An essential, cell cycle-dependent and semi-conservative ORC dimerization cycle critically regulates DNA replication

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Eukaryotic DNA replication licensing, which is a prerequisite for genome duplication and also helps to ensure that all chromosomal DNA is replicated exactly once per cell cycle, involves the recruitment of many replication-initiation proteins by origin recognition complex (ORC) to form pre-replicative complexes (pre-RCs) at replication origins. ORC binds to and marks replication origins throughout the cell cycle. However, some fundamental issues in the regulation of ORC in replication licensing remain unsolved. Here we report that ORC self-interacts and dimerizes (forming a double heterohexamer) before pre-RC formation in budding yeast. Upon S phase entry, each double hexamer ORC separates into two single hexamers to bind each pair of nascent origins until late M phase. The non-chromatin-bound ORC then associates with the chromatin-bound ORC to reform double hexamers at the M-to-G1 transition. Preventing ORC dimerization by depleting non-chromatin bound ORC proteins in M phase, or by Orc6p mutations that disrupt ORC self-interaction, abolishes pre-RC formation, DNA replication and cell proliferation and viability. Similarly, human ORC also self interacts, co-localizing on stretched human chromatin fiber bundles in a cell cycle dependent manner at the M-to-G1 transition. Our findings uncovered an essential, cell cycle-dependent and semi-conservative ORC 'dimerization cycle' that plays three fundamental roles in the regulation of DNA replication: providing a symmetric platform to load the symmetric pre-RCs, marking and protecting the nascent sister replication origins until the next licensing, and providing the first guard against origin re-licensing within the same cell cycle.

Dual-utility NLS Drives RNF169-dependent DNA Damage Responses

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Loading of 53BP1 and RAP80 at DNA double-strand breaks (DSBs) drives cell cycle checkpoint activation but is counterproductive to high-fidelity DNA repair. RNF169 maintains the balance by limiting the deposition of DNA damage mediator proteins at the damaged chromatin. We report here that this is accomplished, in part, by a predicted NLS that not only shuttles RNF169 into the nucleus, but also promotes its stability by mediating a direct interaction with the ubiquitin specific protease USP7. Guided by the crystal structure of USP7 in complex with the RNF169 NLS, we uncoupled USP7 binding from its nuclear import function, and showed that perturbing the USP7-RNF169 complex destabilized RNF169, compromised high-fidelity DSB repair, and hyper-sensitized cells to PARP inhibition. Finally, expression of USP7 and RNF169 positively correlated in breast cancer specimens. Collectively, our findings uncover an NLS-mediated bipartite mechanism that supports the nuclear function of a DSB response protein.

Apical constriction is driven by a pulsatile apical myosin network in delaminating *Drosophila* neuroblasts

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The epithelial to mesenchymal transition (EMT) is a process important for organ formation, tissue homeostasis and tumor metastasis. We used *Drosophila* embryonic neuroblasts delamination from the ventral nerve cord as a model to study EMT events. In the neuroectoderm, for each proneural cluster of cells, one cell undergoes EMT, delaminates from the epithelium and becomes a neuroblast, while the surrounding cells remain as epithelial cells. Apical constriction is one of the key events during EMT, and in our project we aim at dissecting how the actin-myosin network drives apical constriction in a single cell delamination event.

Through imaging live embryos, we observe that a dynamic myosin network of flows and pulses exist in both delaminating neuroblasts and their neighbors. Quantitative analysis shows that medial myosin contractions correlate with apical cell area changes. Although the medial myosin contractile pulses are present in both delaminating neuroblasts and their non-delaminating neighbors, the medial pulses exhibit higher amplitudes and appear at higher frequency in the delaminating cells than their non-delaminating neighbors. Low-dose CytoD injection and *RhoGEF* RNAi are used to deplete medial myosin pulses without affecting junctional myosin, leading to a blockage of delamination. Moreover, we have established a phase field model to simulate the neuroblast delamination process. The mathematical simulation shows that high frequency and intensity of medial myosin are sufficient for the cells to pass over the energy barrier and undergo delamination. The neuroblast fate is set apart from their neighbors by Notch signaling-mediated lateral inhibition. When we inhibit Notch signaling activity in the embryo, we observe that small clusters of cells undergo apical constriction and display an abnormal apical myosin pattern. Together, we demonstrate that a patterned actomyosin network set by Notch signaling activity drives apical constriction in delaminating neuroblasts.

A high throughput approach for discovery of catalytic nucleic acids

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Enzymes are biologic polymers. Catalytic nucleic acids are extremely useful and hence there is constant need for improving and discovering of catalytic molecules for industrial, medical and biotechnological applications.

In vitro evolution has established that single-stranded nucleic acids can display substrate dependent catalysis of specific biochemical reactions. These single stranded oligonucleotides are called aptamers. Mass production and qualitative detection of single-stranded deoxyribonucleic acids are essential for each round of successful in vitro evolutionary pathways for high affinity binding or catalytic aptamers.

In this work we optimized the asymmetric polymerase chain reaction protocol for mass production and subsequently developed a new assay system for detection and quantification of single-stranded deoxyribonucleic acid on the native TBE gel. Further enzyme assay of asymmetric polymerase chain reaction product reflect the quality of single-stranded nucleic acids present in the bulk reaction.

We would further translate the ePCR approach into the most powerful ultrahigh-throughput inexpensive droplet-based microfluidics system for discovery of new catalytic nucleic acid variants having stronger catalytic activity. Isolate variants can further be used in diagnostic assay upon modification of nucleotides for resistance to enzymatic and chemical degradation.

To elucidate the role of the Kinesin motor protein KIF5B in mRNA transport and dendritic spine morphogenesis

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The formation and development of neuronal synapses is crucial for normal brain function. Most of the excitatory synapses are located on the specialized protrusions on the dendritic processes called dendritic spines. The maintenance and maturation of dendritic spine depend on synaptic activity and local protein synthesis. Studies revealed that many dendritic localized RNAs are transported in the form of RNA granules by various molecular motor proteins. However, the functional link between molecular motors and synapse development remains unclear. Previous study has suggested that the Kinesin motor protein KIF5B is responsible for transporting RNA granules. Using dissociated primary culture of hippocampal neurons from *kif5b* knockout mice and RNA-interference, we provide evidence that KIF5B is essential for dendritic mRNA trafficking and dendritic spine morphogenesis. We have also examined how post-translational modification of KIF5B regulates its function in neuron.

The interaction of berberine with chemodrugs on Epstein-Barr virus (EBV)-associated nasopharyngeal carcinoma

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Chemodrugs such as cisplatin and suberoylanilide hydroxamic acid (SAHA) are widely known for their ability to suppress Epstein-Barr virus (EBV)-associated nasopharyngeal carcinoma (NPC) growth by inducing cell-cycle arrest and EBV lytic reactivation respectively. Our previous study has reported that berberine, a pure compound from Chinese medical herbs, can strongly suppress the activation of STAT3 and tumor growth of NPC cells. In this study, we aim to investigate the potential risks or benefits of taking berberine as an adjuvant agent in combination with chemodrugs. We found that berberine sensitized the cells for the cytotoxicity effects of cisplatin and SAHA, although it interfered with the cisplatin-induced G1 arrest and downregulated SAHA-induced lytic reactivation. In the *in vivo* experiments, the tumor growth in nude mice could be suppressed by cisplatin, SAHA or berberine treatment alone. While concurrent use of cisplatin and berberine could further suppress the tumor growth, this combined treatment was detrimental to the healthiness of mice. When the mice were treated with berberine + SAHA, the tumor nodules were swelled up with watery tumour masses. RNAscope of EBV lytic genes (e.g. BZLF1 and gp350) showed that around 10% of cells underwent lytic reactivation. Moreover, H&E staining of the tumor masses suggested the tumors cells were induced into differentiation after treating with berberine + SAHA. Inhibition of STAT3 has suggested to modulate cellular differentiation status for enhancing the cytotoxicity effects of cisplatin and SAHA. Future experiments will be performed to understand the berberine-mediated suppression of STAT3 in affecting the chemodrugs' efficacy.

The role of Nek2 in hepatocellular carcinoma

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Liver cancer is one of the most prevalent cancers over the world and particularly affects people in some East Asia countries such as Korea, Japan and China. In Hong Kong, liver cancer is the third killer among different types of cancer. The NIMA-related kinase 2 (Nek2), which regulates centrosome separation, is a potential player in carcinogenesis. Our evidence showed that the mRNA and protein levels of Nek2 are up-regulated in liver cancer cells. Since Nek2 is pertinent to centrosome cohesion, we speculate that it might take part in carcinogenesis through centrosome regulation. To study the effect of Nek2 in chromosomal instability, centrosome splitting and cell cycle, a stable isoform Nek2A overexpression inducible system was generated in different hepatocellular carcinoma (HCC) and bone osteosarcoma cell lines for investigations through immunofluorescence staining and flow cytometry analysis. In both Nek2A inducible HepG2 and SMMC-7721 cells, a higher proportion of cells with centrosome splitting was observed in the Nek2A overexpressing cells. However, in Nek2A inducible U2OS cells, where p53 was suppressed, induced expression of Nek2A led to a significant increase in cells with multiple centrosomes. Moreover, cell cycle analysis showed an increased content of DNA when Nek2A is overexpressed, suggesting that Nek2A induces more aneuploid cells. To conclude, overexpression of Nek2 exacerbates chromosomal instability. Dysregulation of centrosome may be the key for understanding of regulatory mechanism of chromosomal instability by Nek2.

The Role of Engulfment adaptor protein 1 (GULP1) Theronine-35 Phosphorylation on Amyloid Precursor Protein (APP) Processing

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Alzheimer's disease (AD) is a neurodegenerative disease that causes a progressive decline of cognitive functions. One of the pathologic hallmarks of AD is the presence of neuritic plaques in the disease brains that are microscopic extracellular deposits of amyloid-β (Aβ), a small peptide derived from proteolytic cleavage of amyloid precursor protein (APP). Aggregation and accumulation of Aβ in the brain has been thought to play a critical role in AD pathogenesis. Our laboratory has identified engulfment adaptor protein 1 (GULP1) interacts with APP intracellular domain (AICD) to alter APP processing and Aβ generation. However, the mechanisms by which GULP1-APP interaction is regulated remain unknown. Protein phosphorylation is a common post-translational modification that control many biological processes including protein-protein interaction. In this study, we investigate the role of GULP1 Threonine-35 (T35) phosphorylation on GULP1-mediated APP processing. T35 lies within GULP1 phosphotyrosine binding (PTB) domain, the region that interacts with AICD. Here we show that phosphomimetic mutant (T35D) of GULP1 reduces GULP1-APP binding. The mutation also caused a significant decrease in GULP1-mediated APP processing. Furthermore, we identify protein kinase CK2 as a kinase that targets GULP1 T35, and that this phosphorylation inhibits the effect of GULP1 on APP processing and Aβ generation. Together, these data provide insights into a novel mechanism that regulates GULP1-mediated APP processing.

A change of heart: Neonates to adults and the role of innate immunity in cardiomyocyte functionality

Name of Speaker: CHEN, Francis M.
Supervisor: Prof. WONG, Wing Tak Jack

In 2016, the World Health Organization again recognized that cardiovascular disease was the leading cause of death globally. The greatest hurdle to treating cardiovascular related pathologies, particularly after a myocardial event, is the inability for adult mammalian cardiomyocytes to functionally contribute to the regeneration of heart tissue. Compared to adult cardiomyocytes, neonatal mammalian cardiomyocytes are capable of self-renewal leading to the restoration of functionality following injury or disease. Yet the mechanisms by which neonatal cardiomyocytes shift to a terminally quiescent population are still unclear.

Studies examining the relationship between innate immunity and non-myeloid stem cell populations have expanded the role of type II cytokines in influencing the outcome of regeneration. Due to the required activation of innate immunity for proper resolution of injury, we hypothesize that type II innate signals plays an indispensable role in proper neonatal cardiomyocyte functionality, and that this relationship is abolished in adult cardiomyocyte populations.

This work focuses on dissecting the interplay between the innate immune arm and the heart niche. Specifically, whether interleukin-4, a pleiotropic cytokine which regulates alternatively activated macrophages and implicated in modulating the cell fate and regenerative capacities of a variety of stem cell populations following injury, directly contributes to the regeneration of heart tissue after a myocardial event. IL-4 is hypothesized to play a key role in favoring neonatal cardiomyocyte proliferation and niche remodeling, whereas antagonistically driving pro-fibrotic programs in adults due to the inactivity of adult cardiomyocytes.

Preliminary studies indicate that transcriptional activity of the IL-4R and its downstream transcription factor, STAT6, are significantly increased during the initial phases of development and then abruptly downregulated at seven days after birth. This transcriptional activity mirrors the overall growth of heart tissue basally and after LAD (left anterior descending) ligation to mimic a myocardial event. The aim is to utilize a variety of knockout mice models, cardiomyocytes derived from human iPSCs, and rigorous characterization of the heart niche to shed light on the mechanisms that influence loss of cardiomyocyte functionality in adults with the goal of restoring their regenerative capabilities.

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Exenatide Attenuates Homocysteine-induced Endothelial Dysfunction through Reducing Oxidative Stress

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Hyperhomocysteinemia is an independent risk factor for cardiovascular and metabolic diseases including type 2 diabetes mellitus and atherosclerosis. By inducing oxidative stress, homocysteine impairs endothelial function. Exendin-4, as an enzymatically stable Glucagon-like peptide-1 (GLP-1) analogue, is a form of exenatide identified in the saliva of Heloderman suspectum. Exendin-4 exerts direct protective effects on endothelial cells, through activation of the AMPK/Akt/eNOS pathway. However, the vascular benefits of exendin-4 against homocysteine-induced endothelial dysfunction have been scarcely investigated. Hence, the aim of this study is to explore the vascular effect of exendin-4 in the presence of homocysteine. Functional assays on the rat aortas and porcine circumflex arteries were performed to unravel the vascular benefits of exendin-4. Confocal microscopy was carried out to assess reactive oxygen species (ROS) level in endothelial cells and rat aortic wall. Furthermore, Western blot analysis of Human Umbilical Vein Endothelial Cells (HUVECs) was performed to evaluate the protein expression associated with oxidative stress. The results show that exendin-4 pretreatment rescued the homocysteine-attenuated endothelium-dependent relaxations in rat aortas and porcine circumflex arteries. Moreover, the present study manifests that exendin-4 effectively inhibits ROS overproduction. The Western blot analysis also shows consistent results. In short, our results imply a therapeutic potential of exendin-4 against cardiovascular diseases associated with homocysteine-associated endothelial dysfunction.

Caspase 3 activation and apoptosis facilitates lytic induction of Epstein- Barr virus in epithelial cells

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Epstein-Barr virus (EBV), also known as human herpervirus 4, has an approximately 170-kb double-stranded DNA genome and expresses more than 80 genes during lytic replication. Apoptosis has previously been shown to mediate lytic induction of EBV in lymphoid and gastric carcinoma cells. In contrast to the lytic cycle, only a dozen or fewer viral genes are expressed when EBV establishes a lifelong latent infection. Notably, some latent transcripts of EBV are capable of preventing apoptosis.

Each year, about 200,000 EBV-infected people will develop various types of cancer. Particularly, EBV-associated nasopharyngeal carcinoma (NPC) is prevalent in Hong Kong and adjacent areas. Exactly how lytic replication is induced in nasopharyngeal (NP) epithelial cells and whether caspase 3 activation and apoptosis are involved in this process remain elusive. A better understanding of these questions might shed mechanistic light on EBV-induced NP carcinogenesis.

We found serendipitously that pre-treatment of epithelial cells with pro-apoptotic drugs 5-fluorouracil and etoposide facilitated lytic induction of the M81 strain of EBV. The number of EBV-infected cells was much higher. It was previously shown that caspase 3 activation and apoptosis induction are essential for efficient influenza A virus propagation and replication in host cells. Whether a similar mechanism might operate in NP and NPC cells remains to be clarified.

EBV lytic induction by different pro-apoptotic agents was further assessed and our results suggested that apoptosis plays a critical role in facilitating EBV lytic induction. Specific inhibitors of caspase 3 was employed to suppress caspase 3 activities and the efficiency of EBV lytic induction was significantly reduced in their presence.

Our findings point to an important role of caspase 3 activation and apoptosis in EBV lytic induction in NP epithelial cells. The new knowledge derived might instruct rational design and devlopment of anti-EBV and anti-NPC drugs.

A Role of NOC3 in DNA Replication Initiation in Human Cells

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DNA replication is a fundamental process that occurs in every single cell in any organisms. In order to maintain the genomic stability and hence the well-being of the organism, there are various mechanisms regulating DNA replication and pre-replicative complex (pre-RC) assembly is one of them. Despite the numerous *in vitro* studies of pre-RC assembly done by other groups, we believe that the *in vivo* context is different due to the complex chromatin structure. So, we are still looking for more regulators of pre-RC assembly.

Noc3 (nucleolar complex associated protein 3) was found to be involved in pre-RC assembly in budding yeast by our lab in 2002 through a genetic screen, and its role in pre-RC assembly is separable from its role in ribosome biogenesis. Through sequence similarity, FAD24 (factor for adipocyte differentiation 24) was identified to be the human homolog of Noc3. To determine if NOC3 in human is also involved in pre-RC assembly, various experiments were performed. Here we show that NOC3 associates with the chromatin, particularly on replication origins, throughout most of the cell cycle. It physically interacts with other pre-RC proteins including ORC and MCM subunits. Knockdown of NOC3 abrogates pre-RC formation and hence S-phase entry, and leads to severe apoptosis in HeLa cells. However, knockdown of RPA194 or treatment with specific RNA polymerase I inhibitor CX-5461 doesn't lead to similar pre-RC and cell cycle defects, implying NOC3 in human cells also plays a crucial role in pre-RC assembly, regardless of its potential role in ribosome biogenesis.

High-throughput identification of RNA G-quadruplexes in model species

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RNA G-quadruplex (rG4) is a class of non-canonical secondary structure formed by 4 strands of RNA and stabilized by monovalent cations such as potassium and sodium ions. They are generally identified from guanine-rich sequences and featuring high thermal stability, which discriminates them from canonical RNA structures.

In biological systems, rG4s serve as versatile cis-regulatory elements and are found in both 5'UTR, CDS and 3'UTR of mRNAs. They influence transcription and post-transcription regulations and translations in various manners. Notably, rG4s carry the dual properties as both protein binding motif and stable RNA structures, which allows them to employ many possible mechanisms of action. For instance, they can be interacting with of specific RNA-binding proteins, competing with other functional RNA secondary structures, serving as spatial obstacles to blocking protein actions, and stabilizing the overall conformation of RNA molecules.

In 2016, the RNA G-quadruplex sequencing (rG4-seq) method was proposed by Kwok et al. and was published in Nature Methods journal. rG4-seq combined high-throughput RNA sequencing and rG4-induced reverse transcriptase stalling to achieve in vitro transcriptome-wide rG4 identification. Our analysis of rG4-seq datasets shown that in vitro rG4s can be accurately detected at single-nucleotide resolution. Foreseeing the characterization of rG4s in other model species via rG4-seq, we believe the information will help revealing the significance of rG4 during evolution processes, improving the general computational prediction of rG4 motifs, and associating rG4s to their in vivo biological functions.

MMP14 and Regulation of the Hypertrophic Chondrocyte to Osteoblast Lineage

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During endochondral ossification, cartilage template is degraded by matrix metalloproteinases(MMPs) and replaced by bone. Currently, hypertrophic chondrocytes(HCs) undergo lineage extension and contribute to osteoblasts in trabeculae. However, the role of MMPs in regulating the lineage transition of HCs to osteoblast is unknown. MMP14(MT1-MMP), encoded by *Mmp14*, is a membrane-tethered matrix metalloproteinase which is highly expressed by cells at the chondro-osseous junction where lineage extension of HCs occurs. Here we show that by following the fate of HCs using *Col10a1-Cre* and *Rosa26* reporters, complete inactivation of MMP14 *in vivo* results in loss of trabecular bone and accumulation of HC-derived cells at the chondro-osseous junction. *In situ* hybridization of osteogenic markers such as *Col1a1*, *Mmp13* and *Opn* are decreased in MMP14 mutants. Live cell imaging of endochondral bone explants suggests HC-descendents undergo active migration at the chondro-osseous junction. Conversely, conditional ablation of MMP14 in HC descendent cells causes increased trabecular bone and decreased apoptosis of HCs at the chondro-osseous junction. We found that MMP14 interacts and cleaves parathyroid hormone 1 receptor(PTH1R) which governs the development of chondrocytes, metabolism of osteoblasts as well as fate decision of HCs. Haloinsuffuciency of *pth1r* partially rescues postnatal endochondral ossification in *Mmp14*$^{-/-}$ mice. Our work unravels an important function of MMP14 in lineage progression of hypertrophic chondrocytes.

Lysosomal Ca^{2+} disruption induces autophagy impairment in familial Alzheimer's disease

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Autophagy is an evolutionary conserved cellular pathway for the cell to cope with stress and starvation. Deranged autophagy has been observed in Alzheimer's disease (AD)[1] and it has been reported that presenilin-1(PS1) is essential for the maintenance of acidic lysosomal pH[2]. However, the underlying molecular mechanism for lysosomal alkalization due to PS1 mutation is still obscure. Two-pore channel 2 (TPC2) is a lysosomal Ca^{2+} release channel that was found to play a role in lysosomal alkalization[3]. Here we found that PS1 interacted with TPC2 channel in the human neuroblastoma SH-SY5Y. The lysosomal Ca^{2+} content was significantly lower while its pH was higher in PS1-M146L expressing SH-SY5Y than those of wild-type PS1 expressing cell and GFP-expressing control cell. Intriguingly, reduced lysosomal Ca^{2+} content and alkalization in PS1-M146L can be rescued by treating the cell with TPC2 specific inhibitor, NED-19. Consistent Ca^{2+} disruption and lysosomal alkalization were observed in fibroblasts isolated from AD patients with PS1 mutation and these derangements were corrected by NED-19. Together, our results suggested that lysosomal Ca^{2+} disruption due to PS1 mutation plays a role in the pathogenic mechanism for autophagy impairment in familial AD and this provided a novel target for therapeutic intervention of the disease.

Functional study of Yin Yang 1 in mouse cerebellum development

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The multifunctional transcription factor Yin Yang 1 (YY1), was named after its dual roles of both activate and repress gene transcription. YY1 contains inhibitory domains and activation domain, functions via disrupting DNA-binding of other transcription factors, recruiting co-factors, or changing DNA conformation. YY1 plays complex roles in various fundamental biological processes such as cell survival, proliferation, and differentiation, thus affects embryogenesis. Mouse embryos encounter peri-implantation lethality when YY1 was completely inactivated. Ablation of YY1 in *Xenopus* indicates it is essential for neural induction and patterning. Different studies have revealed YY1 is critical for stem cell development in different tissues. However, studies on the role of YY1 in central nervous system (CNS) development remain limited.

As a model system to study the CNS, cerebellum consists of defined types of cells, and has the well-known functions for motor control and cognition. The cerebellum emerges from metencephalon. The isthmus organizer locates at the mid-hindbrain boundary (MHB) region serves as the signaling center. During embryonic stages, the ventricular zone (VZ) and rhombic lip (RL) give rise to the GABAergic and glutamatergic neurons of cerebellum, which will generate the Purkinje cells and granule neurons respectively. Disruption of the developmental processes may cause severe CNS disorders. YY1 is ubiquitously expressed in the developing cerebellum. Using tissue-specific Cre-LoxP system, we generated MHB YY1 conditional knockout mice. The mutants displayed cerebellar agenesis and truncated midbrain, indicates the crucial role of YY1 in early brain region patterning. YY1 deleted cells showed reduction in cell proliferation and defect in specification.

RNAi-effecting vector construction in "obligate" anaerobic *Salmonella* strain *YB1* as a potentially competent payload in cancer treatment

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By altering the anaerobic properties of *Salmonella* strains, our lab has developed an obligate anaerobic strain *YB1* that confers specificity to hypoxia regions of the tumor, thereby avoiding indiscriminate targeting of these enzymes in aerobic healthy tissue and avoiding side-effects (Yu, Yang et al. 2012). In this study, we tested the efficacy of *YB1* as a novel delivery system for a T7 RNA polymerase (T7 RNAP) positive feedback construct (pIT7S-shRNA), which is theoretically capable of maintaining a high level of T7 RNAP and an ensuing over-expression of the cargo gene—shRNAs against potential cancer therapeutic candidates Sphingosine Kinases (SKs). A more robust and prompt *in vitro* expression of *T7 rnap* in MDA-MB-231 cells after YB1-pIT7S treatment in comparison with chemical transfection was observed, and attested our anticipation on high delivery efficiency of *YB1*. Nonetheless, a counterintuitively elevated expression and absence of RNAi effect of Sphingosine Kinases drew our attention to the bacteria-host interaction and interference of transcription units within the construct. Through transcripts detection across the entire construct region, altogether with pIT7S-EGFP translation verification, we hypothesized that a read-through effect due to overly-high activity of T7 RNAP and insufficient transcriptional termination generated "super-long" transcripts and subsequently inhibited normal processing of shRNAs. While our study keeps going on optimization of the shRNA construct and adaptation of *YB1* vehicle-cargo-host response axis, current findings has provided insights on how to enhance efficacy of treatment by high efficiency transfection and knockdown of SK expression, resulting in cancer cell death with the bacterial delivery system.

Partnering role of FOXM1 and EPS8 in maintaining chemoresistance in human ovarian cancer

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Forkhead box protein M1 (FOXM1) is a transcription factor ubiquitously expressed in proliferating cells. Its levels are overexpressed in various cancers, including ovarian cancer. The promoter activity of FOXM1 was recently found to be unregulated by Epidermal growth factor receptor kinase substrate 8 (EPS8), a substrate for tyrosine kinases. Interestingly, depletion of FOXM1 or EPS8 in cancer cells led to chemosensitivity against cisplatin. Mechanistically, FOXM1 is known to transcriptionally up-regulate copper transporter 1 (hCTR1) and other DNA repair genes, and to enhance β-catenin activation. Our recent finding that FOXM1 and EPS8 physically interacted in yeast two-hybrid and immunoprecipitation assays suggests that EPS8 might mediate its chemoresistant effect in cancer cells via modulation of FOXM1 function. To test whether FOXM1 and EPS8 regulate cancer chemosensitivity synergistically, we studied chemosensitivity in the ovarian cancer cell line ES2 with a series of cisplatin concentrations using XTT assay. First, treatment with FDI6, a small molecule inhibitor of FOXM1 DNA binding activity, was found to increase chemosensitivity and experiments are ongoing to determine the effect of shRNA-mediated EPS8 knockdown. Second, after either FOXM1 inhibition or EPS8 knockdown, more cells were shown to undergo apoptosis under cisplatin treatment as reflected by the increased expression of cleaved PARP-1 protein in immunoblotting analysis. Third, mRNA levels of the DNA repair genes EXO1 and RAD51 were found to be suppressed after FOXM1 inhibition and/or EPS8 knockdown by RT-qPCR analysis. Taken together, our findings support that FOXM1 and EPS8 interact to mediate regulation of chemosensitivity in ovarian cancer cells.

Activation of nucleic acid sensing cGAS-STING pathway by Sindbis Virus

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Cytosolic DNA sensing by cGAS plays an important role in type I interferon mediated antiviral immunity against DNA viruses or retroviruses. Second messenger cGAMP is produced from activated cGAS which binds to STING and leads to subsequent activation of type I interferon production cascade and antiviral response. Currently, only B form and Y form dsDNA as well as DNA:RNA hybrid have been considered as cGAS ligand. The role of cGAS in RNA virus sensing remains unclear. Here we explore the possible mode of cGAS activation by positive-sense single-stranded RNA virus, Sindbis virus. cGAS transcript was induced upon Sindbis virus infection and this induction was type I interferon signaling independent. Reconstitution of cGAS and STING in cGAS- and STING-null cells effectively suppresses Sindbis virus replication. On the other hand, loss of either cGAS or STING augmented Sindbis virus replication and prevented viral induction of type I interferon mediated antiviral immunity, suggesting a possible role of cGAS-STING signaling pathway in RNA viruses sensing. Enrichment of cytosolic nucleic acid species is observed in Sindbis virus-infected cells, indicating the presence of a cGAS ligand. Taken together, our findings reveal an unrecognized role of cGAS-STING pathway for cytosolic DNA sensing in RNA virus infection. Supported by HKRGC (HKU171091/14M and C7011-15R).

Expression of ATP-binding cassette transporter ABCF1 in fetal liver and liver cancer associated with cancer stem cell markers and chemo-resistance

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The importance of understanding stem cell biology in relation to cancer has been made clear since the cancer stem cells (CSCs) notion and recurring deregulated stem cell pathways reported in cancers. ATP-binding cassette (ABC) transporter ABCB1 (also named P-glycoprotein and MDR1) has shown to be CSC marker, mediate multidrug-resistance in model systems, yet, with controversial clinical relevance. Through systemic analysis on the expression profiles of ABC genes in hepatocellular carcinoma (HCC) clinical samples, ABCF1 was found significantly over-expressed in HCCs compared to the adjacent non-tumor liver tissues and associated with recurrence-free survival after curative partial hepatectomy. Recurrences have often been attributed to CSCs. We therefore further explored the significance of ABCF1 in both fetal liver and liver cancer cells in respect to stem cell features. Expression of ABCF1 in fetal, neonatal and adult mouse livers were examined by immunohistochemistry, western blot and flow cytometry. Consistently, elevated ABCF1 expression was demonstrated in early embryonic stages but rarely in neonates and adult livers. Also, ABCF1 co-stained with stem cell-related marker β-catenin and hepatic CSC markers CD133 and GEP in fetal hepatocytes and in HCC cells. ABCF1 suppression by siRNA enhanced chemo-sensitivity of the HCC cells Hep3B compared to control cells on cisplatin and 5-Fluorouracil. Overexpression of ABCF1, conversely, induced chemo-resistance to cisplatin, and doxorubicin efflux ability in Hep3B and HepG2 cells. The current study put together the functional significance of ABCF1 in liver development and liver cancer as a stem cell-related molecule regulating chemo-resistance.

G_q signaling pathway of Melatonin receptor in islets of Langerhans

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Melatonin is a hormone released from the pineal gland and it is involved in many physiological processes. Two G_i-coupled receptors represent the major targets of melatonin in mammalian systems and they are named as melatonin MT_1 and MT_2 receptors. There is increasing evidence to suggest that melatonin may participate in the regulation of glucose metabolism and the pathogenesis of Type 2 diabetes (T2D). A number of genome-wide association studies have recently revealed that single-nucleotide polymorphisms in the gene encoding the MT_2 receptor (*MTNR1B*) are strongly associated with an increased risk of T2D. Since insulin secretion from the islets of Langerhans involves Ca^{2+}-mediated responses, we examined the ability of $MT_{1/2}$ to induce Ca^{2+} mobilization in pancreatic cell lines. Early studies have suggested that MT_1, but not MT_2, is capable of stimulating $G_{q/11}$ but this remains controversial. Herein, we explored the possibility of $MT_{1/2}$ regulating intracellular Ca^{2+} via G_{14}, a member of the G_q family which is expressed in the pancreas. The mRNA levels of $MT_{1/2}$ were determined in both pancreatic αTC1.9 and βTC6 cells by RT-qPCR. $G\alpha_{14}$ expression in these cell lines was assessed by immunoblotting with an anti-$G\alpha_{14}$ antiserum. Our results indicate that although MT_2 may be expressed in βTC6 cells, melatonin cannot mobilize Ca^{2+} as determined by FLIPR assays. Also, MT_2 activation did not inhibit CREB phosphorylation in these cells. (Supported by grants 16103015 and ITCPD/17-9)

Establishment of a NHEJ-mediated visible conditional knockout system in zebrafish

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Conditional disruption of gene of interest in a spatiotemporal-restricted manner is often desirable to precisely dissect its function from the intertwined phenotypes. Conventionally, this is achieved by introducing the loxP-flanked exons into the genetic loci via homologous recombination (HR) within mammalian ES cells. However, this approach is not applicable for zebrafish due to lack of ES cell during its early development. Here, we generated a visible conditional knockout *pu.1* allele (*pu.1*^{KI}) via non-homologous end joining (NHEJ) -mediated knockin method. In *pu.1*^{KI}, GFP signals perfectly mimicked the endogenous *pu.1* expression. Furthermore, we demonstrated that function of Pu.1 in *pu.1*^{KI} allele was maximally retained. After Cre-mediated recombination, coding sequence of *pu.1* exon 4-6 and GFP were excised. With the aid of an artificial splicing acceptor site, sequence of P2A-Dsred were spliced to *pu.1* exon 3 and properly expressed. Phenotypically, we showed that function of Pu.1 was largely disrupted in this knockout allele (*pu.1*^{ckout}). Collectively, we established a visible conditional knockout system that will potentially facilitate further studies in at least 3 aspects: (1) conditional knockout of gene of interest in a spatiotemporal manner; (2) mosaic study of the wild-type and knockout cells with different colors in a single animal; (3) genetic labelling and tracking the cells that express this gene.

Effects of Ginger Extract on TMAO-Induced Atherogenesis

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Trimethylamine-N-oxide (TMAO) has recently been identified as an independent risk factor for promoting atherosclerosis by impairing vascular function, exacerbating inflammation, and adversely affecting cholesterol metabolism through inhibiting hepatic bile acid synthesis. Ginger (*Zingiber officinale*), which is widely used as a spice, has been reported to prevent atherogenesis in many studies due to its potent cholesterol-lowering, vasoprotective and anti-inflammatory activities. The present study was to investigate the effects of ginger extract (GE) on the TMAO-induced atherosclerosis in mice fed with a high-fat-and-cholesterol diet (HCD). After 12-week intervention, results showed that TMAO raised plasma TMAO concentration in mice, resulted in elevated plasma and liver cholesterol levels. GE could conspicuously lower plasma total cholesterol by 24.9% and 21.2% compared to HCD and HCD+TMAO groups, respectively. Slight but not significant declines in liver cholesterol were observed in mice fed with GE. In summary, GE could ameliorate the TMAO-induced cholesterol accumulation in mice.

For future work, fecal neutral and acidic sterols will be quantified to obtain a more comprehensive understanding of cholesterol metabolism and distribution. Plasma inflammation biomarkers including MCP-1, IL-6, IL-1β, TNF-α and IL-10 will be determined by enzyme-linked immune sorbent assay (ELISA). In order to investigate the underlying mechanisms, expression of hepatic and intestinal genes related to cholesterol absorption, regulation and excretion will be analyzed by real-time PCR and western blotting.

Sufu controls the proliferation and differentiation of cochlear prosensory progenitors

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During inner ear development, prosensory progenitors exit cell cycle before E14.5. Later, some progenitors express *Atoh1* and differentiate into hair cells. Loss-of-function analysis suggests that *Shh* controls the timing of hair cell differentiation. However, little is known about the roles of the downstream mediators of Shh signaling in cochlear hair cell progenitors. Gli transcription factors regulate the expression of downstream genes, while Suppressor-of-fused (Sufu) modulates the transcriptional activity, stability and processing of Gli in some tissues. To understand the function of *Sufu* in prosensory progenitors, *Pax2-cre; Sufu*$^{f/f}$ mutant with *Sufu* deleted in the inner ear was generated. By immunostaining, four rows of hair cells were observed at the medial region of E16.5 control cochlea but hair cell was not found at the similar region of *Sufu*-null mutant, indicating severely delayed hair cell differentiation. Since Gli3 repressor level significantly reduced in the absence of *Sufu*, *Gli3*$^{P1\text{-}4/P1\text{-}4}$ mutant that produced no Gli3 repressor protein was studied. The progression of hair cell differentiation was also delayed in *Gli3*$^{P1\text{-}4/P1\text{-}4}$ mutant ear. These showed that *Sufu* modulated the differentiation of hair cell progenitors via Gli3 repressor. As defects in differentiation may be due to impaired progenitor specification and proliferation, immunostaining of Sox2, p27 and BrdU was performed. In the E14.5 *Sufu* mutant, Sox2 and p27 were expressed at the ventral cochlear epithelium. However, loss of *Sufu* resulted in more BrdU^{+} proliferating progenitors in the Sox2^{+} domain. Our data suggest that *Sufu* regulates both proliferation and differentiation of hair cell progenitors.

Investigation on Dendritic Development of Cerebellar Purkinje Cells

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Purkinje cells are essential neurons in cerebellum that integrate multiple inputs through spines on the elaborate dendritic arbors for motor coordination. Dendritic arborization, spinogenesis and synaptogenesis occurs postnatally to help build up the cerebellar circuits. Dysregulation in Purkinje circuits is the recognized phenotype found in ataxias, the symptom featured by uncoordinated movement. We are interested in the detailed molecular mechanisms regulating this postnatal Purkinje cell developmental process. Wnt signaling has been widely studied in other neurogenic processes including dendritogenesis of hippocampal neurons, but little were known about cerebellum. This study focuses on Wnt signaling in Purkinje cell dendritic development to fill up this gap.

Here we summarize and report our study on Wnt signaling during postnatal Purkinje cell development. First, we studied the function of Wnt signaling on dendritic development through Wnt inhibitor treatment on cerebellar slice culture and observed retarded dendritic growth in Purkinje cells. Then we targeted on the molecular level of Wnt signaling in cerebellum and clarified the specific expression of Wntless (Wls) in Purkinje cells. Wls is a conserved Wnt ligand transporting protein, thus indicating the potential role of Purkinje cell to secret Wnt ligands for regulating postnatal cerebellar development. Further, we also identified down-regulation of Wls in conditional Lhx1/5 knockout mutant Purkinje cells with deficit dendritogenesis. Last, we screened and profiled the Wnt ligands and Wnt signaling related molecules expressed in postnatal cerebellum for further study.

Analysis of Protein Sorting at The Trans Golgi Network Through STORM Super-Resolution Imaging

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The trans Golgi network (TGN) is an essential transport hub in the secretory transport pathway. At the TGN, various cargo sorting machineries function to package specific cargo proteins into distinct transport carriers that are targeted to specific destinations. Protein sorting fundamentally relies on spatial segregation, but it remains largely unclear how proteins that participate in the TGN sorting process are spatially related to each other. Here, we utilize 2-color STORM super-resolution localization microscopy (SRLM) to analyze protein sorting at the TGN at high resolution. Using SRLM, we showed that two TGN/endosome-localized cargo adaptors, adaptor complex-1 (AP-1) and epsinR, can form elongated structures with over 250 nm in length in vivo and majority of these structures are associated with clathrin. In contrast, the majority of structures of another cargo adaptor, AP-3, are not associated with clathrin. In addition, we observed that AP-1 is specifically associated with two of the Golgi-localized Arfs, Arfrp1 and Arf1. We also found that AP-1 shows distinct spatial relationships with epsinR, GGA2 and AP-3. Moreover, we directly observed specific packaging of a planar cell polarity signaling receptor, Vangl2, in AP-1- decorated structures upon exiting the TGN. This high-resolution imaging analysis indicates that the TGN membrane is mosaic, where different Arf proteins and cargo adaptors can assemble into large elongated structures on distinct microdomains at the TGN to mediate sorting of specific cargo molecules. Spatially segregated cargo adaptors may function to mediate different cargo sorting process while spatially associated cargo adaptors may cooperatively mediate the cargo sorting process.

Bmal1 deletion in macrophages and monocytes exacerbates atherosclerosis

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Background

The molecular clock is expressed in most cells and plays an essential role in regulating cardiovascular function, immune responses, and metabolism through rhythmic expression of clock-controlled transcripts and their biological functions. Bmal1 is the non-redundant transcription factor in the core molecular clock, and it controls the rhythmic trafficking of inflammatory Ly6c^{hi} monocytes to sites of acute inflammation. We aimed at investigating whether Bmal1 deletion in macrophages and monocytes exacerbates atherosclerosis by enhancing monocyte recruitment to atherosclerotic lesion.

Methods and results

We used the Apoe$^{-/-}$; Bmal$^{Floxp/Floxp}$ as control and Apoe$^{-/-}$; Bmal1$^{FloxP/FloxP}$; LysM$^{Cre/+}$ as myeloid Bmal1-deficient mice on Apoe$^{-/-}$ background for experiments. We found that Oil Red O staining revealed augmented lesion size and lipid content in the aortic root section from mice with Bmal1 deletion in myeloid cells, indicating that Bmal1 deficiency in myeloid cells promoted the formation of atherosclerotic lesion. The number of total lesional macrophages and infiltrating inflammatory monocyte-derived macrophages were increased in Bmal1 deficient mice. Increased macrophage content was accompanied by skewed M2 to M1 macrophage phenotype measured by flow cytometric analysis and quantitative PCR. Enhanced recruitment of Ly6c^{hi} monocytes into the lesion was further confirmed by in vivo labeling of Ly6c^{hi} monocytes by fluorescent beads. In addition, adoptive transfer of Ly6c^{hi} monocytes from myeloid-deficient mice into Apoe$^{-/-}$ mice increased macrophage content in the plaque and lesion size. Meanwhile, Ly6c^{hi} and/or Ly6c^{lo} monocyte subsets in blood, spleen, and bone marrow were not altered in both genotypes.

Conclusion

In this study, we found that myeloid specific Knockout of Bmal1 worsens atherosclerosis by increasing monocytes trafficking. Our findings provide insight into the novel role of molecular clock in the process of chronic inflammation in atherosclerosis.

(Supported by CUHK2/CRF/12G, CUHK464712, CUHK Direct Grant 2015.2.046 and CUHK Lui Che Woo Foundation)

Characterization of KAP1 acetylation and its roles in DNA damage repair

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KAP1, also known as TRIM28, is a heterochromatin-associated protein. It mainly functions in formation of heterochromatin, transcriptional silencing and maintenance of genome integrity. KAP1 also participates in DNA damage repair. In particular, the phosphorylation of KAP1 at pS824 and pS473 is important for its dissociation from the heterochromatin and chromatin relaxation upon DNA damage. However, the biological relevance of other post-translational modifications of KAP1 and how they crosstalk with its phosphorylation remain unknown. Recently, we discovered that KAP1 is a substrate of SIRT6. It can be deacetylated by SIRT6 in vitro. Comparison of wildtype and SIRT6 knockout immortalized MEF also indicates that acetylation of KAP1 is significantly increased when SIRT6 is depleted. We further found that KAP1 can be acetylated by histone acetyltransferases such as MOF, p300, GCN5 and Tip60. The overall acetylation levels of KAP1 will be decreased in response to irradiation-induced DNA damage, suggesting that acetylation of KAP1 plays certain roles in the regulation of DNA damage. This also parallels to our finding that SIRT6 may affect the dissociation of KAP1 from heterochromatin in response to DNA damage repair.

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Antidepressant Reinstates the Plasticity of Vestibular-mediated Navigation in the Adult

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Spatial navigation in dark depends on egocentric cues with inputs from the vestibular system. We have previously revealed a critical period for this behavior in first two postnatal weeks during which adult navigation behavior can be shaped by early sensory inputs from the vestibular nucleus (VN). As a 5-HT reuptake inhibitor, fluoxetine is known to restore the plasticity in the adult visual cortex. We hypothesize that fluoxetine also restores the plasticity of VN-dependent spatial navigation. In the present study, we demonstrated that perturbation in the sensory inputs from the VN at P21 could still lead to deficits in adult navigation when the rats were orally administered with fluoxetine during P21-28. Both long-term plasticity and the ratio between excitation and inhibition in the VN were also restored to a neonatal state after fluoxetine treatment. In addition, there was a significant increase in the number of parvalbumin-expressing (PV^+) cells in those rats treated with fluoxetine during P21-28. With the application of BrdU, new-born PV^+ neurons were then identified in the VN after fluoxetine treatment. Blockade in cell proliferation in the VN rescued the derangement in adult navigation behavior, indicating that PV^+-neurogenesis plays an important role in the restoration of plasticity in the spatial navigation. Taken together, our findings provide an insight on the role of fluoxetine in restoring plasticity in the adult sensorimotor system. [Supported by HKU 761812M]

Modulatory role of orexin on synaptic transmission in the central vestibular system

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Orexin is known to modulate synaptic plasticity in the hippocampus and contribute to social memory in adult rodents. While orexinergic neurons in the lateral hypothalamus project to the vestibular nucleus (VN), the role of orexin in the maturation of vestibular functions remains unexplored. We hypothesized that orexin modulates synaptic transmission in the VN, thereby regulating the expression of vestibular-related behaviors during postnatal development. To understand the role of orexin on synaptic transmission in the VN, we employed in vitro whole-cell patch-clamp technique to study the action of orexin on the excitability of neurons in the medial vestibular nucleus (MVN) of rats at postnatal day 14. Treatment with orexin led to reduction in amplitude and frequency of miniature inhibitory postsynaptic current (mIPSC). This suggests that orexin decreases both presynaptic release of inhibitory transmitters and postsynaptic depolarization within the MVN. We have thus demonstrated that orexin suppresses synaptic inhibition on MVN neurons. We further investigated whether orexin-modulated mIPSC is mediated by GABA-A receptors or glycine receptors. With the use of bicuculline and strychnine, we observed that orexin decreased mIPSC mediated by GABA-A receptors, but not glycine receptors. Taken together, our findings provide us with fundamental knowledge about the modulatory role of orexin in GABAergic transmission within the VN and its impact on postnatal refinement of neural circuit for vestibular-related behavior.

[Supported by N_HKU735/14]

Identification of Non-coding RNA-mediated Gene-regulatory Network in *dachb* Deficient Zebrafish

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In mammals, the body's only blood glucose-lowering hormone – insulin, is secreted by the pancreatic β cells, residing in pancreas. Insulin acts as a central player in the pathogenesis of diabetes, the associated complications of which, including diabetic nephropathy, extremity amputation and heart failure, were the principal contributors of mortality. The dachshund gene family (such as dachb in zebrafish) has been reported playing a role in the production of insulin-producing cell and pancreas development in zebrafish and mice. Long non-coding RNAs (lncRNAs), on the other hand, which serve important roles in gene regulation at multiple levels including transcription, post-transcription and translation, have contribution to the transcriptional mechanisms regulating pancreas development. However, the importance of dachshund gene family-affected lncRNAs has not been investigated. This work aims to investigate how dachb-deficiency leads to lncRNA alterations in zebrafish embryos further to affect pancreas development. High-throughput transcriptome data were sequenced in 8 zebrafish embryos of 3 groups, including dachb and p53 knockdown, p53 knockdown and untreated groups. Here, p53 co-injection was to exclude whether the observation was due to p53 expression-induced off-target effects. Novel differentially-expressed lncRNAs (including those located at antisense strand of protein-coding genes, intergenic and intronic regions) and lncRNA-mediated gene regulations are predicted based on an integrated reference-based and reference-free method in order to maximum the confidence of predictions. Subsequently, validations using public datasets and experiments in zebrafish will also be performed. It is hope that these analyses will contribute to a better understanding of dachb- and lncRNA- mediated pancreas development in zebrafish.

[We thank Prof. Ronald Ma's team at the Department of Medicine and Therapeutics (CUHK) for preparing the knockdown zebrafish and performing the tissue dissection work. This study is supported by a Theme based research scheme (Ref. No.: T12-402/13N) to Prof. Ronald Ma (Department of Medicine and Therapeutics, CUHK)]

Inorganic Polyphosphate Influences Cyclophilin B-Mediated Protein Folding In Osteoblasts

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Evidence is emerging that inorganic polyphosphate (polyP), a ubiquitous polymer of orthophosphates linked by ATP-like bonds, is fundamental to numerous key biological processes in mammalian cells. PolyP's involvement in higher eukaryotes includes chaperoning ability and as modifier of protein misfolding events but several unanswered questions remain regarding polyP's mechanism of action. We aim to identify significant polyP-protein interactions to understand polyP function in osteoblasts. PolyP-specific binding proteins in SaOS-2 osteoblast-like cells were identified using an affinity chromatography approach developed by crosslinking polyP-biotin and immobilising polyP via biotin-streptavidin interactions. We report evidence that cyclophilin B (CypB), an endoplasmic reticulum (ER)-localised chaperone and key enzyme in collagen folding, functionally interacts with polyP at high affinity. Independent binding assays validated that polyP binds tightly to purified, recombinant human CypB. Rotamase assays further revealed that polyP strongly inhibits CypB's peptidyl-prolyl cis-trans isomerase activity. Confocal microscopy confirmed polyP-CypB colocalisation in the ER of SaOS-2 cells. To further study polyP's intracellular activity, we investigated polyP's effects on CypB's collagen folding activity in non-differentiating SaOS-2 cells. Spermine was used to sequester polyP and obstruct polyP-CypB binding. A vector was constructed to express ER-targeted polyphosphatase resulting in reduction of ER-polyP levels. Results suggest that CypB-mediated collagen folding was enhanced by polyP sequestration and depletion in ER. This study provides mechanistic insight into how polyP affects protein folding mediated by CypB function in osteoblasts.

Tracking Anthropogenic Pollution in Aquatic Environments using Class 1 Integron-integrase (*intI1*) Gene in *Escherichia coli*

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Detecting all pollutants in aquatic environments is hardly feasible due to their diverse nature. Monitoring the integron-integrase gene of class 1 integrons, *intI1*, could be an alternative. Class 1 integrons can capture gene cassettes from diverse pool of exogenous genes, including genes that confer adaptive capabilities such as antibiotic resistance. Clinical variant of class 1 integrons is hypothesized to have evolved and proliferated in human-dominated ecosystem due to selective pressure imposed by human activities. Previous studies have showed that class 1 integrons are not stably maintained in *Escherichia coli* without such selective pressure. Therefore, we aim to determine the prevalence of clinical *intI1* in *E. coli* isolated from aquatic environments in Hong Kong and further test its use as a proxy for anthropogenic pollution. *E. coli* isolates (n=280, from water, sediment, and periphyton) from 15 sampling sites in Western HK with different levels of human disturbance were tested with clinical *intI1*-specific primers. Clinical *intI1* was detected in 5.7% of the isolates. A weak trend towards higher occurrence of clinical *intI1* among isolates from sites with higher anthropogenic influence was observed. Our result showed that clinical *intI1* in *E. coli*, the current in-use faecal indicator bacteria in HK, could be a promising proxy for pollution monitoring. Sample size will be increased in upcoming sampling campaign to improve statistical accuracy. Similar studies will be conducted along Pearl River Estuary and HK coastal waters to provide a more precise depiction of the *intI1* distribution among *E. coli* in this region.

Characterization of Copper Transportor Genes in a Zebrafish Liver Cell Line, ZFL

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Copper is essential for all organisms to serve as cofactor of many enzymes involving in many different physiological functions. However, Cu homeostasis must be maintained in the body, and at cellular level, because Cu ions are oxidative and could be toxic. Mutations in human ATP7A cause Menkes disease with Cu deficiency, whereas mutations in ATP7B cause Wilson's disease showing Cu accumulation and toxicities. ATP7A and ATP7B transfer Cu^+ to nascent proteins synthesized in the Golgi apparatus and eliminate Cu^+ from the cells expressing them. CTR1 imports Cu^+ and ATOX1 transports Cu^+ from Ctr1 to ATP7A and ATP7B. In human or mammals, ATP7B is highly expressed in liver, kidney and placenta, and ATP7A is expressed in majority of tissues except liver, while CTR1 and ATOX1 is expressed in all type of cell. Zebrafish (*Danio rerio*) is a powerful vertebrate model organism and ZFL is the cell line of zebrafish liver being used in this project to study the molecular mechanism of Cu homeostasis.

The LC50 values of $CuCl_2$, $ZnCl_2$ and $CdCl_2$ in ZFL were first determined. the expression profile of Cu transporters in ZFL is studied by using qPCR. We are also interested in studying the gene promoters of these four Cu transporters. The upstream region of DNA five kilobase to the four Cu transporters are studied *in silico*. Putative metal response elements, TATA box, and numerous nuclear hepatic factors were found by using web tools in both genes, but *atp7a* has an extra GATA4 regulatory element. Transient gene expression assay in ZFL with dual luciferase reporter gene assay will be conducted to confirm the function of these gene promoters in response to metal challenges.

[Research supported by a Direct Grant (4053172) from the Faculty of Science, Chinese University]

Postnatal Expression of Pax1 in the Intervertebral Disc during Postnatal Growth and Response to Spinal Curvature

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Intervertebral discs (IVD) are fibrocartilaginous tissues of the spine that provide flexibility and absorb mechanical stress. Adolescent Idiopathic Scoliosis (AIS) is a multifactorial disease that causes sudden lateral curvature of the spine during the adolescent growth spurt but has no known cause. SNPs near the *PAX1* gene have been associated with AIS patients, and PAX1 is known to be a key transcription activator in the embryonic development of the disc. However, the postnatal expression and function of PAX1 have not been addressed previously. In addition, Pax1-null mice develop spinal curvature, yet it is unclear how the mutation affects the disc to initiate or contribute to progression of the curve. This study provides the first detailed analysis of postnatal *Pax1* expression in the mouse disc. Pax1 is found to be expressed in the NP and AF of lumbar and tail discs from neonatal to adult ages. Next, to study the effect of spinal curvature on Pax1, the tail looping method was applied to 6- and 8-week old mice for 1 and 4 weeks. Pax1 expression was maintained in the NP, but was reduced on the convex OAF and increased in the concave OAF. This indicates that *Pax1* expression can be influenced by mechanical forces in postnatal growth and maintenance. Thus, *PAX1* as a genetic risk factor may contribute to initiation and progression of spinal curvature in AIS from asymmetrical loading of the IVD. Studies of IVD development and curvature in Pax1-null mice are in progress, and genetic interaction with another transcription factor, Runx3 will be examined in the future.

Structural analysis of ATG9 by cryo-electron microscopy

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Autophagy is an evolutionarily conserved pathway for bulk degradation of intracellular proteins and organelles *via* formation of double-membrane vesicles termed the autophagosomes. Multiple core autophagy-related (ATG) proteins are involved in organizing the pre-autophagosomal structure or phagophore assembly site, from which autophagosomes are generated. ATG9 is the only transmembrane protein of the core autophagy machinery whose function is to deliver lipid source to the expanding phagophore in the form of ATG9-containing vesicles. In plants, ATG9 has been demonstrated to play an essential role in promoting the outgrowth of autophagosomes from the endoplasmic reticulum [1], a process that is likely dependent upon the multimerization of ATG9 [2,3]. There are currently no structures available for ATG9, prohibiting detailed understanding of the mechanism of ATG9-mediated autophagosome biogenesis/progression. Here, we propose to use single-particle cryo-electron microscopy to elucidate the structure of *Arabidopsis thaliana* ATG9 (AtATG9). AtATG9 was overexpressed in *E. coli* and purified by tandem affinity purification method. Electron microscopy of the purified, detergent-solubilized AtATG9 revealed monodisperse particles suitable for single-particle analysis. We anticipate that our findings will shed light upon the universal role of ATG9 in autophagy, as the tertiary structures of ATG9 are generally conserved among different species.

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MicroRNA in Mitochondria

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Mitochondria consist of a unique genome which can encode proteins involved in the electron transport chain (ETC) for energy production. Numerous cellular pathways are under the tight regulation of mitochondria. Mitochondrial dysfunction can lead to cancer formation.

MicroRNAs (miRNAs) are evolutionary conserved, non-coding RNA molecules. miRNAs together with Argonaute (Ago) protein are essential for post-transcriptional gene regulation. It is widely understood that miRNAs can affect the functions of mitochondria by down-regulation of the mitochondrial proteins originated from nuclear DNA.

Growing evidence suggested that nuclear-encoded miRNAs are imported to mitochondria. Despite their existence, their biogenesis and action mechanisms remain unexplored.

This project aims at investigating the roles of miRNAs in mitochondria. In particular, we are going to examine whether these miRNAs can regulate the mitochondrial-encoded proteins in ETC by altering the rate of oxidative phosphorylation (OXPHOS) and their contribution to the development of multidrug resistance (MDR) in hepatocellular carcinoma (HCC). Previous study indicated that ATP depletion can hamper the efficiency of multidrug resistance-associated proteins (MRP).

Doxorubicin resistant HepG2 (R-HepG2) cell line has been developed in our lab. Luciferase assay validated that R-HepG2 cells produced more ATP than HepG2 cells. Highly enriched mitochondrial fraction was determined by Western blotting analysis. miRNAs were isolated from the mitochondria for subsequent RT-PCR assay and miRNA sequencing. More work is needed to dissect the role of miRNAs in mitochondrial functions.

This work is partially supported by direct grants (3132798, 4053100) from the Faculty of Science, CUHK.

Understanding the contribution of Lgr5+ interzone progenitor cells in articular cartilage development and regeneration

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Osteoarthritis (OA) is one of the major joint disease worldwide and the main feature of OA is the lost or damage of the articular cartilage (AC). AC known to have a poor healing ability but cells with progenitor markers expression were found in adult AC. However, the function and origin of these progenitors remain largely unknown. During the formation of the synovial joint, dedifferentiation of cells at the future joint site form a pool of progenitor cells namely the interzone. Leucine-rich repeat-containing, G-protein-coupled receptors (Lgr) 5 is a downstream target of Wnt signaling and a novel progenitor cell marker in hair follicles, intestinal crypt and various tissues. Data from our lab shows that Lgr5 is specifically expressed in the developing joint marking the interzone cell population, while lineage tracing of these Lgr5+ cells in the developing joint suggest that they differentiated into cells in AC, meniscus, ligaments and other joint compartments. Our hypothesis is that these Lgr5+ progenitors may have a potential to contribute the regeneration of AC. Genetic differences in wild type mice have a huge effects on healing ability in different strains of mice, MRL, good healer strain can fully heal an ear puncture and partially heal punctured AC while C57, bad healer strain cannot. By using these genetic differences in mouse and cross breed them with the mouse carrying a Lgr5 promoter controlled GFP reporter, it will give us a tool to understand these progenitor in growth and development and also their contribution during regeneration by inducing articular cartilage defects using a needle puncture model.

Role of FE65 Phosphorylation in APP Processing

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Alzheimer's disease (AD) is an irreversible and fatal neurodegenerative disorder which is currently affecting 46.8 million people worldwide. Despite the long history of discovery, effective treatment against AD is still lacking. One major pathological hallmark of AD is the deposition of neuritic plaques in brain, which comes from the aggregation of amyloid β peptide (Aβ) generated from amyloid precursor protein (APP). APP is a type I transmembrane protein. Upon proteolytic cleavage by β- and γ-secretases, the neurotoxic Aβ is produced. Up to now, amyloidogenic processing of APP is believed to be regulated by numerous APP-interactors, including FE65. FE65 is a multifunctional brain-enriched adaptor protein. However, the mechanism by which FE65 modulates APP processing remains unclear. Previous studies showed that FE65 is a phosphoprotein and many phosphorylated residues were identified by mass spectrometry. By various *in silico* prediction tools, we have identified FE65 T579 as a putative target for Glycogen Synthase Kinase 3β (GSK3β). Dysregulation of GSK3β is highly implicated in AD pathogenesis. In this study, we demonstrated that FE65 was phosphorylated at T579 *in vivo* and *in vitro* by GSK3β. Furthermore, we evaluated the effect of FE65 T579 phosphorylation on APP processing. Our data indicated that FE65 T579 phosphorylation increases both γ- and β-cleavages of APP as shown in the APP-GAL4 reporter assay and CTF analysis respectively. Above all, Aβ liberation is promoted by the FE65 T579 phosphorylation. Collectively, the current findings suggest that GSK3β phosphorylates FE65 at T579 and this serves as a novel regulatory mechanism of APP processing.

Saikosaponin D, a novel autophagy inhibitor, potently inhibited Enterovirus 71(EV71)-induced cell death

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Autophagy is a conserved physiological process which degrades misfolded cellular proteins and damaged organelles for cell survival. Enterovirus 71 (EV71) is a major agent of hand, foot and mouth disease in children that can cause severe central nervous system disease. Defective autophagy is associated with a number of diseases. As intracellular parasites, during the course of an infection, viruses encounter autophagy and interact with the proteins that execute this process. Autophagy serves either as an antiviral defense mechanism or, alternatively, as a pro-viral process during virus infection. We previous found that Saikosaponin D is a potent autophagy inhibitor. Now, we found that Saikosaponin D could inhibit EV71-induced cell death in a concentration-dependent manner in Hela cells. Also, EV71 could induce autophagy. Rapamycin, a well known autophagy inducer, enhanced EV-71 induced autophagy and increased VP1 (virus coat protein) production, then promoted cell death after EV71 infection. Furthermore, VP1 production decreased after EV71 infection in ATG5 knockdown Hela cells. Thus, autophagy play important role in EV71 infection. We are currently exploring the mechanisms underlying anti-virus function of Saikosaponin D and exact role of autophagy in EV71 infection process.

Identification and Mechanistic study of SRPK1 docking groove inhibitors

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Serine/arginine-rich proteins (SR proteins) are a family of essential non-snRNP splicing factors. SR protein kinases family (SRPK) represents a class of enzymes that can phosphorylate SR splicing factors. The prototypic member of SRPK family, SRPK1, shows an important role in the regulation of splicing factor trafficking and subsequently splicing, abnormal expression of SRPK1 has been found to associate with different types of cancers such as leukemia, breast, colon, pancreas, and lung. SRPK1 is implicated in cancers by regulating the activation of Akt/PKB and the splicing of vascular endothelial growth factor during angiogenesis.

Structural studies show that docking interactions between the substrate and a unique docking groove on SRPK1 are critical for the high- affinity binding and the mode of phosphorylation of substrates. Based on these findings, our lab aim to screen for small molecule inhibitors that could block the docking groove of SRPK1. One hit compound and a 7mer peptide have been discovered to show inhibitory activities against SRPK1. However, the mechanisms of how they interact with SRPK1 remain unclear. The compound is found to be a racemic mixture. Therefore, I will first separate the optical isomers of the compound, and then study the biological effects of the enantiomers to identify the active isomer. I will also attempt to solve the structure of the complex of SRPK1 with the active isomer and the 7mer peptide respectively to further elucidate the detailed mechanism of how they inhibit SRPK1 and provide information on further optimization of the compound and 7mer peptide.

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Fission yeast mitochondria are distributed by dynamic microtubules in a motor-independent manner

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The cytoskeleton plays a critical role in regulating mitochondria distribution. Similar to axonal mitochondria, the fission yeast mitochondria are distributed by the microtubule cytoskeleton, but this is regulated by a motor-independent mechanism depending on the microtubule associated protein mmb1p as the absence of mmb1p causes mitochondria aggregation. In this study, using a series of chimeric proteins to control the subcellular localization and motility of mitochondria, we show that a chimeric molecule containing a microtubule binding domain and the mitochondria outer membrane protein tom22p can restore the normal interconnected mitochondria network in mmb1-deletion (mmb1Δ) cells. In contrast, increasing the motility of mitochondria by using a chimeric molecule containing a kinesin motor domain and tom22p cannot rescue mitochondria aggregation defects in mmb1Δ cells. Intriguingly a chimeric molecule carrying an actin binding domain and tom22p results in mitochondria associated with actin filaments at the actomyosin ring during mitosis, leading to cytokinesis defects. These findings suggest that the passive motor-independent microtubule-based mechanism is the major contributor to mitochondria distribution in wild type fission yeast cells. Hence, we establish that attachment to microtubules, but not kinesin-dependent movement and the actin cytoskeleton, is required and crucial for proper mitochondria distribution in fission yeast.

Defective cell adhesion and cell migration in Sox10 mutant neural crest

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The enteric neural crest cells (ENCCs) are derived from migratory vagal neural crest cells (NCCs). Transcription factor Sox10 is crucial for ENCC multipotency maintenance. Sox10 mutant ENCCs fail to colonize the entire gut due to premature differentiation and migration defects. A $Sox10^{NGFP}$ mouse where EGFP is fused to the N-terminal domain of Sox10 was generated to visualize the migration behavior of mutant ENCCs. $Sox10^{NGFP/+}$ mutant ENCCs form aggregates on different extracellular matrices in gut explant culture. We hypothesized that cell adhesion properties were affected in Sox10 mutant neural crest. To gain insight into the cell adhesion and molecular pathways underlying cell migration processes in ENCCs, we performed transcriptome and gene expression analysis as well as immunofluorescence analysis to study cellular phenotypes. E9.5 pre-ENCCs and E12.5 ENCCs from Wnt1-Cre:Z/EG and $Sox10^{NGFP}$ mutant were sorted out and performed RNA sequencing. Bioinformatics analysis indicated that among the differentially expressed genes, the expression of biological adhesion and locomotion genes were significantly affected during ENCC development. Selected differentially expressed genes were further verified by qRT-PCR. The immunostaining results of cultured explants showed that the distribution of cadherins, vinculin and FAK were affected in Sox10 mutant NCCs and ENCCs. Further cellular behavior such as real-time actin filament dynamics and intensity will be assessed by live cell imaging using Wnt1-Cre:Z/EG:Lifeact-mRFP and Sox10NGFP:Lifeact-mRFP mice, which had the ENCCs labelled by EGFP and F-actin . Our results suggest that Sox10 mutation alters the expression of cell adhesion molecules and ECM reporters, thereby may affect ENCCs cellular behavior and migration.

Trafficking of Heparanase 1 is regulated by neuronal activity during homeostatic synaptic plasticity

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Activity regulated protein trafficking in neurons comprises an important mechanism of synaptic plasticity. In this study, we found the lysosomal enzyme heparanase 1 (HPA1) which cleaves heparan sulfate (HS) moiety undergoes axonal and dendritic trafficking in an activity-dependent manner. Persistent increase of neuronal activity permitting the induction of homeostatic weakening of synaptic transmission leads to an elevation of synaptic accumulation of the enzyme despite of no overall expression change. Knock down of HPA1 significantly reduces excitatory synaptic density in vitro and abolishes the induction of normal homeostatic synaptic scaling down. The regulatory role of HPA1 in synaptic plasticity could be achieved by enzymatic modification of synaptic HS. Our study reveals a previously undefined role of the mammalian endoglycosidase HPA1 in nervous system and also highlights the importance of extracellular matrix modification for induction of homeostatic synaptic plasticity.

Effects of tea water extracts on body weight reduction and gut microbiota modification in C57BL/6J mice fed a high-fat diet

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Tea is one of the most widely consumed beverages in the world. Teas can be classified into green tea, oolong tea and black tea on the basis of fermentation degree. Accumulative evidence has shown that tea consumption has benefits on body weight reduction, diabetic prevention and metabolic syndrome (MetS) alleviation. The C57BL/6J mice were fed with 1% of water extracts of green tea, oolong tea and black tea for 28 weeks. Oral glucose tolerance tests (OGTTs) were performed at week 0 and then repeated during intervention at indicated weeks. After sacrificed, the serum analysis and organs test will be conducted to further determine the indicators related to MetS. And fresh feces of mice in different groups will be collected for sequencing of microbiota through 16s rRNA gene sequencing. The current results showed that the calorie consumption (14 ± 1.3 kJ/day/mouse) was stable with insignificant difference among all the groups. Compared with the HFD group, mice ingesting the green tea, oolong tea and black tea diet had significantly lower body weight. At week 16, the oolong tea and black tea diets showed significantly better oral glucose tolerance at individual time points and with respect to area under the blood glucose response curve (AUC) in comparison with mice consuming the HFD diet. The serum analysis, organs and feces test will be further conducted to determine how these three types of teas change the gut microbiota and subsequently attenuate high-fat diet-induced MetS.

Role of TRPC7 in regulating the functions of embryonic stem cell-derived cardiomyocytes

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Ion channels are vital molecular device to maintain the electrophysiological function and calcium homeostasis of cardiomyocytes. Although classical sodium, potassium and calcium channels are intensively studied, many other channels that may also contribute to the subtle regulation of cardiomyocytes remain largely unexplored. Canonical Transient Receptor Potential channel (TRPC) is non-selective cation channel activated by G protein coupled receptors. TRPC channel widely expresses in different tissue, playing an important role in the maintenance of normal cell function. The aim of this project is to study the function of TRPC7 in cardiomyocytes. Western blotting showed TRPC7 is expressed in mouse heart, and detection of multiply bands indicating more than one isoforms may exist in heart. Immunocytochemistry experiments showed that TRPC7 locates at plasma membrane in early differentiation stage mouse embryonic stem cell-derived cardiomyocytes (mESC-CMs), but translocates from membrane to the sarcomere during the maturation of mESC-CMs. Translocation of TRPC7 occurs during the whole process of maturation; TRPC7 preferentially locates near M-line in intermediate differentiation stage mESC-CMs while it locates in Z-line in late differentiation stage mESC-CMs. We speculated that both full-length-TRPC7 and truncated-TRPC7 (which lacks the transmembrane pore region) may exist in cardiomyocytes. The full-length-TRPC7, with its ion channel function, may participate in the regulation of electrophysiological function of cardiomyocytes in early differentiation stage, while the truncated-TRPC7 may insert into sarcomere to facilitate the stability of sarcomere.

The roles of *Irx3* and *Irx5* in inner ear neurosensory patterning

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Iroquois (*Irx*) genes encode evolutionary conserved homeodomain transcription factors that are involved in multiple developing processes. *Irx3* and *Irx5* are *IrxB* cluster genes, linked in chromosome from fly to mammal. In mouse embryo, both *Irx3* and *Irx5* are expressed in the central nervous system, limb bud and otocyst, but their functions in inner ear development are poorly understood. In this study, $Irx3^{LacZ/+}$ and $Irx5^{EGFP/+}$ were analyzed to examine the expression pattern of these two genes, respectively. Both *Irx3* and *Irx5* were expressed in the otic placode. In otic vesicle, the *Irx* genes shared a broad expression domain in the otic epithelium and periotic mesenchyme. In the cochlear epithelium, *Irx3* was expressed in both sensory and non-sensory domains, while *Irx5* became gradually restricted to non-sensory regions, accompanying with the progression of hair cell differentiation. To further understand the functions of *Irx3* and *Irx5* during inner ear development, phenotype analyses have been done in the $Irx3^{LacZ/LacZ}$, $Irx5^{EGFP/EGFP}$ and $Irx3/5^{-/-}$ mutants. The gross morphology of $Irx3^{LacZ/LacZ}$ and $Irx5^{EGFP/EGFP}$ inner ears were relatively normal, while $Irx3/5^{-/-}$ mutant displayed shortened cochlear duct, enlarged cochlear lumen and lack of the non-sensory structure that segregate the vestibule and cochlea. Furthermore, the vestibular macular of saccule and the cochlear sensory structures were fused together in the compound mutant. In addition, spatial expansion of neurosensory competent domain and temporal elongation of neurosensory competence could be observed in $Irx3/5^{-/-}$ otic epithelium. In conclusion, our study suggests that *Irx3* and *Irx5* are required for inner ear patterning and neurosensory fate determination.

Determination of dendritic spine morphology by the striatin scaffold protein STRN4 through interaction with the phosphatase PP2A

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Most excitatory synapses are located in dendritic spines of the postsynaptic neuron. Mature spines appear as mushroom-shaped with large heads, whereas, immature spines include stubby spines or filopodia, which do not possess a distinct spine head, and thin spines which contain elongated necks and small heads. Spine maturation requires local dendritic protein synthesis and spontaneous activity of N-methyl-D-aspartate (NMDA) receptor. Dysregulated mRNA trafficking and local protein synthesis can lead to altered spine morphology in neurodevelopmental disorders such as Fragile-X syndrome and autism. How different subtypes of dendritic spines are selectively maintained along development is still poorly understood. *strn4* was identified in recent transcriptomic studies as an mRNA transcript present in hippocampal neuropil and putative cargo of the RNA-binding protein FMRP. STRN4 belongs to the striatin family of scaffold proteins, and some of the potential striatin-interacting proteins are encoded by autism risk genes. Although previous studies have demonstrated their localization in dendritic spines, the function of various striatin family members in neuron remains unknown. Here we demonstrate that *strn4* mRNA is present in neuronal dendrites and the local expression of STRN4 protein depends on NMDA receptor activation. Notably, STRN4 is preferentially expressed in mushroom spines, and STRN4 specifically maintains mushroom spines but not thin spines and filopodia through interaction with the phosphatase PP2A. Our findings have therefore unraveled the local expression of STRN4 as a novel mechanism for the control of dendritic spine morphology.

ISM1 in mammary gland morphogenesis and its malignant transformation

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Isthmin 1 (ISM1) is a secreted protein dynamically expressed from early embryonic stages throughout adulthood (Osorio et al., 2014). In the mouse, ISM1 is present in particular epithelial cell populations of the respiratory, digestive and reproductive systems, suggesting that ISM1 may contribute to organ ontogeny and homeostasis. The biological relevance of ISM1 remains largely unexplored. ISM1 has been described as an angiogenesis inhibitor that controls endothelial cell survival through integrin signaling (Xiang et al., 2011; Zhang et al., 2011). No other studies have addressed the role of ISM1 in epithelia and/or cancer biology.
In the present work we aimed at determining the function of ISM1 during mammary gland (MG) morphogenesis and its malignant transformation. ISM1 is present in the mammary tissue all through its ontogeny. ISM1 expression occurs in the basal myoepithelial cells of the mammary epithelium as well as in the surrounding stromal cells. Analyses of BAC ISM1-IRES-EGFP transgenic females (ISM1 Tg) show a defective branching morphogenesis. We then examined ISM1 in breast cancer using the MMTV-PyMT mouse model. We found an increased amount of hyperplastic lesions in PyMT;ISM1 Tg at 4 weeks old. Consistently, both the number and total weight of tumors per mouse is increased by more than 2-fold in PyMT;ISM1 Tg mice at later stages. In addition, tumor cells in PyMT;ISM1 Tg mice are easier to metastasize to the lungs. We are currently investigating the mechanism(s) mediating the function of ISM1.

Potential clinical significance of downregulation of MAPK pathway components' mRNA expressions in head and neck squamous cell carcinoma (HNSCC)

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We previously showed that the MAPK pathway can be mutationally activated, and may implicate drug sensitivity in HNSCC. Here, we report that RNA expression of MAPK pathway components may be associated with HNSCC patient outcome based on the TCGA data. We found that loss of *DUSP4*, an MAPK pathway negative regulator, was correlated with poor overall survival (P=0.0219) and disease free survival (DFS) in HNSCC (P=0.00137) which can potentially be explained by activation of oncogenic MAPK pathway upon DUSP4 underexpression. Interestingly, we identified a group of HNSCC patients with homozygous loss and mRNA downregulation of MAPK pathway scaffold protein components (*GRB2*, *SHC2* and *SHC3*) with significantly poorer DFS (P=0.00231). RPPA analysis showed a trend of decreased phospho-RAFs, phospho-MEKs and phosphor-MAPKs protein expression, supportive of an overall decreased MAPK pathway signaling in this subset of patients with reduced MAPK scaffolding gene expression.

Unexpectedly, downregulation of multiple MAPK pathway kinases (*RAF1*, *MAPK1* and *RPS6KA1*) which normally support inactivation of the pathway was found to be significantly associated with poorer DFS (P=6.464x10^-5) with median time to progression of 18.17 months vs. 71.22 months in the unaltered group. Subsequent proteomic analysis of the respective patient tumors from the TCGA cohort (N=357 with RPPA data) showed that these patient tumors had elevated levels of E2F1 protein expression (P=9.383x10^-3). As E2F1 is involved in cell survival upon DNA damage, upregulation of E2F1 protein expression may enable cancer cells to survive after DNA insults by radiotherapy or chemotherapy, and contributes to disease relapse.

Suppressor of fused regulates neural progenitor cell cycle progression during mammalian hindbrain neurogenesis

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Hedgehog signaling is known for its mitogenic function. *Suppressor of fused (Sufu)* is a mediator of mammalian hedgehog signaling, majorly by negatively regulating Gli activities. However, the role of *Sufu* in the cell cycle of neural progenitors is unclear. It was previously observed that when Sufu was conditionally ablated in mouse hindbrain rhombomere 4 (r4), there was an expansion of Sox2+ neural progenitor pool. To explore possible contribution of the phenotype by any abnormalities in r4 neural progenitor cell cycle, a cumulative BrdU labelling scheme was applied to analyze cell cycle kinetics of hindbrain r4 at E11.5 in control and *Sufu* mutant. Since the expanded Sox2 domains in *Sufu* mutant primarily occupied the dorsal *Pax6+* and *Pax7+* regions, r4 was sub-divided into 4 progenitor domains along its dorsal-ventral axis to assess cell cycle individually. Preliminary data showed that *Sufu* mutant dorsal progenitors tended to extend their cell cycle length, with a lengthening of S-phase and a shortening of Gap phases compared to control. To temporally characterize the progenitor proliferation, 1hr BrdU labelling was performed to visualize S phase cells from E10.5 to E12.5. Normally the proportion of neural progenitors in S phase would decrease at E12.5 whereas *Sufu* mutant displayed an accumulation of S phase cells, especially in *Pax6+* and *Pax7+* domains, suggesting that *Sufu* might be required for the shortening of S phases during hindbrain neurogenesis. In summary, the data implied that *Sufu* is involved in the regulation of proper cell cycle progression in mammalian hindbrain neural progenitors.

Genome-wide identification of active enhancers in the developing mouse nucleus pulposus

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Notochord cells (NCCs) and their derivative nucleus pulposus cells (NPCs) are major cell types in nucleus pulposus (the central part of intervertebral discs) at different development stages. Progressive differentiation of NCCs to NPCs is reported to correlate with the onset and development of disc degeneration.

To genome-wide identify active enhancers in NCCs and NPCs, to help unveil the molecular mechanisms underpin development and maintenance of intervertebral discs, NCCs, Neonatal NPCs and Adult NPCs were harvested from *Foxa2*^{mNE}*-Cre x Z/EG* mice for ChIP-Seq. NCCs, Neonatal NPCs were also collected for RNA-Seq. H3K27ac was selected to be the active enhancer marker for ChIP.

By comparing the enhancer profiles at three development stages, we observed higher degree of overlapping of enhancer regions between Neonatal and Adult NPCs, which indicates they share common regulatory mechanisms in development. Functional annotations on active enhancers revealed that TGF-beta signaling pathway is significantly enriched in NCC/NPC enhancer nearby genes, corresponds to findings in mouse RNA-seq analysis. In addition, Smad family member motifs are significantly enriched in NCC and NPC enhancers. Mice with Smad4 mutant are with twisted tails and deformed intervertebral discs, which supports the importance of Smad4 in intervertebral disc development. With integrative analysis of ChIP-Seq and RNA-Seq data we have associated highly expressed nearby genes with putative enhancers especially those with Smad motifs. Further experimental validations of putative enhancers are undergoing in zebrafish and mouse model.

Transplantation of embryonic spinal cord derived cells to prevent muscle atrophy after various peripheral nerve injuries

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Every human has 45 miles long nerve network that together with the spinal cord is susceptible to traumatic injuries, leading to a loss of motor and sensory functions. Different surgical repairs are available for peripheral nerve injuries happening on either distal or proximal site. However, the speed of axonal regeneration after the repair is limited and therefore, target muscles atrophy severely during that time, making them incapable of receiving axons after too long time. Neuronal replacement strategy through cell transplantation is a potential method to prevent muscle atrophy. In our study, we tested E14.5 rat embryonic spinal cord derived fetal cells versus neural progenitor cells (NPCs), as well as cells from 3 different spinal cord segments, for their ability to reduce the muscle atrophy after the complete musculocutaneous nerve transection. We showed that fetal cells were more efficient compared to the NPCs, whereas lumbar cells were slightly better compared to thoracic and cervical cells. Therefore, fetal lumbar cells were next used in clinically more relevant injury models that included delayed surgical repair. After long-term crush injury, these cells helped to preserve the muscles, resulting in an earlier functional recovery, while no effect was seen after the nerve transection with delayed end-to-end repair. Next, spinal root avulsion injury that includes the damage to both PNS and CNS, was used. Here, these cells slightly prevented the muscle atrophy, resulting in earlier and better functional recovery after the delayed ventral root reimplantation, while GDNF treatment helped the host motoneuron cell bodies to survive.

FGF21 Up-Regulates Glycolytic Proteins for Hypertrophic Chondrocyte Survival under ER Stress

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Perturbation of endoplasmic reticulum (ER) homeostasis is associated with various diseases. In a mouse model (13del-tg) for metaphyseal chondrodysplasia type Schmid (MCDS), misfolded mutant Collagen X proteins accumulate in hypertrophic chondrocytes (HCs) triggering ER stress was shown as the molecular basis of the disease. Interestingly, under ER stress, HCs activate a survival signal that also alters the chondrocyte differentiation program in the growth plate, impairing endochondral ossification causing dwarfism. How HCs survive ER stress in this context is unknown. Previously, we showed there is an altered metabolic machinery favoring glycolysis in 13del-tg HCs using a label-free quantitative proteomic approach. Recently, transcriptome analysis identified a marked upregulated expression of FGF21, a novel ER stress target and a key metabolic regulator involved in various metabolic diseases in 13del HCs. However, the role of FGF21 in energy flux maintenance for HCs survival under ER stress have not been studied. Here, in a label-free quantitative proteomic analysis in mice, we showed that FGF21 is required to maintain higher levels of glycolytic proteins in HCs under ER stress for survival, and its inactivation in 13del-tg mice will lead to apoptosis. We found that FGF21 is not required for normal chondrocyte differentiation. Our findings support a switch to rapid energy supply is necessary to sustain pro-survival pathways in HCs to survive under ER stress, and FGF21 have a key role in this switch in activating and/or sustaining an up-regulated glycolytic process required for survival.

Activation of PKA-dependent transcription factors by RGS19 upregulates metastasis suppressor Nm23-H1/2

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Tumor metastasis is the major reason for deaths in cancer patients. The metastasis suppressor Nm23 has the ability to reduce metastasis formation in different cancers, but the molecular mechanisms that regulate the expression of Nm23 is still poorly understood. It is known that Nm23-H1/2 can be upregulated by RGS19, a regulator of G protein signalling protein, through the activation of several transcription factors including AP-1, SRE and CREB. In the present study, we examined whether agents that can increase cAMP levels and CREB phosphorylation are able to upregulate the expression of Nm23-H1/2 in HEK293 and several cancer cell lines. H89, a protein kinase A (PKA) inhibitor, decreased the expression of Nm23-H1/2, suggesting a PKA-dependent mechanism of Nm23-H1/2 regulation. However, cAMP assay did not show elevated levels of cAMP in HEK293 cells stably expressing RGS19 as compared with parental cells. Additionally, the RGS19 stable cell line did not have a significantly higher expression level of phosphorylated CREB at basal condition or after forskolin treatment to stimulate intracellular cAMP formation. Interestingly, reductions in phosphorylated CREB levels were observed with increasing amounts of forskolin, but this phenomenon is less apparent in cells stably expressing RGS19, thus indicating that other mechanisms are probably involved in CREB regulation. (Supported by ITCPD/17-9)

Mapping Interaction Between Influenza RNA Polymerase Subunit PB2 And Nucleoprotein NP

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Influenza virus has long been a threat to mankind. Due to its highly contiguous nature, influenza virus has caused epidemics and occasional pandemics. The transcription and replication of influenza virus requires RNA-dependent RNA polymerase (RDRP). It is a trimeric polymerase (PA, PB1 and PB2), with the presence of nucleoproteins (NP) and viral RNA, a ribonucleoprotein complex (RNP) is formed for the polymerase to perform transcription and replication of the viral genome. Previous studies showed that NP binds to the PB2 subunit of the polymerase. This interaction is thought to be essential for proper functioning of the polymerase. We further identified the key residues for the binding by NMR chemical shift perturbation using the NP peptide as ligand and confirmed their role in interaction by biochemical assays including, SPR, RNP reconstitution, primer extension and immunoprecipitation. Our group found that the NP interacts with a domain (aa 320-483) of PB2. This domain revealed a cap-snatching ability, which binds the methyl cap of RNA. Upon the addition of RNase which degrades RNA, the interaction was abolished. This observation suggested a role of RNA in the interaction. Moreover, the C-terminal domain (aa 538-693) of PB2 was reported to interact with NP. This domain is also known as '627' domain. We discovered a peptide from NP (aa 146-185) shows binding with the domain *in vitro*. RNP activity was reduced significantly upon mutation of identified residues 602-606 from the results of RNP reconstitution and primer extension assay. Mutation of the key residues abolished binding with NP as shown by SPR. The study identified residues aa 602-606 interacted with NP and played different roles in RNP activity. We also suggested that the interaction between NP and cap-binding domain of PB2 required RNA. Since NP interacts with multiple domains of PB2, further elucidation of the interaction is necessary for thorough understanding of RNP mechanism.

Endothelin-1 induced cofilin rod formation in hippocampal neurons via endothelin type B receptor and NADPH oxidase-mediated oxidative stress pathway

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High expression level of endothelin-1 (ET-1) has been detected in the post-mortem brains of Alzheimer's disease (AD) patients. Two ET receptor (ET rec) subtypes are expressed in neurons. However, the direct effects of ET-1 on mature neurons have remained elusive. ET-1 mediated oxidative stress production via NADPH oxidase (NOX) in endothelial cells, and regulated actin in astrocytes. Previously, oxidative stress-mediated aggregation of cofilin was identified as a key mediator of neuronal degeneration in AD models. Formation of cofilin rods was proposed to mediate the loss of synapses and distal dendrites in primary neurons. Here, this study investigated the effect of ET-1 in mediating cofilin rod formation in 14 days *in vitro* primary mouse hippocampal neurons. The 24h treatment of ET-1 significantly increased the percentage of neurons with cofilin rods, which was inhibited by pre-treatment of ET_B rec antagonist (BQ788), but not by ET_A rec antagonist (BQ123). Neurons treated with ET_B rec agonist (IRL1620) formed cofilin rods and showed significant reduction of distal dendritic extension. Co- or pre-treatment of antioxidant (N-acetylcysteine) or NOX inhibitor (VAS2870), respectively, inhibited IRL1620-induced cofilin rod formation, indicating ET_B rec activation mediated cofilin rod formation via oxidative stress pathway. In short, our findings presented a novel neurotoxic role of ET_B rec, associating the ET-1 system dysregulation with AD pathogenesis.

Functional Characterization of Heteromerization effects of Melatonin Receptors

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G protein-coupled receptors (GPCRs) are the largest family of cell surface receptors in humans that has been considered as attractive drug targets for diverse diseases. Upon activation by extracellular signals, GPCRs turn on corresponding G proteins to regulate numerous physiological processes. An increasing number of studies has shown that GPCRs are capable of forming homo/hetero-dimers and higher-ordered oligomers, resulting in cross-talks that are often essential for modulation of receptor functions. However, the implication of such cross-talk between GPCRs is not fully understood. Herein, the effects of heteromerization between melatonin receptors type I (MT_1) and type II (MT_2), and with other GPCRs including GPR50 orphan receptor (GPR50), dopamine D_2 (D_2), μ-opioid (MOR), serotonin 2A (5-HT_{2A}) and 2C (5-HT_{2C}) were investigated. These receptors are enriched in brain tissues and are therapeutically valuable in treating central nervous system disorders such as insomnia, schizophrenia, and depression. Receptors were transiently transfected into HEK293 cell line, alterations in signaling pathway upon receptor heteromerization were characterized by calcium mobilization, cAMP and cAMP response element-binding protein (CREB), which were measured by Fluorometric Imaging Plate Reader (FLIPR), cAMP accumulation assay and western-blot analysis, respectively. The heteromerization effects of MT_1/5-HT_{2A}, MT_1/5-HT_{2C}, MT_2/GPR50, MT_2/5-HT_{2A} were firstly reported in this study, whereas the previously suggested G_q pathway of MT_1/MT_2 heteromer was not observed. (Supported by grants 16103015 and ITCPD/17-9)

Molecular basis of the nematode volatile sex pheromone perception

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Both sexually matured *C. remanei* virgin females and self-sperm exhausted *C. elegans* hermaphrodites release a long-range volatile sex pheromone to attract adult males from afar. This chemotaxis behavior requires CEM, AWA and AIZ neurons. In AWA, a GPCR SRD-1 has been demonstrated as the receptor of this pheromone. Ectopic expression of *srd-1* in AWB in *srd-1* male elicited distinct repulsive behaviour towards pheromone; this result indicated the sufficiency of the SRD-1 receptor acting in AWA. From calcium imaging, we further confirmed the receptor was necessary for the excitation of AWA upon pheromone induction. Male *C. elegans* is the least attracted to pheromone in comparison to *C. briggase*, *C. remanei*, and *C. brenneri*. Based on qPCR results, the expression level may not be the key factor contributing to the difference in chemosensitivity. Transformation rescue of the male *srd-1* mutant in *C. elegans* by *cre-srd-1* cDNA was shown to be more responsive to pheromone than those rescued by *srd-1* cDNA. SRD-1 receptors across four nematode species were highly conserved in terms of their sequences and structure, except their C-terminal region (CT) and ECL polymorphisms. The ECL polymorphisms substituted versions of SRD-1 cDNA cannot elicit a higher responsiveness to this pheromone. CT truncated *cre-srd-1* and *srd-1* cDNA are unable to rescue *srd-1* chemotaxis mutant phenotype, suggesting the CT region to be critical for SRD-1 function. CT domain was swapped between different species SRD-1, in the *srd-1* mutant background. Other species CT with *srd-1* cDNA led to male animals more strongly attracted to the sex pheromone than those carrying *srd-1*-CT with *cre-srd-1* cDNA. CT region in GPCR is usually related to the intracellular signal transduction, we envision that the intracellular signal transduction relay component may account for the differential response of different species in sex pheromone perception.

Identification of nematodes volatile sex pheromone and the molecular basis of its perception

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Nematodes rely on their sensory modalities to locate mating partner. Volatile sex pheromone attracts potential mating partners from afar. Intriguingly, sexually matured females C. remanei and self-sperm exhausted hermaphrodites C. elegans produce a volatile attractant that is detectable by both con-specific and its relative adult males, qualifying this attractant as a long-range sex pheromone. We previously reported a class D GPCR serpentine receptor (SRD-1), a receptor in AWA neuron, is responsible for detecting this pheromone and several components in the olfactory pathway are involved in this pheromone signal transduction.

The GC-MS results of chemo-attractants from female C. remanei verified several volatile chemicals. Two of them exhibited sex-, stage-, and species-specific attractiveness on wild-type males. Noticeably, these compounds could neither attract receptor-defective mutant nor pheromone-perception-defective mutant males. We also visualized AWA neuron excitation by employed a calcium indicator, GCaMP5. The excitation of the AWA neurons was observed only in wild-type males, but not in srd-1 mutants, upon induction by both candidates separately. We concluded that these two chemicals are the active components of this pheromone through the GC-MS analysis, signal transduction pathway mutant strain studies and also the demonstration of ligand-receptor relationships in calcium imaging and behavioural assays.

DNA Polymerase Delta Catalytic Subunit Is a Nucleus and Cytoplasm Shuttling Protein

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The DNA polymerase delta catalytic subunit (PolD1), plays an essential role in DNA replication and repair to maintain genome stability. It modulates cell proliferation and cell cycle progression. We have revealed that PolD1 localizes both in the cytoplasm and in the nucleus. In addition, PolD1 has important functions in the cytoplasm. However, the nuclear import and export mechanisms of PolD1 remain poorly understood. Here, we examined the import and export properties of PolD1. The immunostaining results reveal that PolD1 contains the nuclear localization signals at amino-terminal and carboxy-terminal regions. The amino-terminal signal is much stronger than the carboxy-terminal one. Besides, we find that the PolD1 export from the nucleus is mediated by the exportin CRM1. Collectively, these results reveal a mechanism that controls PolD1 nuclear import and export. This nucleocytoplasmic shuttling mechanism of PolD1 has important significance for performing its functions in the nucleus and the cytoplasm.

The role of Notch signalling during mouse pharyngeal arch development

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During multiple tissue development, Notch signaling pathway plays essential roles in regulating cell proliferation, differentiation and cell fate determination. The Notch receptor interacts with ligands such Jagged and Delta on the adjacent cells, and subsequently, are cleaved and translocated to the nucleus, where it interacts with its co-activator RBPJ to transcriptionally regulate Hes and Hey. In humans, it has been reported that *NOTCH2* and *JAG1* mutations lead to craniofacial malformations in Alagille syndrome. In mice, haploinsufficience of Notch1 leads to abnormal cleft palate. However, how Notch signalling factors lead to the craniofacial abnormalities is not clearly understood. To study the function of Notch signalling during craniofacial development, we investigated the expression patterns of Notch signalling factors during pharyngeal arch development from E8.5 to E9.5, and examined the phenotypes of the *RBPJ* knockout and *Notch1* overexpression mice. We found that *Notch1*, J*ag1*, *DLL1*, *Lfng*, *Hes1* and *Hey1* were expressed in the different populations of pharyngeal ectoderm progenitors, indicating that the Notch signaling pathway may contribute to the cell type specification in the developing pharyngeal arch. Using the *Pax2-Cre* to conditionally knock-out *RBPJ* in the pharyngeal ectoderm, we found that the epibranchial placodal cells committed to neurogenesis prematurely. However, overexpression of Notch1 intracellular domain by *Pax2-Cre* maintains the progenitor status of the pharyngeal epithelium. In summary, our results suggest that Notch signalling is critical in controlling the cell type specification of pharyngeal ectoderm, which would lead to the abnormal pharyngeal arch development in Notch signalling mutants.

Transcriptional and post-transcriptional regulation of NME1/2 in cancer metastasis

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Tumor metastasis remains a huge obstacle in cancer treatment and is responsible for the majority of cancer-related deaths. The metastasis suppressor NME1 was one of the first identified metastasis suppressors with the ability to inhibit the metastatic potential of different cancers. Numerous clinical studies have also shown the value of NME1 expression in predicting patient prognosis. It was previously demonstrated that the regulator of G-protein signaling RGS19 is able to elevate the expression of NME1 and mediate carcinogenic processes including cell proliferation, tumor formation, and metastasis. However, the exact mechanisms that alter NME1 expression remain incomplete. MicroRNAs have recently emerged as post-transcriptional regulators of gene expression that bind to complementary sequences in the 3'UTR of target mRNAs and eventually cause disruption of gene translation. Bioinformatics analysis have revealed potential miRNAs downregulating the expression of RGS19 and NME1. To further investigate the involvement of miRNAs, other experiments including shRNA knockdown of Dicer and overexpression of target genes 3'UTR, are expected to induce upregulation of target genes. Furthermore, analysis of the genomic promoter region of NME1 and the second isoform NME2 by computational methods predicts a great number of binding sites for transcription factors. Subsequent analysis of promoter regions in luciferase reporter, EMSA, and ChIP assays allow the identification of major regulatory elements and provide strong evidence for transcriptional activity. The identification of regulatory miRNAs and transcription factors allows a better understanding of the regulation of NME1/2 expression.
(Supported by ITCPD/17-9)

Protective effects of oxyresveratrol on motor functions in 6-OHDA-Induced neurodegeneration

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Parkinson disease (PD) is the second most common neurodegenerative disorder, associated with progressive loss of dopaminergic neurons in the substantia nigra. Majority of PD is due to environment factors. PD affects approximately 1% of the population over age 55 and 2% of the population over age 65 worldwide and this number is increasing (Dauer et al, 2003). One of the most typical symptoms of PD is motor deficit. Parkinsonian mimetic 6-hydroxydopamine (6-OHDA) can be served as toxin agent in experimental model for PD. Oxyresveratrol (OXY) is a stilbene extracted from mulberry. OXY has been shown to exert antioxidative effects against cerebral ischemia. Our previous studies have shown that OXY elicits neuroprotective effects against experimental PD (Chao et al, 2008). In our *in vivo* study, male Sprague Dawley rats were fed with OXY, resveratrol (RES) as a positive control and pinostilbene (PINO) as a negative control at a dose of 1 mg/kg for 7 days prior to stereotactic injection of 6-OHDA. Rats were fed for 14 days more after surgery. Apomorphine-triggered rotation was used to test the effectiveness of 6-OHDA to induce dopaminergic neuronal loss and rotarod test were used to assess motor function. We found that OXY attenuated motor deficit induced by 6-OHDA 16.7% ± 0.3 (mean ± standard derivation). We further investigated the effect of OXY (25μM) in MES 23.5 and SH-SY5Y cell lines in the *in vitro* model of PD established by 6-OHDA treatments. There was significantly decreased in the release of lactate dehydrogenase (LDH) in both MES 23.5 ($P<0.0001$) and SH-SY5Y cells ($P<0.05$) after OXY treatment comparing with 6-OHDA groups. However, the cleaved caspase 3 expressions only decreased in MES 23.5 cells ($P<0.05$). In conclusion, OXY displayed protective effect on motor function in 6-OHDA induced PD model.

Acknowledgement: The study is supported by Health Medical Research Fund (02131496) from Food and Health Bureau of Hong Kong SAR Government.

CFTR-associated ATP Release in Neonatal And Adult Skeletal Myoblasts

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ATP release from contracting skeletal muscle cells stimulates extracellular adenosine formation, which contributes to vasodilation. We previously showed that 1) lactic acid (which is formed by contracting muscle) lowers intracellular pH and stimulates ATP release, and 2) cystic fibrosis transmembrane conductance regulator (CFTR) is activated by low pH and it is involved in acidosis-induced ATP release. Patients with Cystic Fibrosis commonly have reduced exercise tolerance. ATP is a large diameter molecule, and CFTR chloride channel is too narrow for passage of ATP: we investigated whether connexins and/or pannexins were involved in CFTR-associated ATP release from rat L6 skeletal myoblasts cell line (a model for neonatal muscle) and human skeletal muscle myoblasts (HSMM; a model for adult muscle). We measured protein expression with western blot, ATP release by the luciferase assay and large-diameter channel opening by Lucifer Yellow (LY) uptake.

LY uptake data suggested that both connexins and pannexins function as membrane channels in L6 myoblasts, whereas HSMM expressed Pannexin 1 strongly. Lactic acid increased LY uptake and ATP release in the 2 cell types, confirming that both models are capable of acidosis-induced ATP release, yet CFTR-stimulated ATP release was mediated by pannexins but not connexins in both neonatal and adult skeletal muscle cells, although connexins could mediate non-CFTR-associated ATP release. Further investigation is required to determine the mechanism by which CFTR regulates the opening of pannexin channel.

Live attenuated *Salmonella*-based Vaccines delivering SaEsxA by Type III Secretion System Confer Protection against *Staphylococcus aureus* Infection

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Staphylococcus cause a wide range of infection diseases in human and animals. The emergence of antibiotic-resistant strains promote novel strategies for prophylactic vaccine development. In this study, live attenuated *Salmonella* based vaccine against *Staphylococcus aureus* infection was developed, in which *Salmonella* Pathogenesis Island-1 Type 3 Secretion System (SPI-1 T3SS) was employed to deliver SaEsxA, one of ESAT-6-like (Early Secreted Antigenic Target-6) virulence factors of *S. aureus*. Through fusing with the N-terminal secretion and translocation domain of SPI-1 effector SipA, expression and translocation of SaEsxA into the cytosol of macrophages was detected *in vitro*. Oral administration of *Salmonella*-SaEsxA vaccine to BalB/c mice significantly induced SaEsxA-specific mucosal and humoral immune responses, detected by ELISA. Besides, Th1- and Th17- biased cellular immune response was also observed by ELISPOT assay. This multifaceted immune responses further increased the survival rate to 50% for vaccinated mice when challenged with *S. aureus* Newman strain, whereas all mice in control group died. Thus, the newly developed *Salmonella* based vaccine delivering SaEsxA by SPI-1 T3SS could confer protection against *S. aureus* infection. This study provides preclinical data supporting the use of *Salmonella* SPI-1 T3SS to translocate foreign antigens into the cytosol of antigen presenting cells to induce potent immunity against pathogens.

Sodium in The Apical Solution Mediates Downregulation of Ion Transport in Cultured Pig Tracheal Epithelia

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Respiratory epithelium controls transepithelial ion and fluid transport for maintaining homeostasis of airway surface. Whether the respiratory epithelium could sense and respond to surface fluid changes is vastly unknown. To test this hypothesis, the apical surface of cultured and differentiated pig tracheal epithelia in Millicell inserts was gently washed with the Krebs solution for 3 times, followed by adding 40 μl the Krebs solution for 1 to 24 hr incubation. Our data demonstrate that the short-circuit currents (Isc) of epithelia at basal condition (Isc_{-Basal}) and Isc changes by amiloride (ΔIsc_{-Amil}) due to inhibition of the epithelial sodium channel (ENaC) both were gradually decreased at 4, 7 and 10 hr of incubation, compared to that of the control epithelia with no treatment. However, Isc_{-Basal} and ΔIsc_{-Amil} were recovered back to the control values after 24 hr of incubation. Moreover, the Isc changes by forskolin and IBMX or by Cl^- transporter inhibitors GlyH-101, bumetanide and DPC all displayed similar time-dependent attenuation but with mild effects. These data suggest that ion transport of tracheal epithelia was reduced in response to wash and incubation of the apical Krebs solution. To further explore the mechanism leading to decreased Isc_{-Basal} and ΔIsc_{-Amil}, we either washed or directly incubated the apical side of tracheal epithelia with the Krebs solution or with the salt-free mannitol solution. The data indicate that after 7 hr, wash or incubation with the Krebs solutions all decreased Isc_{-Basal} and ΔIsc_{-Amil}, but with the mannitol solutions both treatments were without effect. By removal of individual Na^+, Cl^- or HCO_3^- in the Krebs solution, our data demonstrate that only the Na^+-free solution did not decrease Isc_{-Basal} and ΔIsc_{-Amil}. These data suggest that the underlying mechanism requires apical Na^+ and could be initiated quickly during the time of surface wash. Our further experiments found that pretreatment of cultured epithelia with the ERK inhibitor U0126 or the AMPK inhibitor compound C in the bottom medium largely attenuated the reductions in Isc_{-Basal} and ΔIsc_{-Amil} of tracheal epithelia by the apical Krebs solutions. In addition, the mRNA levels of ENaC-β and -γ subunits rather than other ion transporters were significantly reduced after 7 hr incubation and returned to normal at 24 hr, compared to that of the control with no treatment. Taken together, our data suggest that fluid challenge on the luminal surface of the tracheal epithelium may reduce transepithelial Na^+ transport, which is mediated by Na^+ in the apical solution resulting in activation of the ERK and AMPK signaling pathways and downregulation in the mRNA levels of ENaC-β and -γ subunits.

Effects of TEFM on the Regulation of Transcription Elongation by Mitochondrial RNA Polymerase

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Transcription elongation regulation is one of the key mechanisms employed to control gene expression. Mitochondrial DNA is transcribed by the single-subunit mitochondrial RNA polymerase (mtRNAP). TEFM was recently reported as a potential stimulator of mitochondrial transcription elongation by directly interacting with mtRNAP. However, the mechanism how TEFM interact with the elongation complex to enhance transcription elongation remains unknown. *In vitro* biochemical transcription elongation assay verified that TEFM can resume elongation from pauses and pre-termination. To quantitatively understand the impact of TEFM on transcription elongation, single-molecule optical tweezers transcription elongation assay was performed. We observed that the dynamics of mtRNAP transcription elongation is comparable with that of nucleus RNA polymerase II. Furthermore, we demonstrated that TEFM does not change the pause-free velocity of transcription elongation process, but significantly decreases the pause frequency and the length of pauses. Our finding explains how TEFM enhances transcription elongation and reveals the potential function of TEFM to provide deeper understanding of transcription elongation regulation.

Structural insights into BAF47 and BAF155 complex formation

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Mammalian BAF complexes are a subfamily of SWI/SNF ATP-dependent chromatin remodelers that dynamically modulate chromatin structure to regulate fundamental cellular processes including gene transcription, cell cycle control and DNA damage response. So far many distinct BAF complexes have been identified with polymorphic assemblies of up to 15 subunits from 29 genes. The evolutionarily conserved BRG1/BRM, BAF47 and BAF155/BAF170 form a stable complex that carries out essential chromatin remodeling activity and therefore have been regarded as the core components of BAF complex. Here, we first confirmed that SWIRM domain of BAF155 is responsible for its interaction with BAF47, then narrowed down the SWIRM-binding region in BAF47 to the Repeat 1 (RPT1) domain. We further presented the high-resolution crystal structure of SWIRM/RPT1 complex. Extensive mutagenesis experiments together with ITC and NMR titrations were performed to corroborate the interactions observed in crystal structure. Overall, we demonstrated that BAF155 SWIRM is a modular domain involved in BAF47 interaction, which is functionally distinct from other characterized SWIRM domains that possess DNA binding activity.

Extracellular histones induce apoptosis in human erythrocytes and release 'Find-me' signal through Pannexin-1

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Pannexin 1 (PANX1), a connexin family protein, was known as an ATP releasing channel in various cell types including human erythrocytes (RBCs). Recent literatures revealed that PANX1 releases ATP into extracellular space as phagocytic signals during apoptosis of Jurkat T cells and regulates the formation of apoptotic bodies. Human RBCs, though lacking nucleus and mitochondrion, undergo apoptosis in a way similar to nucleated cells with a process known as eryptosis. However, the role of PANX1 in eryptosis is still unclear. Histones are structural proteins that organize DNA in cells and are normally confined to nucleus, but in conditions like sepsis, histones are released out of the dying cells and enter blood circulation, leading to endothelial dysfunction, organ failure and death. The present study explores whether and how extracellular histones induce eryptosis and whether 'find-me' signals are released through PANX1 channels, hoping that findings from our study can shed light on our understanding of the underlying mechanism and provide new insights to the treatment of sepsis. Practically, phosphatidylserine exposure at the cell surface was estimated from the binding of Annexin-V-RFP using flow cytometer as an indication of eryptosis and extracellular histones was found to be a potent apoptotic-inducing agent (≥20 μg/mL) in human RBCs with rapid effect (3 h). Moreover, as PANX1 is a target of effector caspase-3 and a specific caspase-cleavage site within PANX1 was essential for PANX1 function during apoptosis, we have examined the caspase-3 activity of histones-treated RBCs using flow cytometer. A dose-dependent increase in caspase-3 activity was observed and the effect of histones on PS exposure was significantly blunted by a caspase-3 inhibitor. Furthermore, a does-dependent increase in ATP release from histones-treated RBCs was detected.

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Tracking low turnover somatic stem and progenitor cells in vivo using a lineage-specific cell cycle counter

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Somatic stem cells, or adult stem cells, have the ability to replenish tissues throughout the body. Adult stem cells are able to self-renew and differentiate. In particular, stem cells play important roles in tissue maintenance and regeneration. However, the regenerative capacity of somatic stem cells declines dramatically in some diseases and ageing. Recent finding suggests the notion that stem cells spontaneously proliferate and subsequently are depleted in old tissues. As somatic stem cell is only a small percentage of total cell number within the tissue and heterogeneity exists within this population, it is a hurdle to identify and isolate these cell populations from adult tissues. Traditionally, identification of low turnover somatic stem cells relies on the label retention technique. However, detection of DNA labels requires cell fixation and the purity of somatic stem cells is questionable. Even though fluorescence activated cell sorting (FACS) is a powerful method to separate them from a heterogeneous mixture of biological cells in vitro, it requires known markers and available antibodies for specific stem cell populations. To lineage trace a specific somatic stem cell population in vivo, inducible recombinases and multicolor fluorescent reporters have been extensively used. In this study, we aim to generate a transgenic mouse line that can serve as both a lineage tracer and a cell division counter up to two rounds of cell cycle. Lineage tracing of specific cells along with cell cycle history of the low turnover somatic stem cells will provide a better understanding of how they behave during homeostasis, pathological conditions and ageing.

Linnorm: Improved statistical analysis for single cell RNA-seq expression data

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Linnorm is an R package for the analysis of single cell RNA sequencing (scRNA-seq) data based on a novel normalizing transformation method. Previous works on scRNA-seq analysis methods have focused on downstream analysis methods by utilizing the conventional relative expression units and/or the log plus one transformation. Moreover, RNA-seq normalization methods are still commonly used for scRNA-seq. We present a scRNA-seq data oriented normalization and transformation method. It allows precise normalization and transformation by filtering of the dataset with or without spike-ins. Our assessments showed that Linnorm performs better than existing methods (edgeR, DESeq2, voom, Seurat etc) in terms of false positive rate control, differential gene expression analysis, clustering analysis and speed. Moreover, we show that existing methods can benefit from Linnorm's normalization and transformation methods.

Evaluating the implication of silica nanoparticles in neurodegeneration

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Silica nanoparticles (SiO_2-NPs) are typical and major components of mineral dust and many other airborne pollutants in the ambient air. Although evidence shows that nano-sized particles may have neurotoxicity and induce neuroinflammation. Whether exposure to SiO_2-NPs results in neurodegenerative-like behavioral and pathological changes is not known. In this study, we employed fluorescein isothiocyanate-tagged SiO_2-NPs (FITC-SiO_2-NPs, NPs) to investigate the effects of SiO_2-NPs on affective and cognitive behaviors in mice. Effects of NPs on synapse were studied in primary culture of cortical neurons and mice. We exposed 3-month-old male C57BL/6N mice to NPs suspension through intranasal instillation, and subjected the mice to a battery of behavior tests after 1 or 2 months treatment. We found that NPs decreased social activity, 2-month treatment-induced anxiety, and impaired memory in novel object recognition test and spatial learning and memory in Morris water maze test. We found deposition of NPs in the medial prefrontal cortex and the hippocampus. Meanwhile, we found neurodegenerative-like pathological changes in the frontal cortex, including reduction in Nissl bodies, increased phosphorylation of tau, and neuroinflammation. In both frontal cortex synaptosome and primary culture of cortical neurons, we found impairment in exocytosis function, accompanied with a decrease of synapsin I and an increase of synaptophysin. Taken together, intranasal instillation of NPs results in mood dysfunction and cognitive impairment in mice, and it may attribute to the neurodegenerative-like pathological and synaptic changes.

Acknowledgement
The study is partly supported by HKU Seed Funding for Basic Science Research (201311159171), and generous donation from Ms KW Chow.

Transcriptomic analysis of wild-type *E. coli* growing in the pore water of marine sediment

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Esherichia coli can adjust their physiology and metabolism to adapt to diverse microenvironments. In small intestine of warm-blooded hosts, the abundance of preferred sugars provided by anaerobes determines the niche of *E. coli*. The utilization of short peptides and amino acids as carbon sources supports *E. coli* growth in bladders or kidneys. Here the probabilities of carbon sources for *E. coli* in coastal marine sediment which nowadays is considered as a "secondary habitat" were investigated with wild strains from different sources. They were cultured in M9-glucose medium and pore water extracted from coastal marine sediment respectively, and their RNA-seq was analyzed for differentially expressed genes. The most differences in two media happened in the carbon metabolism and amino acid metabolism. There is no clear evidence showing the preferred sugar in pore water, while *E. coli* expressed plenty of genes associated with amino acids and peptides transport and utilization in two media. The results showed that all eight strains expressed more stress response genes to adjust themselves to pore water than M9-glucose medium. Also *E. coli* strains in pore water have a higher level of expression of curli biosynthesis. These findings suggest that carbon sources for *E. coli* in coastal marine sediment may not only be simple sugars provided by anaerobic residences but also amino acids, peptides which are microbial metabolites.

Hnrnpa1 Mutations Cause Congenital Heart Defects

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Incomplete penetrance of the congenital heart defects (CHDs) was observed in our mouse model. We hypothesized the contribution of another major genetic locus may regulate the manifestation of the CHD phenotypes. We performed genome-wide linkage mapping, fine mapping and high-throughput targeted sequencing and finally identified a recessive CT deletion in exon 5 of the heterogeneous nuclear ribonucleoprotein A1 (*Hnrnpa1*) gene. Following studies demonstrated the *Hnrnpa1*$^{ct/ct}$ homozygous mutation alone was responsible for the pathogenesis of the CHDs. Whole mount *in situ* hybridization showed *Hnrnpa1* was expressed in both the first heart field (FHF) and second heart field (SHF) cardiac lineages at cardiac crescent stage. After heart tube formation, the expression of *Hnrnpa1* was only maintained in SHF progenitors. At E9.5, Q-RT-PCR and western blot analyses demonstrated loss of *Hnrnpa1* expression in both mRNA and protein levels in *Hnrnpa1*$^{ct/ct}$ mutants. Dysregulation of a series of cardiac genes was also observed. *Hnrnpa1*$^{ct/ct}$ homozygous mutation changed the expression pattern of *Nkx2.5* and *Isl1* and caused cardiac defects at different developmental stages. Lastly, two rare heterozygous mutations of *HNRNPA1* were detected in human CHD patients. These findings suggest novel roles of *Hnrnpa1* in embryonic cardiac development in both mice and humans.

Functional Characterization of Cytoplasmic Polyadenylation Element Binding Protein during Muscle Stem Cell Activation

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Muscle satellite cells (MuSCs) stay quiescent in resting muscles. While activated by muscle injury, MuSCs perform myogenic progression to form regenerated fibers. MuSCs activation is regulated transcriptionally and post-transcriptionally. Cytoplasmic polyadenylation element binding protein 1 (CPEB1) was first identified to regulate Xenopus oocyte maturation through cytoplasmic polyadenylation. Furthermore, nuclear CPEB1 regulates alternative 3'UTR formation of its targets many of which are cell cycle-related genes. We hypothesize that CPEB1 functions to regulate MuSCs activation. Here, we found that CPEB1 was upregulated and translocated into the nucleus in activated MuSCs. Loss of function analysis showed that CPEB1 knockdown delayed MuSCs activation. Furthermore, we demonstrated that CPEB1 was phosphorylated rapidly during MuSCs activation. Aurora A, which phosphorylated CPEB1, was increased dramatically in activated MuSCs. In addition, the highly selective Aurora A inhibitor MK5108 affected activation negatively by inhibiting CPEB1 phosphorylation. On the contrary, MuSCs can be activated via CPEB1 phosphorylation by Insulin pathway. Our immunostaining data indicated that CPEB1 regulated translation efficiency does not depend on the pathways of p-body and stress granule. The FRAP experiments showed that CPEB1 can form dynamic granule suggesting that CPEB1 regulates translation efficiency through dynamic mRNP puncta. To specifically identify targets of CPEB1, we found that myogenic regulatory factors, Myf5 and MyoD, harbor CPEs in their 3'UTRs. Therefore, we suppose that CPEB1 maintains MuSCs quiescence through repressing Myf5 or MyoD translation. Upon stimuli exit, insulin activates Aurora A and induces CPEB1 phosphorylation to kick start the translation of Myf5 or MyoD mRNA for MuSCs activation. The phosphorylated CPEB1 is translocated into nucleus to regulate alternative 3'UTR formation, resulting in shortened 3'UTR globally for increased protein output and to promote MuSCs activation.

In vivo targeted imaging of atherosclerotic plaques: a nanomedicine approach

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Cardiovascular diseases (CVD) are the leading cause of death globally, causing 17.3 million deaths per year (1). Atherosclerosis, characterized by persistent inflammation and accumulation of lipid-rich plaques in arterial walls, is the major cause of CVD (2). Currently it is challenging to accurately identify at-risk CVD patients, as conventional imaging tools can only determine the degree of artery stenosis but not to detect early lesions or analyze plaque size, composition, activity or overall disease burden. However, few of the nanomaterials have been approved in clinical trials for imaging atherosclerosis due to the lack of specificity, long waiting time and slow clearance rate. Here, we report a DNA-containing magnetic nanoparticle-based platform for cell-specific imaging atherosclerotic lesions *in vivo*. We studied their cellular uptake, pharmacokinetics, biodistribution, and accumulation in the plaque by near-infrared fluorescence (NIF) imaging. Our nanostructures show translational promise in *in vivo* imaging of atherosclerotic plaques.

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Dscam 4 Is Required for Elimination of Polarity-deficient Cells in *Drosophila*

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Cell competition is an intrinsic tissue surveillance mechanism that functions to eliminate unfit cells. Loss of cell polarity can trigger cell competition. The Scribble polarity complex, which is located at the basolateral region of epithelial cells, is a well-established model to study cell competition. The Scribble complex is composed of three proteins: Scribble (Scrib), Discs-Large (Dlg) and Lethal giant larvae (Lgl). Homozygous mutants of the Scribble group genes lead to lethal and invasive tumor formation. When the *scribble* or *dlg* mutant cells are generated as mosaic clones, these mutant cells undergo cell competition-induced apoptosis.

In order to identify the molecular network that regulates the elimination of *scribble* mutant cells during cell competition, we compared the transcriptomes of imaginal discs harboring *scribble* mutant clones and those harboring control clones. We identified that *Down syndrome cell adherin molecule 4 (Dscam4)* was significantly upregulted in imaginal discs harboring *scribble* mutant clones in comparison with those bearing control clones. We further found that depletion of Dscam4 could rescue *scribble* mutant cells from elimination. DSCAM family proteins are known as neuronal recognition molecules that function to facilitate self-avoidance during dentrite tiling. Here we identified a novel role of *Dscam4* in the epithelia to maintain tissue fitness.

Mitochondrial transport defect in zQ175 Knock-In Mouse Model of Huntington's Disease

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Huntington's disease (HD) is an autosomal genetic neurodegenerative disorder caused by the expansion of CAG in the huntingtin gene. Mutated Huntingtin proteins (mHtt) induce neuronal death in cortex and striatum regions followed by other brain regions. Mitochondrial dysfunctions are thought to be a key pathogenic mechanism of HD. However, the transport of mitochondria in HD is not clearly understood yet. Our data indicated that the spatial distributions of mitochondria were affected in HD crossed mice. Furthermore, the live cell imaging data showed that the percentage of moving mitochondria significantly decreased in HD. In the primary striatal neurons of HD, the instantaneous speed, travel speed and net speed of total moving mitochondria significantly increased, while still% of moving mitochondria significantly decreased. In HD primary cortical neurons, mitochondrial reverse% significantly increased. Surprisingly, we found that more small moving mitochondria, whose transport behavior was significantly different from large moving mitochondria, existed in HD with an age-dependent manner. Our results propose that the mutant huntingtin protein possibly can work as blockages on microtubule tracks of neurons. Also, the small mitochondria possibly are mitochondrial-derived vesicle, which indicates that mitochondria are under more stress in HD. In summary, mitochondrial transport was altered in both striatal and cortical neurons of HD, which implied mitochondria as potential therapeutic targets of HD.

Anti-influenza Virus Effect of Aqueous Extracts from Traditional Chinese Medicine

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Influenza is a contagious respiratory illness causing annual epidemics and occasional pandemics. The death toll of influenza epidemics is between 250,000 to 500,000. Many strains of influenza virus had developed resistance against the existing anti-influenza drugs, thus it is essential to develop new drugs against this virus. Traditional Chinese Medicine (TCM) has been a good source of therapeutic agents. Empirical knowledge based on the ethnomedical benefits of the herbs, coupled with bioassay-guided fractionation and isolation, has the potential to identify novel antivirals that can be used against influenza.

In this study, I have used a range of bioassays, including cytopathic assay, plaque reduction assay and real-time reverse transcription-PCR assay, to screen aqueous extracts of fifty herbs for antiviral activity against influenza virus. Now, two potential herb extracts have been found exhibiting inhibition against A/WSN/33 (H1N1) and A/Puerto Rico/8/1934 (H1N1). Specific fractions of the two potential extracts isolated by D101 macroporous absorptive resin and silica gel column had an increased potency.

Till now, fraction F1-4 isolated from extract B shows the strongest effect against WSN, as it inhibits WSN replication to around 100% at the concentration of 7.5μg/ml. The combination of fraction F2, F3 and F6 isolated from extract F, show synergistic effect. Together, they can inhibit WSN replication to around 100% at the concentration of 3μg/ml. Work is being carried out to isolating pure compounds for cell assay and *in vivo* efficacy using influenza virus infected mouse model. Also, the inhibitory mechanism will also be studied.

Vinegar protects against high-cholesterol diet-induced hypercholesterolemia in hamsters

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Dietary vinegar has long been regarded as not only a food condiment, but also a healthy nutraceutical due to its capability of preventing metabolic and cardiovascular disease. Vinegar is a very complex food matrix, and contains abundant organic acids and phenolic compounds. The composition of vinegar mainly depends on the raw materials and production process. Previous studies showed that vinegar supplementation reduced blood cholesterol concentrations in rats and mice, however, the underlying mechanisms remained unexplored.

In the present study, five groups of hamsters were administrated with one of the five diets, namely, none-cholesterol diet (NCD), high-cholesterol diet (HCD, 0.2% cholesterol added), HCD + Balsamic vinegar (BV), HCD + Shanxi vinegar (SV), and HCD + acetic acid water solution (Ac), respectively. HPLC was used to analyze the organic acid compositions in vinegars. BV, SV, and Ac were prepared to obtain the concentrations of acetic acid all at 20mg/mL, and were orally gavaged at 8mL/kg body weight daily. After 8 weeks, it is observed that BV and SV but not Ac significantly lowered plasma concentrations of total and non-HDL cholesterol concentrations compared with HCD diet. BV and SV also remarkably reduced liver cholesterol contents while they increased fecal excretion of neutral and acidic sterols. Real-time PCR analyses demonstrated that BV and SV supplementation led to markedly up-regulated liver mRNA expression of low density lipoprotein receptor (LDLR) and cholesterol 7 alpha-hydroxylase (CYP7A1). In addition, SV supplementation also significantly down-regulated the intestinal mRNA expression of microsomal triacylglycerol transfer protein (MTP), thus suppressing chylomicrons excretion and cholesterol absorption in the small intestine.

In conclusion, both Balsamic and Shanxi vinegar were effective in reducing plasma total and non-HDL cholesterol concentrations in hypercholesterolemic hamsters. Rather than acetic acid, other bioactive components in vinegar are probably accountable for the cholesterol-lowering activity of vinegar.

Synthetic Multicellular Systems for Spontaneous Pattern Formation

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Multicellular organisms present amazing examples of self-organized patterns. As a hallmark of the self-organization process, coordinated cellular behavior, commonly orchestrated at a population level, regulates the dynamic spatial arrangement of specialized cell types to generate tissue patterning and form complex body layout. Such coordination often relies on reciprocal interactions among different cell species during morphogenesis. However, the nature of the potential interaction and how such interaction can achieve coordinated spatial patterning and self-organization of multiple cell types still remain elusive. Here we describe a periodic stripe patterning process emerging from a synthetic multicellular system with programmed population interaction. The interaction enables coordinated out-of-phase spatial oscillation of cell densities of two engineered populations of *Escherichia Coli*. Such pattern arises autonomously from reciprocal density-dependent activation of mobility between the two cell species independent of any preexisting positional cues. Moreover, by manipulating the interaction, the original out-of-phase spatial oscillation rhythm can be accordingly turned into in-phase oscillation. The occurrences of out-of-phase and in-phase spatial oscillating patterns suggest density-dependent control of mobility as a simple and general strategy for spatial patterning of run-and-tumble cells even when multiple cell species (over two) were incorporate in the synthetic systems.

Cholesterol Analogs with a Branched Side Chain but not a Straight Chain Possess a Cholesterol-lowering Activity

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Hypercholesterolemia is one of the major risk factors for coronary heart disease (CHD). The present study was conducted to test the effects of β-sitosterol (SI) and three cholesterol analogs on plasma total cholesterol (TC) in hamsters fed a high cholesterol diet. The cholesterol analogs used include CA0 (no side chain), CA3 (a side chain of 3 carbons) and CA14 (a side chain of 14 carbons). CA3 and CA14 are straight side chain analogs of cholesterol while SI is an analog of cholesterol having an additional ethyl group on the side chain at 24 position. Results showed that SI at a dose of 0.15% could effectively reduce plasma TC by 17.7%. SI was not detected in both plasma and liver of the hamsters, indicating that it was poorly absorbed in the intestine. All three analogs, CA0, CA3 and CA14, had no effect on the plasma TC. CA3 and CA14 were found to be accumulated in both plasma and liver, proving that they were well absorbed in the intestine. In conclusion, the study illustrated that analogs having branched side chains possessed plasma TC-lowering activity, while analogs with a straight side chain did not have plasma TC-lowering activity.