter, which contains different number of tetR binding site, in HEK 293 cell. To measure protein production, a red fluorescent protein mCherry has been fused to C-terminal of tetR. Moreover, to increase nuclear repressor concentration, a nuclear localization sequence has been fused to tetR::mCherry fusion protein C-terminal. Consistent with previous work, our data indicate that comparing to non-auto-regulation, N.A.R. dramatically decrease the steepness of the dose-response curve and reduce gene expression noise. Interesting, our data suggest that repressor binding site number in promoter region could significantly affect N.A.R noise reduction effect. For instance, when there are only two repressor binding sites inserted in the promoter region, noise of the regulated gene expression is relatively high, in non-induced state, while in induced state the noise is low and stay at a low level in a wide input dynamic range. Increasing the binding site number in the promoter region decrease the gene expression noise in non-induced state. However, in induced state, the noise of the regulated gene increase as the inducer concentration increase. These results is useful for synthetic biologist to design genetic circuits to achieve precisely gene expression and tightly noise repression.

16 **Activation of Gq without subunits dissociation**

Liu Wen (HKUST)
(Supervisor: Prof. YH Wong, HKUST)

Heterotrimeric G proteins are one of the most well studied signaling proteins. With hundreds of different coupled receptors and multiple downstream effectors, G proteins constitute a major hub in signal transduction, sorting extracellular signals into proper second messengers. According to extensive in vitro evidence, the activation of G proteins requires the dissociation of GTP-bound Gα subunit and Gβγ dimer. However, the question of whether all G proteins follow such a model in vivo remains difficult to confirm due to technical limitations. We have observed a subtype-dependent G protein dissociation by using flag-tagged Gβ1 subunit to pull down different Gα subunits upon GTPγS or AlF4- activation. Most members of the Gi family showed obvious dissociation. On the contrary, Gαq and Gα16, both from the Gq family, remain associated with Gβ even in the activation state. We further confirmed this tight association in Gq activation by constructing artificial Gβ-Gα fusion proteins. The Gα subunit was linked to the Gβ1 subunit by a Leu linker or a Phe-(Gly4 Ser)2- Gly4-Phe linker, providing a tightly stuck condition or a more flexible connection. Our functional results showed that Gβ-Gαq fusion protein activated PLCβ as efficiently as the wild type non-fusion proteins. Gβ-Gαs fusion proteins are also functional. But Gαi3 protein lost its function when tightly linked with Gβ. These results indicate the subtype-dependent variance of activation of G proteins and that Gq proteins may not dissociate upon activation. (Supported by 2013CB530900 and T13-607/12R).

Poster Number

17 **Structural and Biochemical Analysis of Transportin-SR2**

LONG, Yun Xin (CUHK)
(Supervisor: Dr. NGO, Chi Ki, CUHK)

Transportin-SR2 (TRN-SR2) is an importin-family protein. It is initially identified as the nuclear import receptor for SR proteins, which are a essential family of splicing factors. SR proteins contain one or two N-terminal RRM(s) that provides substrate specificity. On the other hand, their C-terminal RS domains aid protein-protein interactions and facilitate spliceosome recruitment. TRN-SR2 is capable of importing the SR proteins to the nucleus by specifically interacting with the multisite phosphorylated RS domains. SRp20 is a member of the SR protein family and is constituted of one N-terminal RRM followed by an RS domain at the C-terminus. It is extensively phosphorylated by SR protein kinases (SRPKs) on the serine residues within the RS domain. Besides playing an important role in pre-mRNA splicing, SRp20 is also involved in other important cellular functions. Aberrant functions of SRp20 have been implicated in multiple forms of human or animal diseases.
In this project, we aim to study the molecular basis of interaction between TRN-SR2 and SRp20, and to decipher the regulatory mechanism of SRp20's nuclear import. To first identify the regions important for interaction, we have performed pull-down experiments using recombinant TRN-SR2 and SRp20 constructs with different lengths of RS domains. We also investigated the requirement of phosphorylation in their interactions. Our results suggest that the electrostatic nature of the RS domain significantly influence the interaction between SRp20 and TRN-SR2. This sheds new light on the mechanism of how TRN-SR2 binds its cargoes to mediate their nuclear import.

18 **Characterization of Porphyromonas gingivalis exopolyphosphatase: implications for bacterial nucleotide and polyphosphate metabolism**

Lu Bingtai (HKU)
(Supervisor: Dr. Rory M Matt, HKU)

Inorganic polyphosphate (poly-P) is a linear biopolymer comprised of tens to hundreds of phosphate residues. Poly-P is ubiquitous in nature, and plays a variety of important physiological roles. In bacteria, it is involved in cellular survival under stressful conditions and resistance to antibiotic agents. It has been proposed that Exopolyphosphatase (PPX) proteins are responsible for the majority of poly-P hydrolysis in bacteria. However, PPX proteins have highly variable sizes and compositions. Our research aims were to characterize the biochemical activities of the PPX protein encoded by the bacterium Porphyromonas gingivalis, which is strongly associated with periodontitis in humans. Multiple bioinformatic approaches were first used to analyze the PG-PPX protein sequence. P. gingivalis ATCC 33277 genomic DNA was purified and the PG-ppx gene was PCR-amplified, cloned, and expressed in Escherichia coli. The biochemical activities of the purified PG-PPX enzyme were characterized. Size exclusion chromatography indicated that PG-PPX forms a stable dimer in solution. Results from phosphate release assays revealed that the PG-PPX protein had potent exopolyphosphatase activities, and could hydrolyze a wide range of poly-P chain lengths: from 14-700 phosphate residues. Its ability to hydrolyze purine and pyrimidine nucleotides was characterized by analyzing reaction mixtures by anion exchange chromatography. Results showed that PG-PPX could remove the 5'-phosphate group from ATP, ADP, GTP, GDP, CTP, UTP and ITP. Our initial results suggest that PG-PPX may play several physiological roles in P. gingivalis, related to nucleotide and polyphosphate metabolism. Further biochemical and biological investigations are underway to establish its full range of functions.

Poster Number

19 **Role of BRCC36 Deubiquitylase in Choice of DNA Double-Strand Break Repair**

Ng Hoi Man (HKU)
(Supervisor: Dr. Michael SY Huen, HKU)

DNA double-strand breaks (DSBs) are perhaps the most lethal type of DNA damage. Inability to repair DSB compromises genome integrity and leads to a spectrum of human disorders, including neurodevelopmental defects, cancer-predisposition and premature aging syndromes.

To circumvent the deleterious effects of DSBs on genome stability and cell homeostasis, mammalian cells have evolved two major DSB repair pathways, namely non-homologous end joining (NHEJ) and homologous recombination (HR), for timely repair of DSBs. Intriguingly, emerging evidence indicate that a good balance between NHEJ and HR is key to genome integrity protection, and that tilting this balance leads to accumulation of chromosomal aberrations and jeopardises cell and organismal survival.

The tumor suppressor BRCA1 exists in multiple protein complexes and is emerging as a master regulator of choice between NHEJ and HR. In particular, the BRCA1-A complex, comprised of RAP80, Abraxas, BRCC45, MERIT40 and BRCC36, is targeted to DSBs and favors NHEJ over HR. Notably, mechanistically how this is accomplished remains obscure.

To dissect how the BRCA1-A complex restrains HR, we were particularly interested in BRCC36, a deubiquitylase (DUB) endowed with substrate specificity for lysine63-linked ubiquitin polymers. We found that BRCC36 suppressed HR in manners that required its DUB activity, and that inactivation of its enzymatic activity compromised retention of the BRCA1-A complex at DSBs. Our preliminary findings highlight a key role of BRCC36 in BRCA1-A complex function, and implicate its DUB activity in choice of DSB repair. Given the aberrant expression of BRCC36 amongst invasive breast cancers, targeting of the BRCC36 DUB may represent a new therapeutic intervention for the treatment and diagnosis of BRCC36-associated cancers. Our study will also provide a mechanistic basis to rationalize how inactivation of the BRCC36 DUB compromises genome stability and contributes to human tumorigenesis.

20 **Characterization of the regulation of gamma tubulin ring complex during cell cycle**

Shen Yue Hong (HKUST)
(Supervisor: Dr. Robert Z. Qi, HKUST)

As a principal microtubule (MT) nucleator, the γ-tubulin ring complex (γTuRC) contains members of GCPs. Recently several proteins have been identified as γTuRC component through the mass spectrometry. Among these proteins, CDK5RAP2 and GCP-WD play key roles in centrosomal attachment of γ-tubulin, MT nucleation, and mitotic spindle organization. Despite the functions above, CDK5RAP2 is crucial for astral MT nucleation during mitosis, while GCP-WD plays an important role in chromosome-mediated MT nucleation.
In our current studies on the regulation of γTuRC, we have uncovered CDK5RAP2 and GCP-WD exist in different γTuRC populations, and their binding with γTuRC is controlled by some regulatory proteins. For the functional study, we found these two populations of γTuRC have different MT nucleation activities both in vivo and vitro. Moreover, we found the dynamic change of CDK5RAP2 and GCP-WD-bound γTuRC composition during cell cycle. When examining the MT nucleation activity of interphase and mitotic γTuRC in vitro, these two types of γTuRC have different nucleation abilities.
Our data suggests that CDK5RAP2 and GCP-WD may be under distinct control for interacting with the γTuRC and the composition of γTuRC is changed in a cell cycle-regulated manner. It indicates different populations of γTuRC may be regulated not only by its binding proteins but also by composition change during cell cycle in order to achieve distinct functions.

Poster Number

21 **Analysis of long-range chromatin interaction and how transcription factors are involved to regulate gene expression**

Wang Yan (HKU)
(Supervisor: Dr. Junwen Wang, HKU)

DNA sequences in nucleus are highly condensed to form chromatins. Due to the compact three-dimensional conformation of chromatin, DNA sequences with large genomic distance are possible to be physically close. The spatial proximity between distant genomic regions is informative in explaining how distant cis-regulatory elements affect gene expression. With the advance of biological technologies we are able to detect the three-dimensional structure of chromosomes and long-range interaction of high resolution. Here we process human Hi-C data to draw a whole-genome interaction map with a resolution of 10kb. Next we explore how different transcription factors are involved in the long-range chromatin interaction. We define that one transcription factor mediates the interaction if it has peaks binding to both bins of an interacting pair and these peaks are enriched with Hi-C reads. We find the highly active transcription factors involved in long-range interaction are informative for the cellular function. In addition, from the colocalization of binding peaks of different transcription factors in long-range interaction sites we detect transcription factor complex. Finally, by the analysis on the enrichment of important biological marks in long-range interaction sites, including CAGE tags, histone modifications, DNA methylation and DHS sites, we find in different types of cell the most influential active regulatory marks participating in long-range interaction are differing from each other. In conclusion, we employ Hi-C data and multiple transcription factor binding profiles in different types of cell to study the role of transcription factors and histone modifications in long-range interaction and gene expression regulation.

22 **Further evidence that the 3'Untranslated Region of the CHOP mRNA possesses mRNA destabilizing effect.**

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The transcription regulatory factor known as Chop is commonly induced to express in stressed cells. In most cases, the expression of CHOP is associated with apoptosis. Although the regulation of CHOP expression at the transcriptional level has been studied to considerable details, the involvement of post-transcriptional mechanism such as mRNA stability has not been much addressed. We previously provided indirect evidence that the 3'UTR of CHOP mRNA had an mRNA destabilizing effect. The objective of this study was to provide more direct evidence to support this hypothesis. A reporter plasmid was constructed so as to express an EGFP mRNA that has its original 3'UTR replaced with CHOP-3'UTR (EGFP1-mRNA) upon transient transfection into Hela cells. The expressed EGFP1-mRNA was extracted from the transfected cells, reverse transcribed to produce the cDNA, and quantified by real time PCR. Results demonstrated that the intracellular level of EGFP1-mRNA was significantly lowered than that of the control (unmodified EGFP mRNA). To see if the relatively lower steady state level of EGFP1-mRNA were due to decreased transcription of the reporter plasmid, or more rapid degradation of EGFP1-mRNA, the degradation rates of EGFP1-mRNA and its control mRNA were measured. The results showed that the rate of degradation of EGFP1-mRNA was higher when compared to the control, hence providing further support that the 3'UTR of CHOP mRNA had an intrinsic destabilizing mechanism to prevent inadvertent expression of the CHOP gene in unstressed cells. Further experiments are being performed to confirm such a hypothesis.

Poster Number

23 **Using Polyanionic Heparin Mimetics for the treatment of COPD**

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COPD patients suffer from sustained inflammation leading to airway damage and deteriorating lung function caused by unopposed protease activity resulting from a1-antitrypsin resistant supramolecular complexes of neutrophil elastase and shed syndecan-1 in the airway. It was further shown that the action of the GAG digesting enzyme heparanase facilitates syndecan-1 shedding.

We hypothesize that the use of a polyanionic heparin mimetic would 1) displace neutrophil elastase from syndecan-1 rendering it susceptible to a1-antitrypsin inhibition and 2) inhibit heparanase activity in the airway.

In vitro tests show that the heparin mimetic successfully displaced neutrophil elastase from high molecular weight supramolecular complexes with syndecan-1. The dissociated neutrophil elastase was then rapidly inhibited by endogenous a1-antitrypsin. A similar degree of complex dissociation and resultant neutrophil inhibition was also shown to be present in BA samples of COPD patients. We also demonstrate that the heparin mimetic was capable of reducing heparanase activity and heparanase-induced syndecan-1 shedding using an air-liquid interface model of the lung.

To assess the feasibility of using the mimetic as a treatment for COPD, we treated cigarette smoke-induced COPD rats with an aerosol preparation of the mimetic. Preliminary results indicated that the number of neutrophils and neutrophil elastase amount in the lung was significantly decreased compared to rats treated with carrier alone. This suggests that our preparation can serve as a potential drug for the treatment of COPD.

24 **Timeless interacts with Parp1 to promote homologous recombination repair**

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Both Parp1 and Timeless have been implicated in DNA damage response, while there is no report that Parp1 could function together with Timeless. We have, for the first time, provided the evidence that Parp1 binds to Timeless both in vitro and in vivo. In addition, we present the crystal structure of Timeless in complex with Parp1, and demonstrate that Timeless-Parp1 complex facilitates homologous recombination repair.

Poster Number

25 **Heterochromatin Protein 1γ safeguards histone H3K27me3**

Xiong Feng (Peking Union Medical College)
(Supervisor: Professor Pinchao Mei, Peking Union Medical College)

Trimethylation of histone H3 lysine 27(H3K27me3) is associated with silenced developmental genes. The steady state level of H3K27me3 results from an interplay between the opposing function of catalytic polycomb complexes (PRCs) and the demethylases KDM6 (Utx and Jmjd3). Here we describe a role of mammalian heterochromatin protein 1 gamma (HP1γ) in safeguarding histone H3K27me3 by suppressing the demethylase activity of UTX in an interaction-dependent manner. In embryonic stem cells (ESCs), transient depletion of HP1γ abolishes global H3K27me3 levels. Moreover, primordial germ cells (PGCs) at embryonic day 10.5 exhibit a sudden loss of HP1γ that triggers genome-wide H3K27me3 demethylation through elevated UTX activity. Our findings reveal that cross-talk between HP1γ and UTX is critical to dynamic regulation of histone H3K27me3, thereby delineate an important, but, until now, unappreciated mechanistic cue in germ cell epigenetic reprogramming. Methodologically, our studies establish an ideal cellular system in which dynamic regulation of H3K27me3 levels can be further investigated without genetically and pharmacologically impairing the activities of PRCs. Furthermore, our findings provide novel insights into the biological process that enables the totipotency acquisition of PGCs.

26 **Transmembrane and Coiled-coil Domain Family 1 Is a Novel Protein of the Rough Endoplasmic Reticulum**

Zhang Chao (HKUST)
(Supervisor: Dr. Robert Z. Qi, HKUST)

The endoplasmic reticulum (ER) is a continuous membrane network in eukaryotic cells comprising the nuclear envelope, the rough ER, and the smooth ER. The ER has multiple critical functions and a characteristic structure. In this study, we identified a new protein of the ER, TMCC1 (transmembrane and coiled-coil domain family 1). The TMCC family consists of at least 3 putative proteins (TMCC1–3) that are conserved from nematode to human. We show that TMCC1 is an ER protein that is expressed in diverse human cell lines. TMCC1 contains 2 adjacent transmembrane domains near the C-terminus, in addition to coiled-coil domains. TMCC1 was targeted to the rough ER through the transmembrane domains, whereas the N-terminal region and C-terminal tail of TMCC1 were found to reside in the cytoplasm. Moreover, the cytosolic region of TMCC1 formed homo- or hetero-dimers or oligomers with other TMCC proteins and interacted with ribosomal proteins. Notably, overexpression of TMCC1 or its transmembrane domains caused defects in ER morphology. Our results suggest roles of TMCC1 in ER organization.

Poster Number

27 **The funcitonal characteristics of a Alu-derived noncoding RNA, TIFm71**

Zhang Li (CUHK)
(Supervisor: Professor Cheung Wing Tai, CUHK)

Noncoding RNAs which do not encode proteins are involved in many crucial biological processes and are increasingly important. The transcript of a novel CXC chemokine TIF (Tumor-Induced Factor) which was identified originally from mas- induced xenograft has a long 3'-UTR containing an antisense Alu element. The Alu elements are conserved repeat sequences that belong to the SINE family of retrotransposons found abundantly in primate genomes and their functions remain elusive. Our study showed that a 71nt fragment named as TIFm71 embedded in the middle of Alu element was processed out from TIF mRNA transcript and played regulatory role independently. TIFm71 was predicted to fold into a stem loop structure which is similar to pre-miRNAs. Using TIFm71-overexpressing stable cell line, we found that TIFm71 overexpression induced epithelial-mesenchymal transition (EMT) and the stable clone displayed higher cell mobility. Western protein analysis showed a higher phosphorylated ERK protein level, which suggested that TIFm71 induced EMT through ERK pathway. In addition, a GFP reporter system was used to probe the regulatory role of TIFm71 on gene expression. Comparing to the control, a significant reduction of GFP protein was noted, suggesting that TIFm71 negatively regulated the expression of the coding transcript. Our study may provide a new direction to explore functions of Alu element.

28 **Cyst formation: Mathematical Model and Data Analysis**

Zhao Yong Feng (HKU)
(Supervisor: Dr. Huang Jiandong, HKU)

Organisms always grow and develop in a precise way. It is precise in both temporal and spatial aspects. It is believed that the development of organism is under precise and complex controlling, but the underlying mechanism is still not known. A lot of efforts have been made in studying the development of branching tubular structure in lung and kidney, but little study has focused on the formation of cyst, which is a hollow spherical structure made of one layer of epithelial cells. However, some diseases such as polycystic kidney disease are related to the production of cyst. The knowledge of cyst formation will not only help us deal with these diseases, but may enable us to manipulate and engineer the epithelial tissue into certain shape and size in synthetic biology.

We are approaching this topic by investigating the process of cyst formation from both experimental and theoretical aspects. We embedded Caco-2 cell, which is an epithelial-like cell line, in jelly-like Matrigel to grow into cyst for further measurements and experiments. Our previous work has found that the mechanical force may have important role in the cyst formation process, so we attempt to build mathematical model to understand the process and propose new experiments. The model can be based on continuum mechanics, or some abstract assumptions of a discrete system. We will also apply computer vision techniques to analyze the large amount of image data from experiments, like 3D reconstruction, edge detection, and cell tracking.

Poster Number

29

Csi1p recruits alp7p/TACC to the spindle pole bodies for bipolar spindle formation

Fan Zheng (HKU)
(Supervisor: Dr. Chuanhai Fu, HKU)

Accurate chromosome segregation requires timely bipolar spindle formation during mitosis. The transforming acidic coiled-coil (TACC) family proteins and the ch-TOG family proteins are key players in bipolar spindle formation. They form a complex to stabilize spindle microtubules, mainly dependent of their localization to the centrosome (the spindle pole body/SPB in yeast). The molecular mechanism underlying the targeting of the TACC-ch-TOG complex to the centrosome remains unclear. Here, we show that the fission yeast Schizosacchromyces pombe TACC ortholog alp7p is recruited to the SPB by csi1p. The csi1p interacting region lies within the conserved TACC domain of alp7p while the carboxyl-terminal domain of csi1p is responsible for interacting with alp7p. Compromised interaction between csi1p and alp7p impairs the localization of alp7p to the SPB during mitosis, thus delaying bipolar spindle formation and leading to anaphase B lagging chromosomes. Hence, our study establishes that csi1p serves as a linking molecule tethering spindle stabilizing factors to the SPB for promoting bipolar spindle assembly.

Theme 2: Cancer & other diseases

Poster
Number

30 Role of FE65 phosphorylation on APP processing

Chow Wan Ning Vanessa (CUHK)
(Supervisor: Dr. Lau Kwok Fai, CUHK)

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by accumulation of amyloid-β peptide (Aβ) and hyperphosphorylated tau protein in the brain. Genetic and biochemical research revealed that the generation of Aβ from its precursor protein, amyloid precursor protein (APP), is crucial to AD pathology. APP is a type I transmembrane protein with a large ectodomain. Sequential cleavage of APP by β- and γ-secretases yields the toxic Aβ fragment, and the process is known to be regulated, at least in part, by a number of APP-interacting proteins including FE65. FE65 is a brain-enriched adaptor protein that binds to the APP intracellular domain (AICD). Although it is known that the interaction between APP and FE65 modulates APP processing, the molecular mechanisms by which the interaction is regulated remain elusive. Since FE65 is a phosphoprotein, and protein phosphorylation is a common post-translational modification that modulates protein-protein interaction, we therefore hypothesized that phosphorylation of FE65 plays a role in regulating FE65-APP interaction. In the study, we evaluated the effect of a reported phosphorylation site of FE65 on FE65-APP interaction and APP processing. Here, we demonstrated that phosphorylation of FE65 alters its binding affinity to APP, the APP processing pathway and APP turnover rate. Our study reveals a novel mechanism by which APP processing is being modulated.

31 Use of Myoglobin Crystals to Enhance the Sensitivity of Radiation Therapy

Heater Brad (CUHK)
(Supervisor: Professor Michael Chan, CUHK)

Radiation therapy is still one of the most effective treatments for malignant tumors, however, severe side effects are associated with this therapy and some tumors are radiation insensitive, making them resistant to such treatments. This is partially due low cellular oxygen, and past experiments have shown that increasing oxygen in tissues increases radiation effectiveness. In this study, we develop a novel method with the potential to treat tumors by delivering myoglobin crystals to cancer cells, making them more sensitive to radiation therapy by increasing the cellular oxygen concentrations. Myoglobin is fused to Cry3A, a crystal forming protein which will improve cellular uptake and prevent proteolytic cleavage of myoglobin, and we report these myoglobin crystals to be properly folded, and to bind and release oxygen similar to wild type. Then, the uptake of human liver cancer, human liver wild type, and human lung carcinoma cells were examined by flow cytommetry using GFP crystals. We also tested the liver cells radiation sensitivities using the standard MTT assay. Next, we must test if myoglobin crystals will increase this sensitivity. If successful, this treatment would allow patients to receive a lower dose of radiation, which would reduce harmful side effects like hair loss and nausea. This strategy takes advantage of an already effective therapy in treating cancer, and such treatments are needed to circumvent the problem until a more permanent solution is discovered.

Poster Number

32 **RGS20 as a modulator of tumorigenesis and angiogenesis**

Manton LEUNG, Lei YANG, Yung H. WONG
Division of Life Science, State Key Laboratory of Molecular Neuroscience, and the Biotechnology Research Institute, HKUST

RGS (regulator of G protein signaling) limits G protein signals by accelerating GTPase activity on the G subunit. RGS20 was found to be expressed or up-regulated in ovarian cancer cell lines or metastatic melanoma (Riker et al., 2008, BMC Medical Genomics, 1:13; Hurst et al., 2009, Cell. Mol. Biol. Lett., 14:153). These observations suggest that RGS20 may play a role in cancer formation. However, the role of RGS20 on tumor development remains unclear. To investigate the actions of RGS20, Yang has stably overexpressed RGS20 in MDA-MB-231 human breast cancer cells. MDA-MB-231/RGS20 cells exhibited elevated cell migration and survival rate, implying increased tumor metastasis and perhaps tumorigenic potentials. Furthermore, RGS20 can apparently interact with vascular endothelial growth factor B (VEGF-B), a potential angiogenic factor, in the yeast two-hybrid assay. Interestingly, RGS20 enhanced VEGF-B expression while its close relative, RGS19, failed to do so. Taken together, these results support the notion that RGS0 may participate in cancer development and angiogenesis. (Supported by 2013CB530900 and HKUST 663412)

33 **In vitro anti-tumor effect of jacaric acid, a conjugated linolenic acid isomer, on human eosinophilic leukemia EoL-1 cells by inducing apoptosis and differentiation**

Liu Wai Nam (CUHK)
(Supervisor: Professor Leung Kwok Nam, CUHK)

Conjugated fatty acids (CFA) refer to the positional and geometric isomers of polyunsaturated fatty acids with conjugated double bonds. Examples of naturally-occurring CFA include conjugated linoleic acid (CLA) from ruminant meats and dairy products and conjugated linolenic acid (CLN) from plant seed oils. Previous studies from other groups have demonstrated both in vitro and in vivo anti-tumor activities of CFA on various human cancers. Nevertheless, the anti-tumor effects and action mechanisms of CLN on human myeloid leukemia cells remain poorly understood.

In the present study, the growth-inhibitory effect and molecular action mechanisms of jacaric acid, one of the CLN isomers, were examined using the human eosinophilic leukemia EoL-1 cells. Our results showed that jacaric acid exhibited anti-proliferative effect on EoL-1 cells in a time- and concentration-dependent manner. Flow cytometric analysis indicated that jacaric acid could trigger cell cycle arrest at G0/G1 phase, accompanied by a decrease in the protein expression levels of cyclin E and CDK2. In addition, we found that jacaric acid could trigger apoptosis in EoL-1 cells through the induction of DNA fragmentation, phosphatidylserine externalization and mitochondrial membrane depolarization. Up-regulation of Bax and caspase-3 proteins and down-regulation of Bcl-2 protein might account for the induction of apoptosis. Interestingly, jacaric acid also induced morphological differentiation in EoL-1 cells and enhanced the expression of two eosinophil-specific proteins, EPO and MBP.

Collectively, we showed that the anti-proliferative effect of jacaric acid on EoL-1 cells might be due to the induction of cell cycle arrest, tumor cell apoptosis and leukemic cell differentiation.

Poster Number

34 **Use of a Tunable Resistive Pulse Sensing System to Detect Drug Response from the Mitochondria at Organelle Level**

Loo Fong Chuen (CUHK)
(Supervisor: Professor Kong Siu Kai, CUHK)

Single organelle analysis from cancer cells provides a better understanding of the drug effects from the isolated organelles for anti-cancer drug development. Since mitochondria play a key role in regulating the life and death of cancer cells, we have developed a nanometer-scale sensing technique using the Tunable Resistive Pulse Sensing (TRPS) system to compare the drug response from single mitochondrion (diameter around 500 nm) isolated from the liver cancer cells with or without multidrug resistance (MDR).

This TRPS technique is based on the detection of blockade events when mitochondria pass through a tunable nano-pore for the change in time duration and current magnitude after drug treatment. In this presentation, the use of the TRPS system for the detection of mitochondrial response upon drug treatment will be highlighted. We first optimized the experimental conditions and verified the system by gold nanoparticles, and then applied the system to detect the drug response from single mitochondrion isolated from cancer cells. We found that more mitochondrial damages include mitochondrial swelling and change in surface charge were observed in the cancer cells with MDR when compared to that without MDR after anti-cancer drug candidate polyphyllin D treatment.

35 **The enzymatic mechanism mediating the breakdown of glycogen in hypoxic tumor cells**

K.L.Mung[1] , N.S.Wong[1]
1.Department of Biochemistry, The University Of Hong Kong

Glycogen has been recently implicated as a major source of metabolic fuel for hypoxic tumor cells. The biochemical mechanism underlying the breakdown of glycogen to produce the substrate for glycolysis though is not fully understood. The aim of this study is to demonstrate the potential role of glycogen phosphorylase or α-glucosidase in mediating breakdown of intracellular glycogen in hypoxic tumor cells. The deprivation of glucose in normoxic tumor cells over a period of 6 hours resulted in marked reduction of intracellular glycogen level. Imposing hypoxia (1% O2) on these cells during the last three hours of glucose deprivation accelerated the decrease of intracellular glycogen content. The co-incubation of cells with a specific glycogen phosphorylase inhibitor (GPI) but not a α-glucosidase inhibitor (miglitol) prevented the loss of intracellular glycogen seen in glucose-deprived cells under normoxic condition. However, GPI or miglitol alone or together had no effect on the enhanced loss of glycogen seen in the hypoxic cells. Results of in vitro enzyme assays for glycogen phosphorylase activity extracted from hypoxic cells showed that both the AMP-independent and the AMP-dependent component of the enzyme activity could be effectively inhibited by GPI. Our data suggest that the degradation of intracellular glycogen in hypoxic tumor cells is unlikely to be mediated by either glycogen phosphorylase or α-glucosidase. Further confirmation of this hypothesis will be achieved by knockdown experiments of glycogen phosphorylase expression decreased after hypoxic treatment.

Poster Number

36 **A Novel Aptamer-Based Enzymatic Assay for the Diagnosis of Malaria**

Roderick Dirkzwager (HKU)
(Supervisor: Dr. Julian A Tanner, HKU)

Malaria causes nearly 1 million annual fatalities in lesser developed regions in South East Asia and Africa. Symptoms of malaria are unspecific (fever, vomitting, headache etc.) meaning without laboratory equipment reliable diagnosis is problematic. Point-of-care antibody-based dipsticks are available on the market but the cost and instability of antibodies in heat and humidity limits their widespread use. Aptamers are short oligonucleotide sequences which selectively bind their targets and present as stable and affordable alternatives to antibodies in point-of-care tests. Aptamers against the malaria antigen Plasmodium falciparum lactate dehydrogenase (pLDH) have been developed in our lab with nanomolar Kd affinities and are specific enough to not interact with human LDH isoforms. We have incorporated these aptamers into a novel 96-well plate based malaria test called the aptaMAL. Surface bound aptamers capture pLDH out of blood samples and after wash steps and reagent addition, the solution turns blue if the test is positive. The colour is achieved by coupling a colour changing reaction to the enzyme activity of pLDH. The test has been found to be effective on rat blood samples spiked with the pLDH antigen at very low concentrations (aptaMAL limit of detection 10 ng/mL, typical pLDH concentration in infected blood 3000 ng/mL). This aptaMAL mechanism shows potential to be incorporated into a point-of-care device format which would be affordable, reliable and have no need for any complex equipment or unstable reagents.

37 **Nuclear translocation of HDAC4 in Alzheimer's disease human brain**

Shen Xuting (HKUST)
(Supervisor: Professor Karl Herrup, HKUST)

Histone deacetylases (HDACs) play a crucial role in histone modification whose inappropriate function has been linked to Alzheimer's disease (AD). HDAC4, as a member of the family of HDACs, presents predominantly in the cytoplasm of the neurons of the human brain. As our lab has shown previously, its nuclear translocation is mediated by a decreased level of ATM (ataxia telangiectasia [A-T], mutated), a multi-functional protein kinase whose deficiency leads to A-T. Based on the fact that both A-T and AD are neurodegenerative disease involving modification of the histone code, there arises the possibility that ATM may play a role in AD. Using the nuclear translocation of HDAC4 as a marker, previous work has proved the involvement of ATM deficiency in AD diagnosed with clinical dementia rating (CDR) system. Extending that work, we performed immunohistochemistry on the human brain samples grouped by Braak stages. This staging scheme is based largely on the extent and location of deposits of hyperphosphorylated tau. We found a significant decrease in ATM activity in hippocampus between groups of age-matched controls with normal cognition, mild cognitive impairment (MCI) and AD. Consistent with previous work, we found that the CA2 sub-region was the area with the highest levels of nuclear HDAC4. Extending this work, we show for the first time a substantial correlation of this phenotype with the presence of ectopic cell cycle markers. Taken together, the results confirm the importance of the decrease in ATM activity during the pathogenesis of AD.

Poster Number

38 **Molecular and Cellular Consequences on Osteoblast Function of an IFITM5 Mutation Causing OI Type V**

Wong Ka Wai (HKU)
(Supervisor: Dr. Michael To, HKU)

Osteogenesis imperfecta (OI) is a group of hereditary skeletal disorders with varying severity and phenotypes, mostly casued by mutations of type I collagen biosynthesis. OI type V shows co-occurrence of typical OI phenotypes including osteopenia and frequent bone fractures, together with over-calcification phenotypes including formation of hyperplastic callus, calcification of forearm interosseous membrane, etc, during bone fracture repair.
All OI type V share a heterozygous dominant mutation (c.-14C>T) of interferon-induced transmembrane protein 5 (IFITM5). This creates an in-frame upstream start codon which adds five amino acid residues to the N-terminus of protein product, bone-restricted interferon-induced transmembrane protein-like protein (BRIL). Since BRIL is highly enriched in osteoblasts, and in vitro Ifitm5 knockdown inhibits bone mineralization, IFITM5 is suggested as a novel candidate involved in regulation of bone formation and mineralization, with unknown molecular mechanism for its functions. Thus, the pathogenesis of this mutation is largely unknown.
This study focuses how molecular and cellular consequences of BRIL mutation would alter patient osteoblast activities responsible for OI type V phenotypes using in vitro approaches. Preliminary analyses on osteoblasts derived from Hong Kong OI type V patient are reported. OI type V osteoblasts show reduced and mis-localized BRIL immunofluorescence. They are closer to pre-osteoblast status and have delayed onset of in vitro mineralization upon induction when compared to control. These suggest possible gain-of-function nature of IFITM5 mutation that alters OI type V osteoblast maturation and mineralization events as consequences. Interaction network of mutant BRIL and molecular events in patient osteoblast differentiation would be crucial to understand the molecular impact brought by the mutation.

39 **Immuno-modulatory effect of photodynamic therapy in cancer treatment**

Yeung Hing Yuen (CUHK)
(Supervisor: Professor Wing-Ping Fong, CUHK)

Photodynamic therapy (PDT) is a kind of cancer treatment. It involves the injection of photosensitizer into the patient's blood stream, followed by the application of light on the tumor site. The photosensitizer itself is non-toxic without activation. In the presence of light, it is capable of converting molecular oxygen into reactive oxygen species (ROS). Thus, the precise application of light onto the tumor site could kill the cancer cells via the generation of ROS in the local vicinity without affecting the healthy tissue. Similar to chemotherapy, PDT mediated direct cytotoxic effect on the cancer cells could lead to the eradication of localized tumor. However, PDT regime focusing on the localized cytotoxic effect could not treat the patient when the tumor has already metastasized or patients may sufffer from cancer relapse after the treatment. Nonetheless, these are the main causes of death of cancer patients. Recent studies demonstrated that some PDT regimes could enhance the immunogenicity of the dying cancer cells. Also, the immuno-competent mice whose tumors have been cured by PDT were shown to resist cancer relapse in tumor re-challenge tests. Hence, PDT regime that flavours the generation of anti-cancer immunity is under investigation. Here we show that performing PDT with unsymmetrical bisamino silicon(IV) phthalocyanine BAM-SiPc, a novel photosensitizer synthesized recently, could enhance the immunogenicity of the cancer cells. In addition, tumor bearing Balb/c mice cured by BAM-SiPc could resist cancer relapse in the tumor re-challenge test. Therefore, BAM-SiPc is a novel photosenstizer worth further investigation into its potential clinical application.

Theme 3: Development & stem cells

Poster Number

40 **Sequential expression of Lgr5 and Col22a1 in developing synovial joints marks the progressive differentiation of progenitor cells to articular chondrocytes**

Feng Chen (HKU)
(Supervisor: Professor Danny Chan, HKU)

Healthy articular cartilage in synovial joints provides a smooth, wear-resistant structure that reduces friction and absorbs impact forces. They are enclosed in joint capsules, containing a fibrous connective outer layer and a synovial inner layer, and stabilized by ligaments and tendons. Degenerative joint diseases involve destruction of the articular cartilage. Damaged articular cartilage is difficult to heal due to their poor regenerative capacity, leading to widespread suffering from arthritis and joint injuries. A clear understanding of how a synovial joint develops and the progenitor cells that contribute to its formation and maintenance is essential for the development of therapeutic strategies for degenerative joint diseases. We identified a stem cell marker, Lgr5, expressed specifically in interzone cells at the earliest stage of joint formation. We showed that Lgr5-expressing (Lgr5+) interzone cells are progenitors that contribute to the formation of the supporting tissues such as the ligaments and synovial membrane, and more importantly to specific regions of the articular cartilage surfaces. We further identified that cells co-expressing Lgr5+ and an extracellular matrix gene, Col22a1 (Lgr5+/Col22a1+ double-positive cells) are progenitors committed to becoming articular chondrocytes. Available mice with molecular tags for Lgr5 and Col22a1 expression will allow the isolation of these specific cell pools in a developing joint to gain an understanding of the molecular signature and the signals that regulate this lineage progression and the maintenance of the interzone cells.

41 **Quantitative characterization of mesenchymal cell aggregation process**

Hu Jianjiang (HKU)
(Supervisor: Professor MH Sham and Dr. Wei Huang, HKU)

Mesenchymal cell aggregation (or named ‘condensation’ in developmental biology) is a fundamental phenomenon in a wide variety of physiological and pathological processes. Although the mechanisms of mesenchymal cell condensation in kidney, vertebrate and limb bud development are still not clear yet, scientists have found ways to create the mesenchymal cell aggregates in vitro for tissue engineering applications. Mesenchymal cell aggregation with proper size and timing is key to the efficiency of subsequent differentiation and generation of functional cells. Nevertheless, there is still little knowledge on the general principles controlling the aggregate formation and the determination of their size and time. The research aim of this study is to quantitatively characterize mesenchymal cell aggregation process in order to identify its governing principles. By combining mathematical analysis and modelling tools with image analysis tools, several quantitative analysis methods have been established. Based on these methods, we identified a unique aggregation principle, and developed approaches to tune the aggregation process based on this principle. Further research will be focused on the correlations of mesenchymal stem cell differentiation efficiency and tuning of the aggregation process.

Poster Number

42 **The zebrafish scale as a possible model for studying bone / plasma Ca^{2+} exchange.**

Jacky T.S. Hung[1], Alessandro Rubinacci[2], Joseph Kunkel[3], Alan Shipley[4], Sarah E. Webb[1] and Andrew L. Miller[1,5]

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In teleosts such as zebrafish, the elasmoid scales are known to be important reservoirs of Ca^{2+} via the presence of a significant amount of hydroxyapatite, (which acts as the primary store of Ca^{2+} in both bones and scales) although the extent of their contribution to either short or long-term regulation of Ca^{2+} homeostasis, when compared with the bony skeleton, is not yet clear. The scleroblasts that ensheath the scales are proposed to be responsible for controlling Ca^{2+} mineralization (influx) and mobilization (efflux). Indeed, it has been suggested that the scales may play a more significant role in Ca^{2+} homeostasis in fish, than the axial skeleton. In this project, the scanning ion-selective electrode technique (SIET) was employed to measure Ca^{2+} fluxes in zebrafish scale samples at different extracellular [Ca^{2+}]. The SIET system makes use of a self-referenced vibrating microelectrode, thus, it is able to non-invasively measure ion fluxes at the pmole/cm^2/sec range from samples in real-time and with a spatial resolution of ~5 µm. On the episquamal side of scales exposed to a hypercalcemic (3 mM) or hypocalcemic (0.01 mM) bathing medium, a steady Ca^{2+} influx and efflux were measured, respectively. On the other hand, on the hyposquamal side of scales, a steady Ca^{2+} efflux was measured at all concentrations of Ca^{2+} in the bathing medium. These preliminary data suggest that the zebrafish scale might be a useful model for studying mammalian (and ultimately human) Ca^{2+} exchange and plasma homeostasis.

43 **Inorganic Polyphosphate as a Forgotten Molecule in Osteoblasts - From Synthesis to Function**

Khong Mei Li (HKU)
(Supervisor: Dr. Julian A Tanner, HKU)

Mineral and organic constituents crucial for cellular growth, development and survival are generally conspicuous with the exception of inorganic polyphosphate (polyP). PolyP, a linear polymer of many phosphate residues linked by energy-rich phosphoanhydride bonds, remained a largely 'forgotten' molecule albeit being ubiquitous and significantly crucial to the survival of living organisms. In the last two decades, major clues about the role polyP have in a plethora of prokaryotic biological processes unravelled with the introduction of novel enzyme-based assays. However, the origin and precise machinery of polyP in humans remain largely elusive. This research aims to discover the mechanisms of polyP as a fundamental molecule in osteoblasts, from its synthesis to function. The classic Clark's polyP extraction method was modified to successfully extract polyPs of different chain lengths from osteoblast-like SaOS-2 cells, for study of differential physiological effects exerted by varying polyP chain lengths. Unexpectedly, addition of standard-length polyphosphates into SaOS-2 cell extracts yielded a pull-down of polyP-interacting proteins which were subsequently identified using peptide mass fingerprinting and MS/MS sequencing. As a secondary line of evidence, future work is underway towards identification of polyP-interacting proteins by the development of a polyP-specific affinity column. This method will incorporate a facile approach of chemically cross-linking the terminal phosphate group of polyP with a primary amine-linked biotin, via phosphoramidate linkage, for attachment to streptavidin-coated beads. This work will not only present a comprehensive overview of the multi-functional roles polyP has in osteoblasts but also provide a broader perspective of polyP's functions in humans.

Poster Number

44 Using CRISPR/cas9 system to investigate the functions of foxk2 during zebrafish development

Lau Wangchi (CUHK)
(Supervisor: Dr. Kwan Kin Ming, CUHK)

The forkhead factors are a large group of transcription factors that serve various important roles during different biological processes. Despite decades of researches on their functions during development, the functions of only a few forkhead transcription factors have been clearly understood. Foxk2 is a novel forkhead factor that has been proposed to have important roles during cell cycle progression and DNA repairing from cell culture and in-vtiro assays, even though its functions during development are poorly understood. In zebrafish, foxk2 were found to be robustly expressed in the central nervous system (CNS). Knockdown of foxk2 by morpholino led to slight CNS hypoplasia and upregulation of p21, myc and max in the brain. Detailed examinations of the specific neural tissues such as optic tectum indicated the population of neural stem cells was increased in the expense of neurogenesis in morphants. These altogether suggest that foxk2 may regulate the neural stem cell fate decision during zebrafish embryogenesis. To further understand the long term consequences of foxk2 depletion on neural stem cells maintenance, foxk2 knockout zebrafish are generated by CRISPR/cas9 system. Moreover, a GFP tag will be used to fuse with the endogenous zebrafish foxk2 to create a foxk2-GFP allele so that the protein expression of foxk2 can be investigated in detail.

45 Collagen II in native nucleus pulposus (NP) tissue may suppress COL2A1 expression in NP cells

Lee Juliana Tsz Yan (HKU)
(Supervisor: Professor Ken Cheung and Dr. Victor Leung, HKU)

Biomimetic scaffolds have been implicated in tissue engineering. They have been shown superior in inducing stem cell differentiation or promoting native cell phenotype. It is believed that the nucleus pulposus (NP) phenotype can be better maintained or enhanced when NP cells are cultured on a scaffold derived from native NP tissues. However, this has not been proved or disproved directly. With the use of freeze-dried NP sections, we showed that some NP marker expressions of cells on NP sections were suppressed compared with cells directly cultured on plastic, which is against the common belief. We found in in vitro assays with cells on cell culture plastic that the use of collagen I or collagen II coating may reduce the COL2A1 mRNA expression in NP cells. Collagen II is one of the major extracellular matrix components of NP tissue but its expression at mRNA level was suppressed when NP cells were grown on this protein. We speculate that there may be some kinds of negative regulatory mechanisms involved in the expression of COL2A1. Our results demonstrate that biomimetic scaffolds do not necessarily enhance NP marker expressions at mRNA levels. There have been efforts from different groups in creating biomimetic scaffolds using COL2A1 as one of the NP markers to assess the biomaterials but our study suggests the necessity of a better understanding of the underlying regulating mechanisms which is absent in the field currently.

Poster Number

46

The role of Sox10 in enteric neural crest cell migration

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Sox10 is a transcription factor essential for maintaining the multipotency of enteric neural crest cells (ENCCs). Sox10 mutant ENCCs fail to colonize the entire gut due to premature differentiation and migration defects. By time-lapse live-cell imaging analysis, we observed that *Sox10* $^{NGFP/+}$ ENCCs formed abnormal aggregates within the gut. In gut explant culture study, we found that mutant ENCCs displayed abnormal interactions on extracellular matrix with enhanced intercellular adhesion properties. Moreover, microtubules elongation seemed to be disrupted in mutant ENCCs when gut explants were stained with α-tubulin. We hypothesize that Sox10 may regulate ENCCs migration through modulating cell adhesion. To address the roles of Sox10 on cell-cell interactions, we examined the expression of candidate molecules including Cadherin and Eph/Ephrin family members by qPCR analysis. Cadherins engage in cell-cell junction formation. Disruption of Cadherin impaires cell-cell contact and adhesion properties. Ephrins/ Eph receptors are thought to mediate cell-cell repulsion to regulate directional migration of neural crest cells. We found that Cadherin-10/11 and EphA2/A4/B1/B2 expression were significantly changed. To access whether they are transcriptionally regulated by Sox10 involved in ENCC adhesion, we determined EphA2/A4 expression profile by immunostaining and found that they were expressed in both mesenchymal cells and ENCCs. Their ligand ephrinB2 was expressed in ENCCs, suggesting that ephrinB2 and EphA2/A4 interaction may be required for ENCC migration. Altered EphA2/A4 expression affects cell-cell interaction and may lead to reduced repulsion responses with neighboring cells. Our findings suggest a potential role of Sox10 in modulating ENCC adhesion by controlling EphA2/A4 expression.

Poster Number

47

Role of autophagy in chondrocyte differentiation

Lie Yuliana S (HKU)
(Supervisor: Professor Danny Chan and Professor KSE Cheah, HKU)

Maintaining cell homeostasis during cellular differentiation is critical for the cell survival. Therefore, the balance between protein biogenesis and degradation is tightly regulated. The removal of the after-used and unwanted substances is not only important for protein turnover but also in regulating cellular differentiation and developmental process. The degradation of protein relies on two well-known systems, the Ubiquitin-proteasome system (UPS) and Autophagy-lysosomal system (ALS). Here, using the unique organization of the growth plate that depicts temporal and spatial "life time" of chondrocytes during their differentiation process, we investigate the role of protein degradation systems during endochondral ossification. Both degradation systems are active during normal chondrocytes differentiation with different level of activity. Interestingly, ALS, in contrast to UPS, it is dynamically regulated in normal growth function. While ALS-related genes are expressed in all zones of the growth plate, autophagy activity is differentially activated during the differentiation process, being high in proliferating chondrocytes, lowest in hypertrophic chondrocytes, but increases again at the cartilage-bone junctions.

Using a mouse model (13del) for metaphyseal chondrodysplasia type Schmid (MCDS), ALS was further studied to better understand its roles in chondrocytes differentiation under pathological condition. In addition, in 13del mouse (MCDS mouse), autophagy is also found to participate in allowing stressed hypertrophic chondrocytes (HCs) to undergo "cell reprogramming" process, which was observed as a mechanism for HCs to alleviate ER-stress caused by the expression of unfolded mutant type X collagen protein. The full clearance of the abnormal protein is allowing stressed HCs to "complete" reprogram to less differentiated status. To address the role of autophagy in this context, we modulated autophagy activation using Rapamycin administration or by genetic inactivation of p53. Rapamycin enhances autophagy by inhibiting mTOR pathway while p53, which is up regulated in stressed HCs, is also known to regulate autophagy. Rapamycin treatment showed only mild activation of autophagy with no observable differences in HCs differentiation at p10. Inactivation of p53 in 13del mice indicated an effect on chondrocyte differentiation, but changes in autophagy were not observed. In conclusions, our descriptive analysis showed an association of autophagy to chondrocyte differentiation process, but mechanistic insights require more specific perturbation of autophagy pathway with additional drugs and genetic studies such as inactivation of Atg5 at specific differentiation stages of chondrocytes in both wild type and 13del mice.

Poster
Number

48

The roles of Irx3 and Irx5 genes in inner ear sensorineural patterning

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Irx3 and Irx5 are members of the Iroquois family TALE homeodomain transcription factors, which function as patterning genes in multiple developmental processes. In the developing limb bud, Irx3 and Irx5 are critical for establishing early AP polarity and digit specification. In the developing inner ear, we have found that Irx3/5 compound mutant displayed enlarged cochlear lumen, abnormal spiral ganglion, and fusion of inner ear sensory regions. These abnormal phenotypes suggest that Irx3 and Irx5 may have essential role in early inner ear patterning.

The mouse mutant Irx3tauLacZ with β-gal reporter was used to examine the expression of Irx3 in otic vesicle and cochlear epithelium. At E10.5, Irx3-LacZ signal was restricted to medial half of anterior otocyst, which is the neural-sensory region, and extended to posterior-lateral region. At E16.5, Irx3 was expressed in the entire otic epithelium, and became very strong in the lateral wall. At both stages, there were no Irx3-LacZ positive cells in the CVG or spiral ganglion. Irx5 gene showed very similar expression patterns as Irx3. Considering the phenotypes in Irx3/5-/- mutant, and the expression pattern of these two genes, we hypothesize that Irx3 and Irx5 control inner ear patterning from early otocyst stage by regulating neuro-sensory cell competence.

To understand how Irx3 and Irx5 genes affect sensory domain specification and neuroblast delamination, mutant otocyst were analyzed with markers for neuro-sensory fate. Expansion of Sox2-positive domain and loss of posterior Pax2 expression region revealed that the neuro-sensory competent domain was shifted and changed its shape in Irx3/5-/-. Moreover, Irx3/5-/- otocyst showed increased NeuroD positive cell with ectopic stream of delaminating neuroblast. In consequence, Tbx1, which could suppress neurogenesis, became more restricted to the posterior otocyst. These results indicate that Irx3 and Irx5 are required for proper sensory specification and neurogenesis at otic vesicle stage.

Our further study will focus on how Irx3 and Irx5 affect the patterning of the cochlear epithelium and what causes the fusion of saccule and organ of Corti. BMP signaling is a potential regulatory pathway that could maintain proper sensory/non-sensory boundary, and lost of Bmp4 expression domain has been observed in Irx3/5-/- cochlea. Whether BMP signaling is affected in Irx3/5-/- cochlea or other factors will contribute to the abnormal cochlea development in Irx3/5-/- will be further investigated.

Poster Number

49 **Phylogenetic Analysis of Planarian Collagens and their Roles in Regeneration**

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Stem cells are regulated by the microenvironment or niche they reside in, which consists of growth factors, niche cells and the extracellular matrix. The ECM acts as both a structural component and as a reservoir for growth factors that are released upon degradation. During regeneration, stem cells in the planarian are activated to migrate and proliferate; however, the role of the ECM in stem cell regulation is still unclear. Analysis of an EST library of planarian transcripts revealed nine fibrillar-related collagen chains (DjCol1-9). Sequence and structural analysis reveal interuptions in the triple helical domain, the functions of which are unknown. Bioinformatic and phylogenetic analysis reveal that the fibrillar A and B clades are present in the planarian. Proteomics analysis of the blastema of the planarian Dugesia japonica showed that DjCol1, DjCol2, DjCol4 and DjCol6 are dynamically upregulated. Djcol1 and -2 are co-expressed with piwi expressing stem cells in the intact worm but are not co-expressed in stem cells of the regenerating blastema. This is an interesting collagen expression pattern in stem cells, suggesting a potential functional relationship in cell maintenance and regeneration. Indeed, knockdown of Djcol1 and -2 by RNAi showed increased number of piwi expressing cells in the regenerating blastema and evidence of enhanced regeneration capacity. On the other hand, Djcol3 and -4 are expressed in supepidermal differentiated cells, and are expressed slightly later during regeneration, suggesting a differing role in the regeneration process. Djcol5 also shows similar expression pattern to the other four collagens. This study provides the first insight into the role of ECM in the regulation of stem cell property/function, with collagens as a negative regulator.

50 **The role of extracellular matrix in planarian regeneration**

Shen Yun (HKU)
(Supervisor: Professor Danny Chan, HKU)

As an important niche component, the role of extracellular matrix (ECM) in stem cell biology is well recognized, however, its role in tissue regeneration is not well understood. Planarians are able to regenerate any missing parts of the organism and this feat is thought to be contributing by its large population of stem cells, which are distributed throughout the inner mesenchymal region. Here we use planarian as the model system to study the dynamic protein expression changes during tissue regeneration in order to gain insights into the role of ECM in regeneration.

Using a novel time-lapse microscopy, I demonstrated that the process of planarian blastema development is coordinated with the proliferation and differentiation of stem cells and also the reorganization of tissue components. Proteome expression profiling of the blastema during the first 4 days of regeneration revealed that many ECMs were dynamically expressed during this process suggesting the regulation of matrix assembly and remodeling is important.

Expression analysis of two collagen chains, renamed Dj-col1 and Dj-col2 showed that they are co-expressed with piwi expressing stem cells in intact planarians; however, in blastema, their co-expression with piwi positive cells become delinked. Knock-down them by RNAi amputated planarians showed “faster regeneration”. Quantitative analysis indicated there was a general increase in the number of piwi expressing stem cells following RNAi treatment. Together, these findings demonstrated these collagen chains acts as negative regulators of stem cells and regeneration and provide the first insight into the role of ECM in the regenerative processes of planarians.

Poster Number

51 **sPDZD2: A Novel Negative Modulator of Hedgehog Signaling**

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PDZD2 is a multi-PDZ domain-containing protein of unknown function in early development. It is proteolytically cleaved to generate its secreted form, sPDZD2. Human PDZD2 is mapped to chromosome 5p13.2, which co-localizes with the disease-associated gene in a family of Brachydactyly Type A1 (BDA1) patients, suggesting involvement of PDZD2 in limb development. Hedgehog (Hh) is an important morphogen that dictates tissue patterning during embryonic development and recent studies showed that mutations in Indian Hedgehog (IHH) resulted in BDA1. Interestingly, in situ hybridization revealed that Pdzd2 was expressed in the distal mesenchyme partially overlapping with Shh in mouse limb bud. During digit patterning, Pdzd2 was expressed in the interzone that flanked the Ihh/Gli1-expressing phalanx condensation. Moreover, Pdzd2 was expressed in the paraxial mesoderm adjacent to the differentiating neural tube. It is worth noting that PDZD2 protein was detected at the neural tube away from its site of synthesis, indicating a non-cell autonomous role of PDZD2 possibly via sPDZD2. Pdzd2 expression in various Hh-active tissues in mouse and chicken suggested an evolutionary conserved role of Pdzd2 in modulating general Hh signaling during early development.
Functional studies showed that overexpression of sPDZD2 in the chicken neural tube leads to down-regulation of NKX2.2 and OLIG2 expression. sPDZD2 was shown to counteract the ectopic NKX2.2 expression induced by long-range signaling of ectopic HH. Consistently, sPDZD2 exhibited an inhibitory effect on SHH-induced reporter activity in a Gli-luciferase cell line. Taken together, our results provided the first evidence that sPDZD2 is a negative modulator of Hedgehog signaling.

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52 **Sufu and Gli3 repressor mediate the temporal basal-to-apical progression of hair cell differentiation in mammalian cochleae**

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The Sonic Hedgehog pathway plays important roles in mammalian inner ear development. Mutations of Shh, Smo and Gli3 lead to severe defects in mouse inner ear morphogenesis. However, knockout of Gli2 does not affect inner ear morphology or cochlear hair cell differentiation, suggesting that the Gli repressor function may be required for Hedgehog signaling during inner ear development. Sufu is a negative regulator of Hedgehog signaling and it functions to repress Gli activator and enhance Gli repressor activities. To evaluate the involvement of Sufu and Gli transcription factors in mediating cochlear hair cell differentiation, we have analyzed the Pax2Cre;Sufuflox/flox, Gli3P1-4/P1-4 and Gli3Δ699/Δ699 mutants using hair cell marker Myosin7a and supporting cell markers Sox2, P75 and Jag1. At E16.5, only one row of inner hair cells could be observed at the basal region of cochleae in the Pax2Cre;Sufuflox/flox mutants. Nevertheless, normal hair cells appeared at the medial region at E18.5, indicating that deletion of Sufu delays cochlear hair cell differentiation. Gli3 repressor is abolished in the Gli3P1-4/P1-4 mutant, in which cochlear hair cell differentiation was delayed. Interestingly, in the Gli3Δ699/Δ699 mutant with excessive Gli3 repressor, hair cell differentiation was accelerated in the apical region of the cochlear duct. Our results suggest that Sufu and Gli3 repressor are essential factors which regulate the temporal basal-to-apical progression of cochlear hair cell differentiation, supporting that Sonic Hedgehog signaling is required to control the dynamics of hair cell differentiation.

Poster Number

53 **N16, a nacreous protein, inhibits osteoclast differentiation and enhances osteogenesis**

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Osteoporosis is a disease characterized by a significant loss of bone mass which leads to higher bone fragility and is attributed to the imbalance between bone resorption and formation. Organic matrix of nacre has been shown to regulate calcification during shell formation and the water-soluble components facilitate bone repair by inducing osteoblast differentiation and bone formation in vitro and in vivo. In this study, the effects of N16, a nacreous protein, on osteoclast differentiation and osteogenesis were investigated using the pre-osteoclast cell line RAW264.7 and pre-osteoblast cell line MC3T3-E1, respectively. Here we showed that N16 potently suppressed the proliferation, RANKL-induced formation of multinucleated osteoclasts and TRAP activity in pre-osteoclasts in a dose-dependent way. The nacre protein also inhibited actin ring formation and RANKL-induced mRNA expression of transcription factor NFATc1 and osteoclast-associated genes, including TRAP, c-Src, cathepsin K. Besides, the results on pre-osteoblast found that N16 increased ALP activity as well as mineralized nodule formation with concomitant increases in the mRNA expression of osteoblast marker genes, namely osteopotin, osteocalcin and Runx2. Our findings demonstrate this nacreous protein exerts both anabolic and anti-resorptive effects on bone and is a promising anti-osteoporosis agent.

54 **Interplay between ER stress and hypoxia pathways in chondrocytes**

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Interplay between stress signals can regulate cell fate. Potential connections are emerging between hypoxia and ER homeostasis. It is well established that presence of mis-folded protein in the endoplasmic reticulum (ER) triggers the unfolded protein response (UPR) as part of the ER-stress signal for cell survival. Under low oxygen tension or hypoxia, the cell activates the hypoxic stress signal via the activation of Hif genes, and utilize components of the ER-stress pathways for cell survival and adaptation to the stress condition. However, whether activation of ER stress by accumulation of mis-folded protein has a direct relationship with activation of hypoxia and components of the hypoxia stress pathways is unknown. Collagen X is an ECM specifically expressed by hypertrophic chondrocytes. We previously generated transgenic and gene-targeted mouse models for metaphyseal chondrodysplasia type Schmid (MCDS), expressing mutant Collagen X (13del) that cannot folded correctly, and are retained within the ER, activating ER-stress. Interestingly, we showed that components of the hypoxic stress were also activated concomitantly. This is evidenced by the activated expression of two key transcription factors of hypoxia stress, HIF-1and HIF-2expressed and localized within hypertrophic chondrocytes of MCDS mice compared to WT mice. In addition, the oxygen tension of hypertrophic chondrocytes in MCDS mice is significantly decreased when assayed using EF5, a bio-reductive marker of hypoxia. This study suggested, for the first time in vivo, that ectopic expression of mis-folded protein in chondrocytes which induces ER stress can also trigger hypoxia and hypoxia pathways. Understanding the mechanism of this newly discovered relationship between hypoxia and ER stress, and how do they control cell fates, will have important implications to development and many human diseases.

Poster Number

55

Roles of Progenitor Cells for Intervertebral Disc Regeneration in "Healer" Mice

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Introduction: Intervertebral disc (IVD) degeneration is a major cause of back pain that can also lead to sciatica, affecting the quality of life. Current treatments are limited to salvage surgical operations. Biological treatments to relieve symptoms or to restore disc are not available as we know little about the biology of IVD degeneration and its potential to regeneration. While most people will develop disc degeneration with aging, there are individuals who are protected even at the age (older than 50 years) when over 90% of the population would succumb to the problem, suggesting the presence of protective genes. Furthermore, maintenance of progenitor cells within the nucleus pulposus (NP) is thought to play an important role in disc homeostasis. A hypothesis is that genetic factors can confer a protection against disc degeneration via better maintenance of resident progenitor cells. There exist strains of "healer" mice (MRL/MpJ, LG/J) that have better regenerative potentials of cartilage tissues[1,2]. Thus, we propose to address the NP progenitor cell pools in these healer mice in relation to the degeneration and potential repair/regeneration potentials of the disc.

Materials and Methods: Good healer (MRL and LG/J) and poor healer (C57/BL6C, and SM/J) mice were used in this study. Histological comparison of tail disc sections was assessed from 8 to 24 weeks of age. Progenitor cell pools and differentiated NP cells were assessed using immunohistochemistry using specific cell markers, Tie-2 and disialoganglioside (GD2), that were recently identified[3]. Tail looping at 8 weeks of age for a fixed period was used as an environmental perturbation that will induce degeneration. Unlooping the tail after the period of looping can assess healing processes with appropriate controls.

Results: A comparison of MRL and C57 mice showed neither observable histological differences, nor signs of degenerative processes from 8-week to 24-week of age. Following tail looping for 4, 5, 6 and 8 weeks, there were significant distortion of the annulus fibrosus (AF) and NP at the compressed and distended sides; in terms of loss of NP cells, AF tears and ruptures, and cell death in the AF. After the tails are unlooped for 4 weeks, there are restoration of NP and AF structures such as cell number in both MRL and C57 mice. However, superior healing is seen for MRL mice at all time-points studied; especially in TL6/TL7, TL7/TL8 and TL8/TL9 disc levels, in which the disc structure restores better via continuous expansion of NP region, cell repopulation and lamellae orientation recovers in the compressed AF sides with a clear NP AF boundary. In C57 mice, the AF lamellae structure remained disorganized following unlooping. Interestingly, in the absence of tail looping, SM/J tail discs already showed severe degeneration even at 8-week-old, while that of LG/J mice were relatively normal, suggesting an impact on developmental or maturation in SM/J IVDs. Immunohistochemistry analysis of progenitors related marker Tie-2 and GD2 shows different expression pattern from 4 to 24 weeks, in which MRL maintain more Tie-2 negative, GD2 positive cells during aging, indicating a role of this cell pool in maintaining disc homeostasis.

Conclusion: LG/J and MRL/MpJ mice have better IVD structure and maintenance than C57BL/6J and SM/J with aging, indicating genetic variations can significantly influence disc function. MRL/MpJ mice can better maintain a NP and AF boundary than C57BL/6J mice from mechanical loading, suggesting a potential "protective" effect and also MRL/MpJ mice maintain a higher number of Tie-2-/GD2+ cells, suggesting this pool of cells may have better function for disc maintenance. In depth analyses with more time points and molecular markers of IVD cells are needed to gain a better understanding of the "protective" genetic influences in the "healer" mice

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Theme 4: Infection & immunity

Poster Number

56 **The functional study of the N-terminal region of influenza B virus nucleoprotein**

Liu Wai Nam (CUHK)
(Supervisor: Professor Leung Kwok Nam, CUHK)

Influenza B virus infection causes morbidity and mortality worldwide. The nucleoprotein (NP) in this virus (BNP) is a major component of the viral ribonucleoprotein (vRNP), a protein complex for successful transcription and replication of the virus. Although the crystal structure of BNP is available, the structure and function of the N-terminal of BNP (the first 66 residues, N-66) remain unknown. We discovered that the N-66 played an importan role in viral activities. Biochemical and cellular experiments were performed to characterize the function of N-66. As a result, We identified that there are two functional regions located in N-66. The 1-38 sequence plays an important role in the oligomerization of NP. And the sequence 44-47 serves as a nuclear localization signal (NLS), which helps NP to enter the nucleus. Mutating these two functional regions resulted in the loss of RNP activity and defect in viral rescue, showing that these regions are essential in the replication and transcription of influenza B virus.

57 **Identification of inhibitors targeting influenza A nucleoprotein through structure-based virtual screening**

Lo Chun Yeung (CUHK)
(Supervisor: Professor Shaw Pang Chui, CUHK)

Currently, many strains of influenza A virus have developed resistance against anti-influenza drugs, and it is essential to find new chemicals to combat this virus. The viral nucleoprotein (NP) is a major component of the ribonucleoprotein (RNP) complex for the transcription and replication of the virus. In order to maintain a stable RNP structure, NP forms homo-oligomers by inserting its tail-loop to the tail-loop insertion site of another NP. In this study, we have employed structure-based virtual screening on the influenza A NP tail loop insertion site and found two hit compounds number 7 and 16 that can subdue influenza RNP activities.
Subsequently, two analogs from compound 16 were identified which inhibit RNP activities of various influenza A subtypes and viral growth at micromolar levels. These analogs were also shown to directly interact with NP at 12.0 ± 1.25 and 41.6 ± 1.93 μM respectively by surface plasmon resonance assay. These novel anti-influenza compounds would provide a template for designing drug candidates with higher potency.

Poster
Number

58 Roles of a novel splice variant of human IFI16 in innate immune response

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DNA from viral or bacterial pathogens activates innate immune response. The recognition of self-DNA would induce autoimmune diseases such as systemic lupus erythematosus (SLE). In human, AIM2 like receptors (ALRs) including AIM2, IFI16, IFIX and MNDA are DNA binding proteins implicated in DNA sensing. Most ALRs contain an N-terminal pyrin domain and C-terminal HIN200 domains. However, mouse SLE susceptibility locus p202 encodes only HIN200 domains. A human homolog of p202 was not found. Here, we identified and characterized a novel splice variant of human IFI16, which has a similar domain structure as mouse p202. We named it as IFI16β and the original version became IFI16α. We found that IFI16β has its own promoter and is regulated independently of IFI16α. IFI16β was more abundant in human THP-1 monocytic leukemia cells and it was induced substantially by interferon β in HEK293T and HepG2 cells. Expression of IFI16β was significantly higher in peripheral blood leukocytes of SLE patients. IFI16β was also found to interact with AIM2 and STING. Whereas IFI16α was predominantly found in the nucleus, IFN16β localized to the cytoplasm. Interestingly, IFI16β was a potent inhibitor of NLRP3 inflammasomes. Further investigations are required to determine whether IFI16β exerts its suppressive activity on NLRP3 inflammasomes by counteracting AIM2. Taken together, human IFI16β is a novel alternatively spliced variant that might have inhibitory activity on inflammasome activation in the cytoplasm.

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59 Transcriptional activation and biochemical activities of Small Alarmone Synthase (SAS) proteins from *Staphylococcus aureus*

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The stringent response is a key regulatory process exhibited by bacteria in response to amino acid deprivation or other challenging environmental conditions. It is mediated by the nucleotides guanosine 3',5'-bis(diphosphate) (ppGpp) and guanosine 3'-diphosphate, 5'-triphosphate (pppGpp), collectively known as (p)ppGpp. Rel-family proteins (RSH: RelA, SpoT) are responsible for the synthesis and hydrolysis of (p)ppGpp in most bacterial species. However, many bacteria also encode one or two smaller Rel-family proteins, known as small alarmone synthases (SAS), which are also capable of producing (p)ppGpp. In this study, we investigated the transcription and biochemical activities of the Rel-family proteins (SA-Rel, SA-RelP, SA-RelQ) from the notable bacterial pathogen *Staphylococcus aureus*. The respective abilities of the SA-Rel, SA-RelP and SA- RelQ proteins to catalyze the synthesis of alarmones were characterized by analyzing enzymatic reaction mixtures by anion exchange chromatography. Quantitative real-time PCR (qRT-PCR) was used to determine their transcription levels under various 'stress-inducing' conditions. The SA-RelP and SA-RelQ proteins could effectively synthesize (p)ppGpp alarmones, but had no hydrolytic activities. The transcription of SA-Rel, SA-RelP and SA-RelQ was induced by the presence of the antibiotic mupirocin, which is known to induce the stringent response. Transcription patterns for SA-Rel, SA-RelP and SA-RelQ were more variable in response to other stressful environmental conditions. In brief conclusion, our data indicates that all three Rel-family proteins play important roles in helping *S. aureus* alter its cellular physiology to respond to challenging nutritional and environmental conditions.

Poster
Number

60 **Structure of UreG/UreF/UreH complex reveals how urease accessory proteins facilitate maturation of Helicobacter pylori urease**

Yuen Man Hon Nicholas (CUHK)
(Supervisor: Professor Kambo Wong, CUHK)

Urease is a metalloenzyme essential for the survival of Helicobacter pylori in acidic gastric environment. Maturation of urease involves carbamylation of Lys219 and insertion of two nickel ions at its active site. This process requires GTP hydrolysis and the formation of a preactivation complex consisting of apo-urease and urease accessory proteins UreF, UreH, and UreG. UreF and UreH form a complex to recruit UreG, which is a SIMIBI class GTPase, to the preactivation complex. We report here the crystal structure of the UreG/UreF/UreH complex, which illustrates how UreF and UreH facilitate dimerization of UreG, and assembles its metal binding site by juxtaposing two invariant Cys66-Pro67-His68 metal binding motif at the interface to form the (UreG/UreF/UreH)2 complex. Interaction studies revealed that addition of nickel and GTP to the UreG/UreF/UreH complex releases a UreG dimer that binds a nickel ion at the dimeric interface. Substitution of Cys66 and His68 with alanine abolishes the formation of the nickel-charged UreG dimer. This nickel-charged UreG dimer can activate urease in vitro in the presence of the UreF/UreH complex. Static light scattering and atomic absorption spectroscopy measurements demonstrated that the nickel-charged UreG dimer, upon GTP hydrolysis, reverts to its monomeric form and releases nickel to urease. Based on our results, we propose a mechanism on how urease accessory proteins facilitate maturation of urease.

61 **Optimization of novel anti-HIV inhibitors to overcome drug resistance of HIV-1**

Zeng Chuyue (CUHK)
(Supervisor: Dr. Jacky Ngo, CUHK)

Currently, due to the highly error-prone reverse transcription process of viral RNA, HIV-1 acquires resistance to all known inhibitors that hinders the effectiveness of antiretroviral therapy. Therefore, novel anti-retroviral therapies are required.

The integrated HIV-1 viral DNA transcribes a single precursor mRNA to generate more than 40 different mRNAs of key viral proteins by alternative splicing, which is tightly regulated by cellular splicing factors serine-arginine rich proteins (SR proteins). Cellular functions of SR proteins are tightly governed by the phosphorylation state of their RS domains. One main family of kinases, SR protein kinases (SRPKs), phosphorylates SR proteins and has been implicated in HIV-1 replication. This phosphorylation process is regulated by docking interactions between a negatively charged kinase docking groove and basic docking motifs on the substrates. Thus, we propose to target the host cell's splicing machinery by blocking the interaction between SRPK1 and its substrates in order to inhibit the expression of integrated HIV-1 provirus.

Structure-based in silico screening has been performed to identify small-molecule inhibitors that bind to the docking groove of SRPK1. 43 potential candidates have been identified. One of them shows good inhibitory effects on SRSF1 phosphorylation and its nuclear import, and subsequently HIV-1 mRNA splicing. This compound only exhibits moderate cytotoxicity to both T cells and HeLa cells. In the future, the kinase-inhibitor binding kinetics of the derivatives of this compound will be measured. Their inhibitory effects on viral infection and replication of HIV-1, as well as activity of other kinases and splicing of endogenous genes will be investigated.

Poster
Number

62 Molecular interaction of motor switch protein FliM and spermidine synthase in Helicobacter pylori

Zhang Huawei (CUHK)
(Supervisor: Dr. Shannon Au, CUHK)

Helicobacter pylori is the major pathogen causing gastritis, ulcer and gastric cancer. One of the important virulence factors during infection of H. pylori is the flagellum, which is responsible for bacterial motility and colonization. Rotation of flagellar in clockwise or counter-clockwise direction is mediated through the motor switch complex (also known as cytoplasmic ring (C-ring)) which consists of FliG, FliM, FliN and FliY in H. pylori. Chemotaxis is regarded as the main signaling pathway to regulate flagellar rotational switching by the binding of phosphorylated CheY to FliM. Additionally, several protein factors have recently been reported to modulate the flagellar function by binding to the switch complex, such as YcgR in S. enteritidis, EpsE in B. subtilis, and H-NS and fumarate reductase in E. coli.

Here, we report the identification of spermidine synthease (SpeE) as a FliM binding protein in H. pylori. Their molecular interactions have been confirmed by pull-down assay and size-exclusion chromatography. The determination of a 2.9Å crystal structure of FliM-SpeE complex reveals that their association may link to the regulation of motor function and SpeE activity. On the other hands, binding of SpeE to FliMm can reduce the FliG-FliM interaction. Our findings suggest that polyamine metabolism pathway may be involved in the flagellar function besides the chemotaxis signaling pathway in H. pylori.

63 The scaffolding protein RanBPM in IFN-lambda signaling pathway

Zhang Junwen (Peking Union Medical College)
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Cytokines play a critical role in modulating the innate and adaptive immune systems. Interferons (IFNs) are key cytokines in the establishment of a multifaceted antiviral response. IFN-lambda1, also known as interleukin-29 (IL-29), is a member of a new family of cytokines called the type III IFNs, which have similar functions to the type I IFNs, IFN-alpha and IFN-beta. Exploring more interacting proteins with IFN-lambda receptor (IFN-R1) is pivotal for studying IFN-lambda1 function and its molecular mechanism. The Ran-binding protein in the microtubule-organizing center (RanBPM) served as a scaffold protein that had been shown to interact with many proteins, such as MET, Axl/Sky, TRAF6, IFNR, TrKA and TrkB in addition to p75NTR. Recently, great progress has been made in understanding the diversity of RanBPM in regulating various cellular functions. RanBPM has been reported to have proapoptotic activities that regulate cell death pathways and has also been associated with amyloid-beta peptide generation, Ca2+ channels, cell arrangement, cell morphology regulation and titin assembly. In the current study, we have identified RanBPM as a novel binding partner of IFN-R1. The IFN-R1 and RanBPM association was confirmed by co-immunoprecipitation. Under the stimulation of IFN-lambda1 the interaction between RanBPM and IFN-R1 was enhanced. Fortunately, we also detected another protein STAT5 (Signal transducers and activators of transcription 5) in IFN-lambda pathway associated with RanBPM. Additionally, in the presence of the ligand IFN-lambda1, there are more STAT5 and RanBPM binding with the IFN-R1. Moreover, the subcellular localization of STAT5, RanBPM and IFN-R1 was visualized in Huh7 cells, and it was found that IFN-R1 could be localized in cell nucleus. Furthermore, RanBPM together with IFN-lambda1 strengthen the luciferase activity of ISRE. Taken together, our results reveal that RanBPM may modulate IFN-lambda-mediated downstream signaling and biological functions.

Theme 5: Neuroscience

Poster
Number

64 **The localization of ATR (ataxia telangiectasia and Rad3-related) in synaptic vesicles – a super-resolution study**

Cheng Aifang (HKUST)
(Supervisor: Professor Karl Herrup, HKUST)

ATR (ataxia telangiectasia and Rad3-related), is a PI3-kinase involved in DNA single-strand break repair. Mutations that lower the activity of ATR are responsible for Seckel syndrome, a devastating CNS developmental disorder. In addition to its expected nuclear localization, ATR is found in the neuronal cytoplasm where it physically associates with the homologous PI3-kinase, ATM (ataxia telangiectasia mutated) as well as with VAMP2 (synaptobrevin) and synapsin-I. Cytoplasmic ATM has been localized to vesicular structures in the neuronal cytoplasm; a detailed analysis of ATR localization has not been done. We used stochastic optical reconstruction microscopy (STORM), a new technique of optical super-resolution microscopy to gain further insight into the localization and function of cytoplasmic ATR at 20 nm resolution. ATR antibodies clearly labeled vesicular structures in DIV15 neurons. The identity of these structures as vesicles was confirmed by double immunostaining with the CgA vesicular cargo protein. This finding emphasizes that ATR is found not only on neurotransmitter containing synaptic vesicles, but also on dense core vesicles, the precursors of nascent synaptic active zones in neurons. Our double labeling showed that ATR significant co-localized with Bassoon, an active zone localized protein. Curiously, we also found ATR co-localized with MAP2 as well as with PSD95, opening the possibility that ATR is located on both sides of the synapse. We are exploring the hypothesis that ATR is responsible for phosphorylation of [S/T]Q substrates at sites within the dendritic spine itself while its sister kinase, ATM, is localized to and mainly regulates substrates in cytoplasm.

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65 **Differential roles of cytokines in survival and proliferation in Neuroblastoma cell line and mature neurons**

Hui Chin Wai (HKUST)
(Supervisor: Professor Karl Herrup, HKUST)

Cytokines are proteins with a diverse set of functions. They are important in controlling immune response, cell growth, proliferation and maturation. Previous studies have shown that expression of pro-inflammatory cytokines is correlated with neurodegeneration yet also contributes to anti-tumor effects. In the current studies, we investigate the differential functions of cytokines in tumor and neuronal cells.

We used two inflammatory cytokines, tumor necrosis factor alpha (TNFα) and interleukin 1 beta (IL1β) as well as the Toll-like receptor 4 ligand, lipopolysaccharide (LPS) that triggers a broad range of cytokine production. In log-phase (mitotic) neuroblastoma cells and 3-day differentiated (partially differentiated) cells, the stimulations significantly reduced ERK phosphorylation and increased p38 phosphorylation and thus decreased their survival and proliferation. However, MAP kinases respond to each stimulus differently in fully differentiated cells.. TNFα specifically triggered ERK phosphorylation while LPS and IL1β failed to change the phosphorylation of MAP kinases, suggesting a role for TNFα in neuronal survival. This specific effect may be linked to an increase in p50 expression under TNFα treatment. Similar results were observed in cortical neurons where TNFα suppressed neuronal cell cycles while IL1β worked in opposite manner to cause neuronal damage, further suggesting the differential roles of cytokines in mature neurons.

We pursued these findings with the NF-κB inhibitor, celastrol. Celastrol decreased NF-κB protein expression in both differentiated N2a and cortical neurons at 1μM. Cytotoxicity stimulated at high celastrol concentration was related to reduced p50 nuclear localization, stimulated ATM phosphorylation (DNA damage), increased p38 phosphorylation and decreased ERK phosphorylation. Taken together, the present studies suggest that cytokines impose variable functions in tumor and neuronal cells and further studies should be done to study the underlying mechanisms.

Poster
Number

66 **Semaphorin3A, Associated with Perineuronal Nets, Regulates the Development of the Maturation of the Central Vestibular Circuitry**

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During the formative period of neural circuits, perineuronal nets (PN) are established to restrict plasticity of the circuit. The role of PN in vestibular plasticity can be tested by studying the emergence of negative geotaxis with postnatal maturation of the vestibular circuitry for gravity detection.

Using rats as model, we observed that negative geotaxis was mature by postnatal day (P) 9, in correlation with consolidation of PN around GABAergic neurons in the vestibular nucleus (VN). Treatment of the VN at P6 with chondroitinase ABC (ChABC) cleaved chondroitin sulfate (CS) moieties of PN and delayed emergence of negative geotaxis to P13. Delay to P13 was also observed following treatment of the VN with a GABAA receptor antagonist, reinforcing GABAergic transmission as regulated by perineuronal CS is a crucial step for the maturation of negative geotaxis.

Throughout postnatal development of the VN, CS moieties of PN colocalized with semaphorin 3A (Sema3A), a secreted glycoprotein that regulates neuronal polarization. The expression of Sema3A in VN was confirmed by in-situ hybridization. In addition, Sema3A regulated the dendrite growth pattern of GABAergic neurons in VN. We infer that the morphological changes of the dendritic arbor can contribute to the vestibular plasticity.

Our results suggest that, as the VN circuitry undergoes maturation, perineuronal CS moieties retain Sema3A and limit the participation of Sema3A in the modulation of morphological and functional properties of GABAergic interneurons in the VN, thereby contributing to the hardwiring of the central pathway for vestibular behavior. [Grant Supported by HKRGC 774608M & 777911M]

67 **Chondroitinase ABC-I AND -II linked chitosan microbeads increase neurtire length in CSPG-enriched astrocyte culture.**

Kwok Lam Fung (HKU)
(Supervisor: Professor DKY Shum, HKU)

After spinal cord injury, axonal regrowth is often restricted due to upregulation of chondroitin sulfate proteoglycans (CSPGs) at the lesion site. Chondroitinase ABC I and II (recombinant ChABC-I & -II) cleave CS moieties of the PGs enhancing prospects of axonal regrowth through the lesion. We attempted to use glutaraldehyde to immobilize ChABC-I or-II separately on chitosan beads. Immobilized ChABC-I demonstrated CS-cleaving activity both in biochemical assay and in CSPGs-enriched astrocyte cultures that had been activated by transforming growth factor beta (TGF-β). Neurite length was increased in co-cultures of TGF-β activated astrocytes and cortical neurons mixed with immobilized ChABC-I, immobilized ChABC–II or both. Given CSPG enrichment at astrocyte-Schwann cell (A/S) encounters, A/S confrontation co-culture was used in a further bioassay of immobilized enzyme activity. Preliminary data showed that neurite length was increased in ChABC-I treated A/S co-culture. In future, the effect of immobilized ChABC-I and -II on neurite length will be tested in A/S co-culture.

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68 Heparanase 1 and Heparanase 2 expression in Hippocampal Neurons

Lau Wing Yan (HKU)
(Supervisor: Professor DKY Shum, HKU)

Selective modification (weakening or strengthening) of synaptic connections between neurons within the hippocampal circuit contribute to the learning and memory processes in the brain. Enzymatic heparanase-1 (hpa1) was found expressed in hippocampus by both immunohistochemistry and in-situ hybridization. Western blot analysis of neuronal secretions however revealed the pro-form, i.e. proheparanase, which do not have enzymatic activity is secreted by hippocampal neurons. Proheparanase could trigger AMPA receptor internalization upon binding to the Heparin sulphate on the proteoglycan, therefore impact on synaptic strength and long-term potentiation of synaptic efficacy. We further found not only heparanase-1 but also heparanase-2 (hpa2) expression in hippocampal neurons. We hypothesize therefore a partnership of hpa2 with hpa1 in regulating AMPA receptor internalization at glutamatergic synapses. Immunohistochemical staining of hippocampal sections from adult rats found hpa2 expression in neurons of both the hippocampal DG, CA3 and CA1 regions. Like hpa1, hpa2 immunopositivity was found mainly in perinuclear regions of neurons. In addition to proheparanase, hpa2 was also found to be secreted by hippocampal neurons in culture. Secreted proheparanase and hpa2 remained associated with cell surface heparan sulfate. With results that support our hypothesis, we expect to pursue the partnership between proheparanase 1 and hpa2 with the respective recombinant protein.

69 Role of FE65 in neurite outgrowth

Li Wen (CUHK)
(Supervisor: Dr. Lau Kwok Fai, CUHK)

FE65 is an adaptor protein that binds to the amyloid precursor protein (APP). As such, FE65 has been implicated in the pathogenesis of Alzheimer's disease. In addition, evidence suggests that FE65 is involved in brain development. It is generally believed that FE65 participates in these processes by recruiting various interacting partners to form functional complexes. Here, we show that via its first phosphotyrosine binding (PTB) domain, FE65 binds to the small GTPase ADP-ribosylation factor 6 (ARF6). FE65 preferentially binds to ARF6-GDP, and they colocalize in neuronal growth cones. Interestingly, FE65 stimulates the activation of both ARF6 and its downstream GTPase Rac1, a regulator of actin dynamics, and functions in growth cones to stimulate neurite outgrowth. We show that transfection of FE65 and/or ARF6 promotes whereas small interfering RNA knockdown of FE65 or ARF6 inhibits neurite outgrowth in cultured neurons as compared to the mock-transfected control cells. Moreover, knockdown of ARF6 attenuates FE65 stimulation of neurite outgrowth and defective neurite outgrowth seen in FE65-deficient neurons is partially corrected by ARF6 overexpression. Notably, the stimulatory effect of FE65 and ARF6 on neurite outgrowth is abrogated either by dominant-negative Rac1 or knockdown of Rac1. Thus, we identify FE65 as a novel regulator of neurite outgrowth via controlling ARF6-Rac1 signaling.

Poster
Number

70 Role of BMP/Smad signaling in cerebellum neurogenesis

Ma Tsz Ching (CUHK)
(Supervisor: Dr. Kwan Kin Ming, CUHK)

Cerebellum neurogenesis is tightly regulated by transcription factors and signaling molecules that specific types of neurons are produced sequentially from two germinal matrices, ventricular zone and anterior rhombic lip. Although both germinal matrices are equally important to cerebellum formation, our understanding on the maintenance and specification of multipotent neural stem cells in ventricular zone is far uncomparable to that in anterior rhombic lip. Bone morphogenetic proteins (BMPs) are signaling molecules crucial to the maintenance of stem cell identity. We previously demonstrated that Smad1/5 is critical to granule cell production by governing proliferation and specification of neural progenitor cells in anterior rhombic lip. Because BMPs are secreted from rhombomere 1 roof plate during cerebellum development, we proposed that canonical BMP/Smad signaling is involved in neurogenesis within ventricular zone as well. Our En1-cre driven Smad1/5 double conditional knockout mice displayed an enlarged Purkinje cell population at E11.5, implying abnormal neurogenesis from ventricular zone. However, because of the observation of thinner PCNA-positive domain in mutant cerebellum, enhanced Purkinje cell neurogenesis in Smad1/5-null cerebellum is unlikely attributed by an expanded progenitor cell pool. Taken together, our data suggest canonical BMP/Smad signaling regulates the balance between stem cell renewal and specification to Purkinje cells in ventricular zone.

71 Expression of Chondroitin Sulfotransferase in Cranial Motor Neurons for Cell Migration in Rat Embryonic Hindbrain

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Neuronal migration allows the proper positioning of neurons for establishing functional connectivity of defined neural circuits. We and others reported the restrictive role of Chondroitin sulfate (CS) moieties in axonal fasciculation. CS moieties of proteoglycans are therefore hypothesized to control the timely orchestration of cranial motor neuron migration during hindbrain development by the varying sulfation patterns of the chondroitins between the migrating and ready-to-migrate neurons. Hindbrain explants of E11.5 Sprague Dawley rats were maintained in culture for time lapse video recording of individual neuronal somal movements. In control cultures, we observed the advancement of neuronal somata in the direction of the leading process away from the explant core. In test cultures treated with chondroitinase ABC, the neuronal cell bodies lost the direction movement but remained motile. Immunocytochemistry confirmed the presence of CS56 epitopes among Tuj-1-positive neurons not only in the explant core and those advancing beyond the core, but also in the vicinity of the migrating neuronal somata. In situ hybridization revealed chondroitin-4-sulfotransferase 2 (C4ST2) mRNA expression among cells heading away from the core whereas chondroitin-4-sulfotransferase 1 (C4ST1) mRNA was found essentially in the core of the explant. Thus far, the results suggest differential sulfation of chondroitins on proteoglycans expressed by neurons in determining the migratory phenotype.

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72 **Instructive roles of DRG neurons in directing the fate choice of bone marrow derived Schwann cells**

Tai Wing Yin Evelyn (HKU)
(Supervisor: Professor YS Chan, HKU)

The bone marrow offers an autologous source of progenitor cells for use in remyelination therapy. Our success in deriving Schwann cells from bone marrow stromal cells suggests juxtacrine signals provided by dorsal root ganglia (DRG) neurons in the switch of Schwann cell-like cells (SCLCs) to fate commitment.

In our search for the signals, immunocytochemical analysis found the Notch ligands,DLL-1 and Jagged-1, localized on the surface of DRG neurons whereas the receptor, Notch-1, was on bone marrow-derived SCLCs. In cocultures with DRG neurons, SCLCs indicated nuclear localization of the Notch intracellular domain (NICD), suggestive of ligand-activated Notch signaling. Concomitantly, increase in ErbB2/3 expression was revealed among SCLCs by mmunocytochemistry and confirmed by Western blotting. As cells achieved commitment to the Schwann cell fate, nuclear NICD returned to the basal level. When a γ-secretase inhibitor was used to inhibit Notch signaling, the increase inErbB2/3 expression among SCLCs was no longer effected and progress of SCLCs to the Schwann cell fate was significantly retarded. We therefore revealed an emerging role of Notch signaling in the upregulation of ErbB receptors for neuregulin-activated signaling as SCLCs transition into fate commitment.

73 **Chitosan Nanofiber Composed Nerve Conduit for Directing Axonal Growth**

Tung Wing Tai (HKU)
(Supervisor: Professor DKY Shum, HKU)

Schwann cell-seeded guidance channels have been exploited to bridge and guide axonal re-growth across gaps in lesioned nerves. By orienting the Schwann cell growth on aligned nanofibers, we hypothesized that axonal growth can be guided along the designated direction towards the target. Chitosan as the choice scaffold material given its biocompatibility and the tunable susceptibility to biodegradation. Chitosan was dissolved in trifluoroacetic acid/methylene chloride solution and for electrospinning onto a high speed rotating collector drum yielding aligned nanofibers. These aligned chitosan nanofibers directed the growth pattern of Schwann cells. We further seeded dissociated cells of dorsal root ganglia (DRG, E14/15 rats) onto the aligned nanofibers and found that neurons and Schwann cells adapted the uniaxial arrangement of nanofibers. The Schwann cells could also be induced to undergo myelination of the DRG neurons. We then rolled the Schwann cell-seeded or DRG explant-seeded nanofiber sheet into a model of nerve conduit. The Schwann cells remained in alignment and DRG neuron grew along the longitudinal axis of the conduit. These in vitro results provide proof-of-principle for pursuing improvement in post-traumatic recovery from nerve injury with use of Schwann cell-seeded uniaxially aligned chitosan nanofibers as a nerve guidance channel.

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Poster
Number

74 Sox9 mediates glial switch by inhibiting neurogenesis but not determining gliogenic fate in the cerebellum

Vong Keng IOI (CUHK)
(Supervisor: Dr. Kwan Kin Ming, CUHK)

Sequential production of neurons and glia from the neural stem cells is a conserved fundamental phenomenon in the central nervous system (CNS). In the spinal cord and retina, Sox9 is the master regulator that defines glial fate choice by mediating the neuron-to-glia fate switch. Sox9 is also critical for the induction and maintenance of the neural stem cell pool in both embryonic and adult CNS. However, the genetic repertoire governing the maintenance and fate decision of neural progenitors in the cerebellum has remained elusive. Sox9 is expressed robustly in the cerebellar neural progenitor pool at the ventricular zone (VZ) throughout development. Surprsingly, neither the self-renewal capacity nor the multipotency of neural progenitors was perturbed upon conditional inactivation of Sox9 in the cerebellum. Instead, the mutants exhibited an increased number of VZ-derived neurons including Purkinje cells and GABAergic interneurons. Birthdate tracing revealed a continuous production of neurons from the VZ in Sox9-null cerebellum at late gestation, when normally neurogenesis ceases to occur and gives way for gliogenesis. Unlike in other CNS tissues where neurons were generated at the expense of glia populations, glial specification appeared unaffected in Sox9-null cerebellum. Our findings thus give new insights into the current understanding that Sox9 instructively triggers the glial switch by defining glial cell fate. Our data instead suggest that Sox9 may mediate the neuron-to-glia fate switch by preventing the neural progenitors from adopting neuronal fate.

75 Potential Role of Atg16L1 in Autophagic Vesicle Trafficking in Primary Cultured Neurons

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Autophagy is a cellular homeostatic degradation process for the removal of part of the cytoplasm, dysfunctional organelles or protein aggregates via the lysosomal pathway. A number of studies have reported abnormal accumulation of autophagic vacuoles (AVs) in the brains of neurodegenerative disorder patients, especially in the neurites. While the cause of this accumulation is incompletely understood, impairment in the trafficking of well-formed AVs along the neurites has been implicated. However, mechanism of autophagic vesicles trafficking has remained largely enigmatic. Interestingly, we have identified Atg16L1 as a potential regulator of AV movement. Using a tandem construct expressing LC3, a protein that is selectively recruited to AVs, we examined the effect of Atg16L1 knock-down on the movement of AVs using live cell imaging. LC3 puncta movement analysis revealed that Atg16L1 knock-down decreased the trafficking velocity of AVs, especially that of the autolysosomes in control cells. In addition, AVs quantification analysis revealed that Atg16L1 knock-down significantly increased the amount of dendritic autolysosomes. To identify new Atg16L1 interacting proteins that may mediate the regulation of AV trafficking by Atg16L1, mass spectrometry was performed and several candidate proteins have been identified. In summary, this study highlighted a new function of Atg16L1 in autophagic vesicle trafficking regulation, and may shed light on the development of therapeutics against neurodegenerative disorders.

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