Molecular Characterization of Seipin in *Caenorhabditis elegans*

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Lipid droplets (LDs) are conserved organelles for storing and supplying lipid in eukaryotes. They consist of a neutral lipid core that is surrounded by a phospholipid monolayer. Accumulating evidence suggests that the endoplasmic reticulum (ER) is in tight association with LDs both physically and functionally during the biogenesis and expansion of LDs. Seipin, loss of which in humans causes lipodystrophy, is predicted to be an integral membrane protein in the ER. Studies in a number of model systems suggest differential roles of seipin in regulating adipogenesis and LD morphology. However, the exact function of seipin remains elusive. Here we report that a functional GFP fusion protein of the *C. elegans* seipin ortholog (SEI-1) was stably targeted to hitherto uncharacterized "cage" structures around a subset of LDs. Using light and stimulated Raman scattering (SRS) microscopy, we found that loss of SEI-1 reduced the size of a subset of LDs while overexpression of SEI-1 had the opposite effect. Genetic and dietary supplementation experiments revealed a requirement of specific polyunsaturated fatty acids (PUFAs) for SEI-1 targeting. In mammalian cells, SEI-1 enrichment on a subgroup of ER tubules were visualized by electron microscopy. Live cell imaging also revealed the accumulation of SEI-1 to the proximity of nascent LDs upon oleic acid loading. Taken together, our results provide the first characterization of the *C. elegans* seipin ortholog. More importantly, the molecular basis for SEI-1 localization suggests a novel mechanism that enforces organelle heterogeneity, driven by PUFAs-dependent protein targeting.

Dual regulatory effects of SFTS virus NSs protein on interferon signaling

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Innate interferon (IFN) response that inhibits viral replication is the first-line host defense against viral infection. To circumvent this response, viruses have developed various counter-measures to antagonize IFN production and/or signaling. SFTS virus (SFTSV) is an emerging zoonotic pathogen initially identified in China and subsequently found in other parts of the world. SFTSV NSs protein is an IFN antagonist that has been shown to counteract type I IFN induction by targeting TBK1 and IKKε kinases and to impede IFN signaling by interacting with and sequestrating STAT2 in the cytoplasm. In this study, we demonstrated that SFTSV infection and its NSs protein suppresses both production and signaling of type I and type III IFNs by preventing STAT1 phosphorylation and activation whereas augments type II IFN signaling. In contrast, expression of NSs or infection with SFTSV had no influence on the activation of NF-κB signaling. NSs protein not only interacts with STAT1 and STAT2, but also inhibits IFN-β-induced phosphorylation at serine 727 of STAT1 without affecting serine 701 phosphorylation. Furthermore, STAT1 protein was inhibited at the transcriptional level. IRF1 and CXCl10 expression was further induced as a result of the increase in STAT1 phosphorylation in the presence of both IFN-γ and NSs. Taken together, our findings suggested that SFTSV NSs protein is a viral modulator of IFN signaling that has opposite effect on type I and type II IFN signaling. Our work provides new knowledge in SFTSV pathogenesis and has implications in the design and development of anti-SFTSV agents and vaccines. This work was supported by RGC-NSFC JRS (N-HKU 714/12), RGC CRF (HKU1/CRF/11G and C7011-15R) and HMRF (HKM-15-M01).

Loop-mediated isothermal DNA amplification (LAMP) on a lab-on-a-disc (LOAD) platform for the detection of *Mycobacterium tuberculosis*

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Tuberculosis (TB) is a widespread airborne disease transmitted via *Mycobacterium tuberculosis*. Bacterial culture is the standard diagnostic method for TB infection but it remains time-consuming. Recent advances in molecular biology enabled faster TB detection using novel loop-mediated isothermal amplification (LAMP) technique. LAMP is a powerful DNA amplification method that can be completed in an hour without the need of a thermal cycler. Multiple primers hybridize to a distinct region on a gene, making LAMP highly specific. Lab-on-a-disc (LOAD) is a microfluidic device with customisable components to complete laborious detection procedures automatically in a single run. Movement and temperature manipulation of samples and reagents are driven mainly by centrifugal force. LAMP reaction on LOAD is made possible with automated temperature control and addition of primers and enzymes. The present study aimed at combining DNA extraction from sputum samples and LAMP onto a LOAD platform for fast and sensitive TB screening. Our in-house LOAD platform consisted of a bio-disc and a structural LOAD machine. A belt-driven motor provided controllable centrifugal force to the bio-disc. The bio-disc was directly attached to a heater board with wireless temperature and fluorescence signal detection modules. DNA amplification products were monitored real-time with a fluorescent reporting probe. Sputum specimens were donated by healthy volunteers in the Prince of Wales Hospital with ethics approval. Our results showed that bacterial concentration of as low as 10^3 colony-forming unit per millilitre sputum sample could be detected in 60 minutes in the Prince of Wales Hospital. It is believed that further fine-tuning can lead to an automated LAMP on LOAD platform for improving TB infection monitoring and hence disease control.

Differential G protein subunit dissociation mechanisms suggest possible family-wise selective Gβγ signaling

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Heterotrimeric G proteins represent one of the most universal and complicated group of signaling proteins in eukaryotes. The heterotrimer dissociates into two components upon activation by receptors: the GTP-bound Gα subunit and the Gβγ dimer. Four families of mammalian Gα subunits exhibit distinct functions but no functional differences are observed among dozens of Gβγ dimers, which begs the question of the specificity of Gβγ signaling. We have observed that active Gα subunits from different families show differential binding affinities to the $G\beta_1\gamma_2$ dimer; activated Gα subunits from the G_i family dissociate more readily from the Gβγ dimer than those from other families like G_q or G_s. To test whether such affinity difference is related to the function of the G proteins, we constructed a series of Gβ-Gα fusion proteins that prevent complete subunit dissociation of the G protein, and examined their ability to stimulate downstream effectors. Our results suggest that Gα subunits of the G_i family have impaired functions when tightly linked with the Gβ subunit, while the Gα subunits from other families retain their functions despite under such non-dissociable condition. These observations support the idea that only certain subtypes of G proteins can release free Gβγ dimers when activated. Since the major effectors of Gβγ share similar Gβ binding interface with Gα subunits, it is likely that such dissociation differences among G protein families can contribute to the specificity of the Gβγ signaling, resulting in Gβγ signaling activities being closely associated with G_i activation. (Supported by grants 16137516 and ITCPD/17-9)

Sorting of Planar Cell Polarity Signaling Receptor, Frizzled6, at the *trans* Golgi Membranes

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Planar cell polarity (PCP) is mediated by conserved PCP proteins that localize asymmetrically on opposing cellular boundaries. Here, we have investigated sorting of two asymmetrically localized PCP proteins, Vangl2 and Frizzled6, at the *trans*-Golgi network (TGN). Through an *in vitro* vesicle formation assay, we find that Vangl2 and Frizzled6 exit the TGN in separate vesicles. Further analysis indicates that a clathrin adaptor, epsinR, regulate TGN export of Frizzled6 but not Vangl2. EpsinR forms a stable complex with clathrin and this complex directly interacts with a conserved polybasic motif in Frizzled6 cytosolic domain to package Frizzled6 into transport vesicles. Our results suggest that Vangl2 and Frizzled6 are packaged into separate vesicles by different clathrin adaptors at the TGN, which may contribute to their asymmetric localizations.

Canonical BMP signaling is crucial to the maintenance of neural stem cells at cerebellar ventricular zone

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Cerebellum is essential for motor coordination and body balance. The mouse cerebellum contains two functionally distinct germinal matrices, namely the anterior rhombic lip (ARL) and the ventricular zone (VZ). While ARL gives rise to glutamatergic neurons, VZ produces GABAergic neurons and astrocytes in cerebellum. During cerebellar development, ligands of bone morphogenetic protein (BMP) signaling were produced from the ARL, dorsal roof plate and the forth ventricular choroid plexus. Canonical BMP signaling is essential to the generation of granule cell progenitors from the ARL while its importance at cerebellum VZ was unknown. We showed that phosphorylated Smad1/5 are expressed at cerebellar VZ, suggesting that canonical BMP signaling may regulate VZ neural stem cells. We found that conditional knockout of *Smad1*/5 led to reduced cell proliferation at cerebellar VZ. Further analyses suggested that neural stem cells at cerebellar VZ were depleted more prematurely, resulting in precocious production of Purkinje cells and interneurons. Gliogenesis begins at around mouse embryonic day (E) 14. Interestingly, generation of astrocytes and Bergmann glia were impaired by *Smad1*/5-ablation. Altogether, we identified a novel role of canonical BMP signaling in cerebellar development. Our findings suggest canonical BMP signaling helps to maintain the neural stem cells at cerebellar VZ through inhibition of neurogenesis and maintenance of self-renewal capacity.

An Aptamer-Mediated DNA Nano-Switch

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The programmability of DNA has multiple applications including diagnosis, drug delivery and bioelectronics. Recent designs of responsive DNA nanostructure could detect the presence of different nucleic acid targets and other molecules through strand displacement as an indirect method. We have developed the DNA nanotweezers triggered by the presence of *Plasmodium falciparum* lactate dehydrogenase (PfLDH). The closure of nanotweezers will result in the formation of G-quadruplex catalyzing the peroxidase activity of hemin as a colorimetric signal. We also applied the same aptamer to a DNA origami box and used PfLDH as a key to open it. The observations from transmission electron microscopy and fluorescence resonance energy transfer indicated that the opening can be achieved in three hours and it is highly specific towards PfLDH. The success in using protein specific aptamer to control the structural change of DNA nanostructure indicates the potential of developing more diversified diagnostic and drug delivery systems.

Identification and characterization of the interaction between influenza A nucleoprotein (NP) and host heterogeneous nuclear ribonucleoprotein C

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The occasional influenza pandemics and annual epidemics pose not only a global health risk but also an economic burden worldwide. In order to combat this disease, it is important to understand how the virus interacts with and manipulates host factors to during its viral cycle. The nucleoprotein (NP) is an indispensable component of the influenza virus ribonucleoprotein (vRNP) which perform multiple roles such as encapsidating viral RNA (vRNA). We study from structural and biochemical perspectives how NP interacts with host heterogeneous nuclear ribonucleoprotein C (hnRNPC), which was identified by our group as a novel interacting partner of NP using a mass spectrometric approach. Here we describe our recent progress in characterizing NP-hnRNPC interaction. Using a mass spectrometry-coupled proteomic approach, we identified hnRNPC as a potential interacting partner of influenza nucleoprotein. We characterized hnRNPC-NP interaction using co-immunoprecipitation and in vitro pull down assays. Further, we have set off to determine the functional relevance of this interaction by a combination of biochemical techniques. We have confirmed that hnRNPC-NP interaction is a direct interaction and exists across different influenza A sub-types and strains. In vitro pull-downs showed that hnRNPC interact with NP via its highly acidic C-terminal portion peptide. We were able to disrupt hnRNPC-NP interaction by expressing this hnRNPC peptide. Also cellular XIAP level was restored by expressing the same peptide peptide prior to infection. HnRNPC is an essential host factor which is involved multiple cellular functions including mRNA splicing, mRNA packaging, translation initiation and mRNA export. Our data show that hnRNPC is itself anti-apoptotic. Our results also show that interaction between hnRNPC and NP may be implicated in promoting host cell apoptosis upon influenza infection.

The First Wave of T Lymphopoiesis in Zebrafish Arises from Aorta Endothelium Independent of Hematopoietic Stem Cells

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T lymphocytes are key cellular components of adaptive immune system and play a central role in cell-mediated immunity in vertebrates. Despite their heterogeneities, it is generally believed that all different types of T lymphocytes are generated exclusively via the differentiation of hematopoietic stem cells (HSCs). Using temporal-spatial resolved fate mapping analysis and time-lapse imaging, here we show that the aorta endothelium in the zebrafish aorta-gonad-mesonephros (AGM) and posterior blood island (PBI), the hematopoietic tissues previously known to generate HSCs and erythro-myeloid progenitors (EMPs) respectively, produces a transient wave of T lymphopoiesis independent of HSCs. We further show that this HSC-independent T lymphopoiesis occurs early and generates predominantly $CD4^{+}CD8^{-}$ $T_{\alpha\beta}$ cells exclusively in larval stage, whereas the HSC-derived T lymphopoiesis emerges late and produces various types of T lymphocytes continuously from larval stage to adulthood. Our study unveils the existence, origin and ontogeny of HSC-independent T lymphopoiesis and the complexity of endothelial-hematopoietic transition of the aorta.

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Essential role of Sox9 in CSF secretion and establishment of the blood-CSF barrier

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The choroid plexus (CP) is crucial to the development and homeostasis of the CNS by producing cerebrospinal fluid (CSF) and establishing the blood-CSF barrier. However, the genetic mechanisms responsible for CP development and function are poorly understood. Here we identified the transcription factor Sox9 was robustly expressed in mouse hindbrain CP epithelium. Using genetic loss-of-function approach, we show that Sox9 is essential for CP development and function. Sox9 was required for the expression of genes encoding extracellular matrix (ECM) components in the CP. Deficiency of ECM deposition at the CP basal lamina was observed in mutant. Moreover, Sox9 mutant failed to establish the apical-basal polarity in CP epithelia. Formation of junctional components between mutant epithelial cells was perturbed. Loss of Sox9 also led to an abrupt increase in CSF protein content, indicating the leakage of blood-CSF barrier in mutant. Interestingly however, we observed that number of exocytic CD63+ vesicles were remarkably reduced in mutant CP epithelium, suggesting that the regulated secretion across CP was attenuated upon the loss of Sox9. Furthermore, forced over-expression of Sox9 in the CP as well led to defective ECM decomposition and CP dysplasia. Our results conclude that Sox9 proper expression is indispensable for CP development and its proper function.

The p110α Isoform of Phosphatidylinositol-3 kinase Critically Regulates Quiescence Exit and Cell Cycle Reentry in Adult Muscle Satellite Cells

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Adult mouse muscle satellite cells (MuSC) are quiescent. Upon injury, MuSC undergo quiescence exit, re-enter the cell cycle to proliferate, then differentiate to repair the damaged muscles. Although several signaling pathways are known to regulate proliferation and differentiation of MuSC, it remains unclear which pathway regulates quiescence exit in adult MuSC. Here, we demonstrated that MuSC-specific deletion of *p110α*, a catalytic subunit of phosphatidylinositol 3-kinase (PI3K), rendered MuSC unable to undergo quiescence exit, resulting in severely impaired muscle regeneration. Genetic reactivation of mTORC1 in *p110α*-null MuSC partially rescued the above defects. Mechanistically, we found that Jun and FoxOs are two key downstream effectors of PI3K in regulating quiescence exit. Moreover, deliberate activation of PI3K in quiescent MuSC was sufficient for them to spontaneously break quiescence in uninjured muscles leading to their gradual depletion. Thus, PI3K is both necessary and sufficient for MuSC to exit quiescence in response to activating signals.

Role of transient receptor potential vanilloid 1 channels in the regulation of functional properties of embryonic stem cell-derived cardiomyocytes

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Transient receptor potential (TRP) channels are broadly expressed in a variety of tissues and cell types and they are able to respond to a wide range of stimuli in the cellular environment. Among TRP channels, TRPV1 channel is activated by heat, pain inducers and the pungent component of hot chili peppers capsaicin. Previous studies showed that upon stimulation, TRPV1 channel can generate intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) changes by Ca^{2+} entry via the plasma membrane or by Ca^{2+} release from intracellular organelles. As a fundamental property of cardiomyocytes (CMs), $[Ca^{2+}]_i$ plays an important role in different cellular processes such as excitation-contraction coupling, cell proliferation and cell death. However, there is only limited knowledge on the function of TRPV1 channel in the Ca^{2+}-handling properties and electrophysiology characters of CMs. In this study, we used embryonic stem cells (ESCs)-derived CMs as a model to study the role of TRPV1 channel in CMs. Our recent results revealed that TRPV1 agonist, capsaicin decreased the rate and diastolic depolarization slope of action potential of ESC-derived CMs, as well as the amplitude and frequency of spontaneous Ca^{2+} transients of ESC-derived CMs. To study the role of TRPV1 in ESC-derived CMs, TRPV1 will be functionally knocked down by the dominant negative form of TRPV1, TRPV1β. Adenovirus harboring the TRPV1β will be made in the near future. This study will not only give a better understanding of Ca^{2+} homeostasis of ESC-derived CMs, but also provide insights into the future cell replacement therapies.