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<th><strong>Title</strong></th>
<th>MMP14 regulates the lineage progression of hypertrophic chondrocytes</th>
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2013 Hong Kong Inter-University Biochemistry Postgraduate Symposium

15th June, 2013

Mong Man Wai Building

The Chinese University of Hong Kong

Hong Kong, CHINA
Symposium Organizing Committee

Professor Ho Yin Edwin CHAN
School of Life Sciences
The Chinese University of Hong Kong

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The University of Hong Kong

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New Asia College, CUHK
Event Schedule
2013 Hong Kong Inter-University Biochemistry Postgraduate Symposium

8:15 a.m. – 9:00 a.m.  Registration + Poster Setup

9:00 a.m. – 9:15 a.m.  Opening Remarks

9:15 a.m. – 10:35 a.m.  Student Stage Presentation Session I
CHEUNG, HN
FE65 interacts with ARF6 to stimulate neurite outgrowth through Rac1 activation
HO, B
Mechanisms of Sox2 in regulating hair pigmentation
HOR, HH
Sufu Defines the Balance of Hindbrain Progenitor Cells Maintenance and Differentiation
LI, L
Sever Acute Respiratory Syndrome Coronavirus M-protein Induces Cell Death through Disruption of PDK1/Akt Signaling Cascade

10:35 a.m. – 11:35 a.m.  Plenary Talk (Professor Sir Richard Roberts, New England Biolabs, USA)
COMBREX: a project to uncover the biochemical function of unknown genes

11:35 a.m. – 12:20 p.m.  Tea Reception + Student Poster Session

12:20 p.m. – 1:20 p.m.  Plenary Talk (Professor Bill Hunter, University of Dundee, UK)
Targeting folate metabolism in trypanosomatid parasites for drug discovery
1:20 p.m. – 2:30 p.m.  Buffet Lunch + Student Poster Session

2:30 p.m. – 3:50 p.m.  Student Stage Presentation Session II
   LO, CY
   Identification of inhibitors targeting influenza A nucleoprotein through structure-based virtual screening
   NG, SC
   A novel polycation-mode of molecular recognition by the intrinsically disordered Ewing's sarcoma transcriptional activation domain
   SHEN, YH
   Identification and Characterization of Various Populations of the γ-Tubulin Ring Complex
   TANG, J
   Cdk5-dependent phosphorylation of Mst3 regulates radial migration through modulating RhoA activity

3:50 p.m. – 4:30 p.m.  Tea Reception + Student Poster Session

4:30 p.m. – 5:50 p.m.  Student Stage Presentation Session III
   TONG, KK
   BMP/Smad Signaling in Mouse Sternum Development
   WONG, HLX
   Functional Crosstalk among MT1-MMP and ADAMs
   YE, F
   Structures and Target Recognition Modes of PDZ Domains: recurring themes and emerging pictures
   YUEN, KS
   Roles of Epstein-Barr virus-encoded miR-BART microRNAs in viral persistence and transformation of epithelial cells

6:00 p.m. – 6:15 p.m.  Closing Ceremony + Award Presentation
Abstracts of
Platform Presentation
FE65 interacts with ARF6 to stimulate neurite outgrowth through Rac1 activation

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FE65 is a brain-enriched PTB domain containing adaptor protein. It is implicated in many cellular processes, including the regulation of neurite outgrowth during brain development. ARF6, a sole class III member of the ADP-ribosylation factor (ARF) family GTPases, has also been reported to modulate neurite length through remodeling the actin dynamics. Here we report that ARF6 is a novel FE65 interacting protein. FE65 and ARF6 colocalize in neuronal growth cones. Interestingly, FE65 enhances the activation of ARF6, and its downstream effector, the small GTPase Rac1. Since accumulating evidence shows that Rac1 is involved in regulating actin dynamics within growth cones, we suggest FE65 may regulate neurite outgrowth through the ARF6-Rac1 signaling. In fact, the coexpression of FE65 and ARF6 stimulates neurite outgrowth synergistically in primary rat embryonic cortical neurons, whereas the synergism is abrogated when Rac1 dominant negative mutant is coexpressed. Our findings suggest that FE65 activates ARF6 to regulate neurite extension through Rac1 activation.
The roles of Sox2 and Sox18 in hair development and growth

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Signals from the dermal papilla (DP) are essential in regulating hair morphogenesis. Sox2 is specifically expressed in the DP of primary and secondary hair and a recent study has shown the loss of Sox2 in the DP affects hair growth and the migration of progenitor cells. However, the functions of Sox2 in hair type specification and pigmentation remain to be well understood.

To analyze the function of Sox2 in hair type specification, we utilized Sox2¹⁰²/¹⁰² and Sox2¹³¹/¹³¹ allelic mutants which the cis-acting regulatory elements of Sox2 were disrupted due to chromosomal rearrangements. Sox2 was down-regulated in the DP during embryonic development. They displayed lighter hair coat color due to a change in hair type specification, with increased number of tertiary hair and reduced secondary hair without a change in total density. The band of pheomelanin was extended in the mutants as well. We further identified Corin and Pomp as possible downstream targets of Sox2 in hair pigmentation.

These data suggest an additional role of Sox2 in specifying hair types and pigmentation apart from regulating hair growth during morphogenesis.
Suppressor of fused (*Sufu*) was identified as a regulator in Hedgehog signalling. Study shown that *Sufu* knock-out mice were embryonic lethal at E9.5, exhibiting cephalic deformities, open neural tube and ventralized spinal cord resulting from ectopic Shh signalling, implying indispensable role of *Sufu* during development of central nervous system.

Aiming to investigate the functions of *Sufu* in hindbrain neurogenesis, we used B2-r4-Cre to knock-out *Sufu* in rhombomere4 (r4). We observed significant enlargement of mutant r4 size from E10.5, exhibiting more profound expansion in the dorsal region at E12.5. Accordingly, BrdU pulse labelling and sox2 staining showed region specific increased accumulation of proliferative progenitor cells, indicating differential maintenance of progenitor pools along the dorsoventral axis of r4. Tuj1 staining also showed impaired differentiation of the ectopic progenitor cells. Further analysis revealed dramatic dorsal expansion of pMN and p2 progenitor domains in mutant r4. Surprisingly, the FoxA2 positive floor plate, and the dorsal p0 domain were not severely affected, suggesting a novel domain specific regulation of neural progenitor pools by *Sufu*. Intriguingly, we observed spatial upregulation Gli1 and Gli2 transcription factors, selectively at the region that resides highly proliferative cells, implying that the increased cell proliferation could be caused by the changes of Gli transcription factors. Indeed, concomitant deletion of Gli2 in the *Sufu* mutant largely rescued the aberrant phenotypes. These findings clearly showed the requirement of *Sufu* to suppress Gli2 to conduct a domain specific regulation of hindbrain progenitor maintenance and differentiation.

Our study demonstrates novel function of *Sufu* to ensure a tightly controlled progenitor pools maintenance and differentiation, mainly achieve by suppressing Gli2 activation.
Sever Acute Respiratory Syndrome Coronavirus M-protein Induces Cell Death through Disruption of PDK1/Akt Signaling Cascade

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The severe acute respiratory syndrome coronavirus (SARS-CoV), emerged at the end of 2002, is a lethal infectious agent which caused the severe acute respiratory syndrome. A number of viral gene products have been shown to trigger apoptosis through interfering with cellular signaling cascades, Akt kinase pathway included. Our previous studies have demonstrated that a structural protein in SARS-CoV called Membrane (M) protein is pro-apoptotic. A forward genetic screen was performed and led to the identification of phosphoinositidedependent kinase 1 (PDK1) as a genetic modifier of M-protein mediated cell death. Phosphoinositide-dependent kinase 1 is responsible for phosphorylating Akt to mediate survival signaling pathway. In this study, the relationship between M-protein, Akt and PDK1 was elucidated. The C-terminus of M-protein was found to interact with PH domain of PDK1. Since PH domain is a site for Akt to dock onto PDK1, this interaction is thus essential for Akt phosphorylation. M-protein was shown to disrupt the association between Akt and PDK1, which caused Akt hypophosphorylation. Functional assays further confirmed that the activity of Akt was compromised in the presence of M-protein. Consequently, the levels of phosphorylated form of forkhead transcription factor (FKHR) and apoptosis signal-regulating kinase (ASK) were diminished. The phosphorylation status of FKHR and ASK is important for cell survival, and any interference with their phosphorylation level would lead to apoptosis. As expected, caspase activation was observed in cell that expressed M-protein. In summary, our results demonstrated that the SARS-CoV M-protein induces apoptosis through disrupting PDK1/Akt cell survival pathway which subsequently triggers activation through FKHR and ASK.
Identification of inhibitors targeting influenza A nucleoprotein through structure-based virtual screening

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Currently, many strains of influenza A virus have developed resistance against anti-influenza drugs, and it is essential to find new chemicals to combat this virus. The viral nucleoprotein (NP) is a major component of the ribonucleoprotein (RNP) complex for the transcription and replication of the virus. In order to maintain a stable RNP structure, NP forms homooligomers by inserting its tail-loop structure to the tail-loop insertion site of another NP. In this study, we have employed structure-based virtual screening on the influenza A NP tail loop insertion site and found two hit compounds number 7 and 16 that can subdue influenza RNP activities. Subsequently, two analogs from compound 16 were identified which inhibit RNP activities of various influenza A subtypes and viral growth at micromolar levels. These analogs were also shown to directly interact with NP by surface plasmon resonance assay.
A novel polycation-mode of molecular recognition by the intrinsically disordered Ewing's sarcoma transcriptional activation domain

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Intrinsically Disordered Protein Regions (IDPRs) are of emerging importance to protein function and disease. IDPRs commonly bind to their protein partners by an induced folding mechanism. However, some IDPRs remain disordered in the bound state and such interactions have been termed "fuzzy". Fuzzy interactions are often characterized by IDPR polyvalency, sequence-insensitivity and a dynamic ensemble of disordered bound-state conformations. Detailed biophysical models for fuzzy interactions are generally lacking because classical structural analysis is not possible.

The transcriptional activation domain of the Ewing's Sarcoma oncoprotein family (EAD) is an IDPR that exhibits the above hallmarks of fuzziness. EAD most likely interacts with a large number of proteins, although few functionally relevant EAD partners have been identified. Using an in vivo transcriptional (functional) assay to test a range of EAD mutants it was previously established that EAD activity requires multiple dispersed EAD aromatic side chains in a disordered structure. However biophysical basis for molecular recognition by EAD remained to be determined.

Cation-π interactions between aromatic π electrons (in Y, F and W) and cationic amino acids (R and K) are prevalent at protein-protein interfaces and we therefore hypothesized that molecular recognition by EAD involves polycation-π contacts between EAD and basic residues on target proteins. We evaluated this model by functional interrogation of EAD mutants (considering aromatic number, density, distribution and charge perturbations) and our results are strongly supportive. Moreover, functional effects are well captured computationally by molecular dynamic simulations based on interaction of EAD aromatics and surface cations of a generic globular target. Overall EAD-target binding is seen to be governed by a combination of EAD conformational entropy and polycation-π interactions. Such a highly versatile mode of molecular recognition may reflect a more general solution for promiscuous target recognition by polyvalent IDPRs.
Identification and Characterization of Various Populations of
the γ-Tubulin Ring Complex

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As a well-known microtubule (MT) nucleator, the γ-tubulin ring complex (γTuRC) contains members of a protein family called GCPs. It is widely thought there are seven γ-tubulin small complexes (γTuSCs) cooperate with GCP4, 5, 6 to form a γTuRC. Recently CDK5RAP2 and GCP-WD have been identified as γTuRC-anchoring proteins. Despite the elucidation of key roles for these proteins in centrosomal attachment, MT nucleation, and mitotic spindle organization, CDK5RAP2 is crucial for astral MT nucleation during mitosis, which ensures the fidelity of cell division, while GCP-WD plays an important role in chromosome-mediated MT nucleation.

Our aim is to investigate the γTuRC composition change during cell cycle and the regulation of CDK5RAP2 and GCP-WD in binding to the γTuRC. In our present studies, we have uncovered the dynamic change of γTuRC composition during cell cycle. For the γTuRC-anchoring proteins, despite their direct binding to the γTuRC, CDK5RAP2 and GCP-WD exist in different γTuRC populations, and there is a dynamic equilibrium between these two populations. For the functional study, CDK5RAP2-bound γTuRC stimulates MT nucleation both in vivo and in vitro, while GCP-WD-bound γTuRC does not have this function. Our data suggests that γTuRC composition is changed in a cell cycle-regulated manner; CDK5RAP2 and GCP-WD may be under distinct control for interacting with the γTuRC and have distinct functions when associate with γTuRC. For the first time we have found the dynamic change of the γTuRC composition during cell cycle and characterized at least two different populations of the γTuRC.
Cdk5-dependent phosphorylation of Mst3 regulates radial migration through modulating RhoA activity

Jing Tang\textsuperscript{1,2,3,4}, Jacque P. K. Ip\textsuperscript{1,2,3,4}, Weiqun Fang\textsuperscript{1,2,3,4}, Wanting Zhong\textsuperscript{1,2,3,4}, Yu Pong Ng\textsuperscript{1,2,3,4}, Zhenguo Wu\textsuperscript{1,2,3,4}, Lei Shi\textsuperscript{1,2,3}, Amy K. Y. Fu\textsuperscript{1,2,3,4}, and Nancy Y. Ip\textsuperscript{1,2,3,4}* 

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Radial migration of newborn neurons is critical for the lamination of the cerebral cortex. Whereas the migration of neurons requires a precise organization and dynamics of the actin and microtubule cytoskeleton, the underlying signaling mechanisms are not well understood. Here, we identify that Mst3, a serine/threonine kinase highly expressed in the developing mouse brain, is important for radial migration of neurons. Silencing of Mst3 \textit{in utero} resulted in a significant delay in radial migration and aberrant polarity acquisition of neurons in developing mouse neocortex. While the kinase activity of Mst3 is essential for its functions in neuronal morphogenesis and migration, its kinase activity is regulated by the phosphorylation at Ser79 by a serine/threonine kinase, cyclin-dependent kinase 5 (Cdk5). Interestingly, we identify RhoA, a Rho GTPase that is critical for reorganization of actin cytoskeleton, as a novel substrate of Mst3. Mst3 phosphorylates RhoA at Ser26 residue, thereby negatively regulating the GTPase activity of RhoA. Importantly, down-regulation of RhoA restored normal neuronal migration in Mst3-knockdown cortex. Together, our findings suggest that Cdk5-Mst3 signaling regulates neuronal migration process through RhoA-dependent actin dynamics.

This study was supported in part by the Research Grants Council of Hong Kong SAR (HKUST 660808, 660810, 660711 and 661111), the National Key Basic Research Program of China (2013CB530900), the Shenzhen Peacock Plan, the Theme-based Research Scheme of the University Grants Committee (T13-607/12-R), and the Innovation and Technology Fund for State Key Laboratory (ITCPT/17-9).
BMP/ Smad Signaling in Mouse Sternum Development

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The canonical bone morphogenetic protein (BMP) signaling is well known to be important for the endochondral bone development. Previous Col2-Cre driven chondrocyte-specific inactivation of both Smad1 and Smad5 (Smad1/5) in mouse resulted in the loss of most skeletal components, except the sternum. This suggests that the R-Smad may play less important role in the sternum development. To fully investigate the function of R-Smad in the sternum development, instead of utilizing a late expressing Cre, we employed the En1-Cre to inactivate both Smad1 and Smad5 in the early sternal progenitors. The mutants displayed total loss of sternum components except the manubrium, suggesting the importance of canonical BMP signaling in the early sternal development. Besides, loss of Sox9 and Runx1 expression was observed in the sternal progenitors of the Smad1/5 mutants, whereas no significant difference in proliferation and apoptosis was detected. Our results suggest that the transcription network of the early specification processes for sternum development is ablated in the absence of R-Smad. In addition, to investigate the role of co-Smad (Smad4) in the sternum development, En1-Cre driven Smad4 mutant mice were generated. To our surprise, all sternal components were present in the Smad4 mutant and thus the R-Smad could work independent of co-Smad during the sternum development. Together, our data challenges the current working model of the canonical BMP signaling.
Membrane Type 1-Matrix Metalloproteinase (MT1-MMP), also called as MMP14, is a membrane tethered enzyme essential for tissue remodeling and signaling transducing events ranging from growth and development to cancer progression and metastasis. Mmp14−/− mice exhibit severe craniofacial abnormalities similar to those of FGF signaling mutant mice. We found that cranial defects occurred as early as 15.5 dpc. in Mmp14−/− embryos, resulting from compromised FGF signaling that is a consequence of increased FGFR2 shedding mediated by ADAM9. MT1-MMP forms a complex with ADAM9 and FGFR2. Through this complex formation, MT1-MMP proteolytically inactivates ADAM9 to protect FGFR2 from ectodomain shedding. Interestingly, targeted deletion of ADAM9 significantly rescued cranial defects of Mmp14−/− mice in a FGF signaling-dependent manner. These findings reveal a novel paradigm for the regulation of FGF signaling (Chan et al., 2012).

In addition, MT1-MMP can regulate another ADAM family member, ADAM15. MT1-MMP physically interacts with and cleaves ADAM15 to promote its degradation (Wong et al., 2012). Disturbance of this regulatory loop, such as depletion of ADAM15 in Mmp14−/− mice, leads to aberrant neovascularization. These results suggest that the regulation of ADAM15 by MT1-MMP is essential for the maintenance of vascular homeostasis. These findings also highlight the importance of the functional crosstalk between MMP and ADAM families in physiological development and probably other pathological conditions.

References
Structures and Target Recognition Modes of PDZ Domains: recurring themes and emerging pictures

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PDZ domains are highly abundant protein-protein interaction modules and often found in multi-domain scaffold proteins. PDZ-domain-containing scaffold proteins regulate multiple biological processes, including trafficking and clustering receptors and ion-channels at defined membrane regions, organizing and targeting signaling complexes at specific cellular compartments, interfacing cytoskeletal structures with membranes, and maintaining various cellular structures. PDZ domains, each with ~90 amino acid residues folding into a highly similar structure, are best known to bind to short carboxyl tail peptides from their target proteins. A series of recent studies have revealed that, in addition to the canonical target binding mode, many PDZ/target interactions involve amino acid residues beyond the regular PDZ domain fold, which we refer to as extensions. Such extension sequences often form an integral structural and functional unit with the attached PDZ domain, which is defined as a PDZ supramodule. Correspondingly, PDZ domain binding sequences from target proteins are frequently found to require extension sequences beyond canonical short carboxyl tail peptides. Formation of PDZ supramodules not only affords necessary binding specificities and affinities demanded by physiological functions of PDZ domain targets, but also provides regulatory switches to be built in the PDZ/target interactions. Here, we try to summarize structural features and target binding properties of such PDZ supramodules emerging from studies in recent years.
Roles of Epstein-Barr virus-encoded miR-BART microRNAs in viral persistence and transformation of epithelial cells

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Epstein-Barr virus (EBV) infects more than 90 % of the world’s population and is strongly associated with B-cell and epithelial malignancies including nasopharyngeal carcinoma (NPC). It is the first virus reported to encode microRNA and its BART microRNAs are highly expressed in NPC. Using a candidate gene approach, we have previously shown that miR-BART5 and miR-BART3* downregulate the expression of proapoptotic factor PUMA and tumor suppressor gene DICE1 inside infected epithelial cells. These findings suggest that miR-BARTs affect EBV-host interaction and might be important for viral persistence. Exactly how EBV contributes to epithelial carcinogenesis remains elusive. In this regard, miR-BARTs plausibly play a critical role in tumor development.

In order to understand the functions and importance of EBV miR-BARTs in epithelial cells systematically, a nasopharyngeal epithelial cells (NP) infection model is established with recombinant EBV BAC clones in this study. A miR-BART-defective recombinant EBV BAC was constructed by using galK-RED recombination system. The miR-BART promoter was disrupted in this EBV mutant leading to a defect in miR-BART production. The mutant virus production cell line was established with HEK293 cells and used to co-culture with NP460 cell line for EBV infection. NP460 cells were successfully infected with the miR-BART-defective recombinant EBV. A lentiviral library for miR-BART expression was also constructed. Different combinations of miR-BARTs will be re-introduced into miR-BART-defective EBV infected NP cells for further functional assays. This screening platform provides us a systematic approach to analyse and sort out the important miR-BART candidates which contribute substantially to the development of EBV-associated epithelial tumors.

This work was supported by Hong Kong Research Grants Council (HKU7668/09M, HKU1/CRF/11G and AoE/M-06/08), Hong Kong Health and Medical Research Fund (11100602 and 12110962) and S.K. Yee Medical Research Fund (2011).
Abstracts of
Poster Presentation
Characterization of intracellular Ca2+ signaling, as well as changes in gross morphology, cytoskeletal organization and mitochondria localization during differentiation of human embryonic stem cells into ventricular cardiomyocytes

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The possible role of calcium signaling during human embryonic stem (hES) cell differentiation and maturation into ventricular cardiomyocytes (vCMs) was investigated using hES2 cells that were transduced with the jellyfish apo-aequorin gene using a lentiviral vector. Active holo-aequorin was reconstituted from the apo-aequorin expressed, by incubation with f-coelenterazine, the prosthetic co-factor of the holo-aequorin complex. Holo-aequorin is a Ca2+-sensitive bioluminescent complex that emits light at ~470 nm on binding with Ca2+. The temporal nature of the Ca2+ signals generated by a population of these undifferentiated cells as well as by cells at different stages of differentiation into vCMs were then characterized using a luminometer. We also optimized methods to measure changes in the gross morphology, cytoskeletal organization and localization of the mitochondria in hES-vCMs during differentiation. Cells were double-immunolabelled with the α-actinin and mitochondria (COX IV) antibodies, after which images were acquired via confocal microscopy and high-resolution stimulated emission depletion (STED) microscopy. Image analysis protocols were then designed using Image J and Metamorph to measure the extent of differentiation. Supported by RGC TBRS award-T13-706/11-1; HKUST PG studentship-T13-706/11PG and RGC GRF award HKUST662211.
MMP14 regulates the lineage progression of hypertrophic chondrocytes

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It is traditionally believed that chondrocytes and osteoblasts are two separate lineages with hypertrophic chondrocytes (HCs) being the terminal stage of chondrocyte differentiation, culminating in apoptosis. However, we have shown that HCs can contribute to the full osteoblast (Obs) lineage in vivo. MMP14 is a transmembrane matrix metalloproteinase responsible for matrix remodeling that is highly expressed at the chondro-osseous junction which coincides with the transition from HCs to Obs. Knockout of Mmp14 in mice results in impaired endochondral ossification. To test whether loss of MMP14 has an impact on the HC to Obs transition, we have employed a genetic recombination approach to track and compare the fate of HCs in wild-type and Mmp14 conditional and total null mutants. Both complete and conditional deletion of MMP14 activity results in increased number of HC-descendent cells in the trabecular bone. Surprisingly, conditional knockout of Mmp14 in HC-descendent cells results in increased trabecular bone formation. Our results suggest that MMP14 in general negatively regulates HC to Obs transition.
An investigation of the possible function of Two-Pore Channel 2 (TPC2)-mediated Ca\textsuperscript{2+} signaling during the development of slow muscle cells in zebrafish embryos

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Ca\textsuperscript{2+} signaling has been reported to play a key role in the differentiation of many cell types including vertebrate skeletal muscle. Recent work from our lab has characterized the Ca\textsuperscript{2+} signals generated during the development of slow muscle cells (SMCs) in intact zebrafish, and provided evidence that Ca\textsuperscript{2+} release is mediated via both inositol trisphosphate receptors and ryanodine receptors. Here, I present new data, which suggest that in zebrafish embryos, a novel member of the voltage-gated channel superfamily, the two pore channel 2 (TPC2; which is known to be located in the membranes of acidic organelles and is activated by NAADP), also plays a crucial role in the differentiation of SMCs. Using a line of transgenic zebrafish that express the Ca\textsuperscript{2+}-sensitive bioluminescent protein aequorin specifically in skeletal muscle cells, morpholino (MO)-based knockdown of TPC2 resulted in a significant attenuation of the Ca\textsuperscript{2+} signals and gross morphological changes in the trunk musculature. Embryos treated with the inhibitor of TPCs (Ned-19) or of the vacuolar-type H\textsuperscript{+} ATPase that depletes the Ca\textsuperscript{2+} store in acidic organelles (bafilomycin), resulted in a disrupted pattern of Ca\textsuperscript{2+} signals as well as an altered morphology of the SMCs. I also show that TPC2 is expressed in a striated pattern in the SMCs at 24 hours post fertilization. Together, these data provide evidence to suggest that TPC2-mediated Ca\textsuperscript{2+} release might initiate the sarcoplasmic reticulum-generated Ca\textsuperscript{2+} transients shown to be essential for myofibrillogenesis as well as for the overall development of the trunk musculature. Supported by RGC award: HKUST662211.
Authentic human basic fibroblast growth factor produced by secretion in *Bacillus subtilis*

Kwong KKY, Ng KL, Lam CC, Wang YY, Chan AKN and Wong WKR

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Bacillus subtilis is generally accepted as an inborn host candidate employed for secretory production of heterologous proteins. However, this ideal host system has never been employed for commercial production of medically useful proteins. In this communication, we report for the first time the employment of an engineered B. subtilis system, in conjunction with a facile cell-wall destabilization protocol, to successfully obtain an alluring yield of 40 mg/l of secreted human basic fibroblast growth factor (hbFGF) in the culture supernatant. The product was not only shown to exhibit potent bioactivity but also revealed to possess a protein sequence identical to that of mature native hbFGF (Mat-hbFGF). Our findings may pave way for the development of a cost-effective process for producing Mat-hbFGF, which is currently sold at an unusually expensive price of over US $1 million/g, for medical and skin care applications.
Regulation of NRG3 neurotrophic factor causes instigation on neuritic elongation and survival by a novel phosphatase, PTPN21

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Neuregulin3 (NRG3) is a neurotrophic factor which promotes neuronal survival. It is a known risk factor for schizophrenia, however the causes of schizophrenia remain elusive and efforts are being made to identify novel mediators in the hopes of achieving early diagnosis, delayed onset or slowed progression of the disorder. In order to identify the mediator of Nrg3, expression quantitative trait locus (eQTL) analysis on recombinant inbred mouse brain was performed and protein tyrosine phosphatase non-receptor 21 (Ptpn21) was identified as a novel mediator of Nrg3. The subsequent biochemical study demonstrated that PTPN21 induced Elk-1 phosphorylation and activation as well as NRG3 expression in embryonic cortical neurons. Our studies support the Elk-1 activation dependence of PTPN21 can be abolished by dominant negative Ras mutant (N17S) or MEK inhibitor U0126. Furthermore, a novel Elk-1 binding motif was identified in a region located ~1919 bp upstream of NRG3 initiation codon. Coincidently, PTPN21 was also identified as a genetic risk factor recently.

PTPN21 enhances neuron survival akin to NRG3, a well established neurotrophic factor, a time-course trophic factor deprivation assay demonstrated PTPN21 not only promotes cortical neuronal survival but also enhances neuritic length plausible through Elk-1 and NRG3. In summary, we identified the NRG3 gene as a novel target for Elk-1 and characterized the role of PTPN21 in relation to the activation of Elk-1 and hence the expression of NRG3. Our findings placed two genetic risk factors for schizophrenia, PTPN21 and NRG3, in the same signaling pathway and affirmed the prosurvival property of PTPN21.
Detecting Structural Variations using Optical Mapping

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Structural variations have become an important field of study in genomic science for its close relation to disease susceptibility. Due to the complex nature of the human genome, most structural variations are hard to identify simply by next-generation sequencing, which is limited by short read-length (usually of size ~ 100bp). Optical mapping (OM), a specialized imaging technique of DNA molecules treated with restriction or nicking endonuclease for specific sequence recognition, followed by fluorescent labeling, produces specific patterns along a long DNA molecule. In theory, optical mapping could complement sequencing in detecting structural variation effectively. Recently, we generated an optical mapping dataset from NA12878, a reference human DNA sample used in the 1000 Genome project, with previously studied structural variations. We employed this data to (1) confirm and validate our structural variation detection methods; and (2) further identify new structural variations. Our preliminary study reveals promising abilities of OM to detect known inversions. Further investigation in other types of structural variations is still underway.
An investigation on the anti-tumor and immunomodulatory effects of conjugated fatty acids

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Conjugated fatty acids (CFA) refer to the positional and geometric isomers of polyunsaturated fatty acids (PUFA) with conjugated double bonds. CFA have been shown to possess various biological and pharmacological activities, including anti-tumor and immunomodulatory effects. Among all CFA, conjugated linoleic acid (CLA) has been most extensively studied in relation to its occurrence, metabolism and physiological effects. Nevertheless, the occurrence of CLA in natural food is found to be less than 1%. When compared to the low concentration of CLA, conjugated linolenic acid (CLN) which possesses one more conjugated double bond, is found at a higher concentration in natural food. Apart from the relative abundance, it was also reported that CLN shows stronger dose-dependent inhibitory effect than CLA on different cultured cancer cell lines.

Despite the anti-tumor effect of CFA has been well documented, there are very limited studies on the immunomodulatory activity of CFA using immune cells or animal models. Therefore, the immunomodulatory effects of CFA and their action mechanisms on murine macrophages would be investigated. Our results showed that CFA such as jacaric acid and cis-parinaric acid exhibited no significant cytotoxicity on the thioglycollate-induced peritoneal macrophages but markedly increased their cytostatic ability. Moreover, the endocytic activity as well as the capability of murine macrophages to produce reactive oxygen species (ROS) were enhanced after the treatment with those CFA. Last but not least, jacaric acid-treated macrophages also showed an elevated increase in the secretion of nitric oxide (NO) and tumor necrosis factor-alpha (TNF-α).
Influenza B virus infection causes morbidity and mortality worldwide. The nucleoprotein in this virus (BNP) is a major component of the viral ribonucleoprotein (RNP), a protein complex for successful transcription and replication of the virus. Although the crystal structure of BNP is available, the structure and function of the N-terminal of BNP (the first 66 residues, N-66) remain unknown. We discovered that without the presence of N-66, the RNP activity decreased to 50%, showing that the N-66 is crucial to the RNP activities. Experiments were performed to characterize the function of N-66. We identified that there are two functional regions located in N-66. The 1-38 sequence plays an important role in the oligomerization of BNP, and the sequence 44-47 serves as a nuclear localization signal (NLS), which helps BNP to enter the nucleus. Mutating these two functional regions resulted in the loss of RNP activity, showing that these regions are essential in the replication and transcription of influenza B virus.

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Serum metabolomics for the diagnosis and classification of myasthenia gravis

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Myasthenia gravis (MG, 重症肌无力) is an acquired autoimmune disease caused by specific autoantibodies against nicotinic acetylcholine receptors at the neuromuscular junctions. The global incidence rate of MG is about 3-30 cases per million per year. In recent years, the prevalence rate is increasing as a result of increased awareness. However, current diagnostic measures are not conclusive and satisfactory for MG. In the present study, a mass spectrometry-based metabolomic approach was applied to develop a novel measure for the diagnosis and classification of MG with high sensitivity and specificity. Peripheral blood samples from patients 42 MG patients and 16 healthy volunteers were collected. Here, the patients were divided into two groups (early- and late-stages) based on their symptoms to investigate the metabolic differences between MG patients with different grades of MG, instead of traditional five grades, due to the limitation on number of subjects. Sera prepared from the blood samples were monitored by the liquid chromatography Fourier transform mass spectrometry (LC-FTMS). The mass spectral data were analyzed by multivariate statistical analyses, including orthogonal partial least squares (OPLS) and orthogonal partial least squares discriminant analysis (OPLS-DA). By comparing analysis with the healthy volunteers, 142 significant changed ions from serum metabolic profile of MG patients were picked out as the potential biomarkers of MG. The developed OPLS-DA prediction model based on the 142 special ions showed a high sensitivity (92.8%) and specificity (83.3%) in detecting MG. In addition, we found that the changes of serum metabolic profile were different between early and late-stage MG patients. For instance, concentrations of 5, 8-tetradecadienoic acid and cholyglycine were significantly decreased by over 50% in the serum of early-stage MG patients compared with healthy volunteers, in contrast to below 20% in late-stage MG patients. The two metabolites could be considered as the special biomarkers of early-stage MG patients.
Structure-function study of the ligand-binding domain of an Arabidopsis Vacuolar Sorting Receptor

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In plant cells, soluble proteins reach vacuoles because they contain vacuolar sorting determinants (VSDs) that are recognized by vacuolar sorting receptor (VSR) proteins. It is believed that the protease-associated (PA) domain of VSR is involved in sequence-specific recognition of the NPIR motif of cargo-proteins such as aleurain.

However, how VSR proteins recognize their cargo-proteins is still poorly understood. In this study, the PA domain of VSR isoform-1 from Arabidopsis thaliana (AtVSR1-PA) is investigated at both structural and functional levels. We showed that AtVSR1-PA can bind the aleurain peptide in vitro, and have determined the crystal structures of AtVSR1-PA and its complex with peptide ligand. Preliminary structure of AtVSR1-PA reveals that conserved residues of AtVSR1-PA are clustered in one region of the protein. The complex structure showed some conserved residues form a ligand-binding surface for cargo recognition. Moreover, the complex structure showed conformational change upon ligand binding and residues involved in peptide recognition. Site-directed mutagenesis is performed to further confirm the structural determinants for the receptor-cargo interaction both in vitro and in vivo. The rules derived from our studies will be useful in predicting potential cargo-proteins within the genomes of Arabidopsis or other model organisms in the future.
Tumor hypoxia commonly occurs in solid tumors due to abnormal intratumor angiogenesis. Oxygen is the final acceptor of electron released as a result of oxidative phosphorylation. Consequently the synthesis of ATP molecules in tumor cells must rely on glycolysis under hypoxic conditions. To understand how glycolysis becomes up-regulated in tumor cells exposed to hypoxia, the expression of key regulatory enzymes in both the glycolytic and gluconeogenic pathways were examined in the HepG2 and the Hek293 cell lines. The exposure of both tumor cell lines to acute hypoxia for 6 hours resulted in significant increase in cell death in the absence but not the presence of glucose demonstrating an absolute requirement for glucose for survival under hypoxic conditions. Analysis by real time PCR revealed that the mRNA level of the cytoplasmic form of phosphoenolpyruvate carboxykinase 1(PEPCK1) was down-regulated by four and two times respectively in HEK293 cells and HepG2 cells. On the other hand, the mRNA level of hexokinase 2, lactate dehydrogenase A and pyruvate dehydrogenase kinase 1 were up-regulated upon acute hypoxia. Longer term exposure (24hr) of cells to hypoxia resulted in return of PEPCK1 mRNA level to the basal level in HEK293 but not the HepG2 cells. These results suggest that in addition of up-regulation of expression of glycolytic enzymes, the coordinated down-regulation of gluconeogenic enzymes may also be important to ensure a net increase in glycolytic flux for certain tumor cell types to adapt to hypoxia. Gluconeogenesis may thus be a potential therapeutic target for cancer treatment.
Integrative Approach for omic data analysis and gene regulatory network construction

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With the aim to understanding the complex gene regulatory system in various biological processes, a mass of high-throughput omic data have been generated. However, gene regulatory system contains multiple levels, such as transcriptional, post-transcriptional and pre-translational levels. Each omic technology measuring the molecular abundance or behavior at a single level has a limited ability to depict this multiple dimension system. And each data set may produce a large number of false positives. Integrative omic data dissect the gene regulatory system in multi-dimension and reduce the false positives. Thus, we develop ChIP-Array, a web server that integrates ChIP-X and expression data to detect direct and indirect target genes regulated by a TF of interest and to construct the transcription regulatory network. Besides of transcription regulation, post-transcription regulation also needs multiple omic data to improve the analysis, since miRNAs regulate gene expression through translational repression and RNA degradation. We integrate proteomic and mRNA expression data together to infer miRNA-centered regulatory networks using our newly developed web server, ProteoMirExpress. Several published data have been used to assess the quality of our inferred networks and prove the value of our servers. ChIP-Array and ProteoMirExpress are available at http://wanglab.hku.hk/ChIP-Array and http://jjwanglab.org/ProteoMirExpress respectively, with free access to academic users.
Characterization of heterogeneous nuclear ribonucleoprotein C (hnRNP C), a potential telomerase inhibitor, on telomerase function

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Telomeres are repetitive DNA located at the ends of the linear chromosomes for maintaining genomic stability. They undergo shortening after each replication cycle. Telomerase is responsible for the maintenance of the telomere length and its activity is absent in most of the somatic cells, except germ line, stem cells and tumor cells. Thus, it is believed to play a crucial role in both aging and cancer. hnRNP C is a member of the hnRNPs family that has implications in telomere regulation. It has been found that overexpression of hnRNP C can lead to telomere shortening. hnRNP C also can associate directly with the integral RNA template of mammalian telomerase. In our experiment, we identified that hnRNP C can inhibit telomerase activity in vitro. In addition, silencing of hnRNP C resulted in an increase in telomerase activity. The potential inhibitory domain was identified and the interaction between hnRNP C and telomerase catalytic subunit were characterized. It was found that the interaction can be independent of the RNA. These results reveal that hnRNP C has a negative role in regulating the telomerase activity and provide further understanding on its role in the telomere regulation.

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β-Sitosterol (BS) is an active ingredient in Herba Cistanches for protecting against oxidant injury in H9c2 cardiomyocytes and in rat hearts

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Herba Cistanches, the dried whole plant of Cistanche deserticola Y.C. Ma, is a ‘Yang-invigorating’ tonic herb in Chinese medicine. Its beneficial role as an antioxidant has been demonstrated in experimental models of neurological, hepatic and pulmonary disorders. Previous experimental findings showed that HCF1, a semi-purified fraction of Herba Cistanches ethanol extract, induced mitochondrial uncoupling, with a concomitant mitochondrial reactive oxygen species (ROS) generation secondary to enhanced mitochondrial electron transport. The sustained low level of mitochondrial ROS triggered a retrograde upregulation of cellular antioxidant response and protected against hypoxia/reoxygenation-induced apoptosis in H9c2 cardiomyocytes and myocardial ischemia/reperfusion (I/R) injury in rats.

To search for the active ingredient(s), HCF1 was further subjected to high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) analyses. Phytosterols, including BS, stigmasterol, campesterol and brassicasterol, were identified as the major chemical constituents of HCF1. To investigate the possible role of BS in producing pharmacological activities afforded by HCF1, the effects of BS on H9c2 cardiomyocytes and rat hearts were examined. In H9c2 cardiomyocytes, BS induced a retrograde upregulation of cellular glutathione antioxidant response via a mechanism comparable to HCF1, with the effect of HCF1 being more prominent. The protective effect of BS against myocardial I/R injury was also confirmed in female rats ex vivo, with the extent of protection afforded by BS being less than that of HCF1. Consistent with HCF1, the BS-induced cardioprotection was mediated by the enhancement of mitochondrial glutathione redox cycling, possibly through mitochondrial uncoupling. The findings suggested that phytosterols such as BS may be the active principles of HCF1 in protecting against oxidant injury in H9c2 cardiomyocytes and in rat hearts.
Identification and Characterization of Long-range SOX9 Enhancers in Limb Development

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The transcription factor Sox9 is a master regulator of skeletogenesis. Heterozygous mutations of human SOX9 result in Campomelic Dysplasia (CD), in which affected individuals display distinct abnormalities in limbs and other skeletal assemblies. Recently, chromosomal translocations and deletions at >1Mb from SOX9 have been detected in some CD patients, suggesting the requirement of long-range regulatory elements in mediating both spatiotemporal and dosage of Sox9 during limb development. To this end, we exploited several published ChIP-Seq data, and identified nine, evolutionarily conserved, putative limb enhancers of SOX9, namely E1Sox9 to E9Sox9. Transgenic mouse embryos carrying E1Sox9-driven LacZ reporter showed discrete transgene expression at the pre-scapular domain where endogenous Sox9 is also expressed. Bioinformatic analyses on our candidate enhancers result in the identification of several signaling effector binding motifs, and indeed, we revealed that BMP-Smad and Shh-Gli pathways are possible upstream regulatory networks that govern the spatiotemporal and dosage of limb Sox9 expression via our predicted enhancers, respectively. Our results unveil the underlying molecular control in governing the complex patterning of Sox9 expression in the developing limb, and provide new molecular insight to the etiology of CD syndrome.
Structural Characterization of Eukaryotic GTPase Associated Centre

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The elongation cycle of protein synthesis is driven by two elongation factors that bind to overlapping sites at the base of the ribosomal stalk. Both factors have limited inherent GTPase activity and they rely on the GTPase associated centre to activate GTP hydrolysis at appropriate times during elongation. In eukaryotes, this region consists of a 58-base 28S ribosomal RNA, the P0(P1/P2)2 pentameric stalk complex and the stalk base protein eL12. Due to the dynamic nature of the ribosomal stalk, this region remains as a missing piece in the high-resolution structural studies of the eukaryotic ribosome. In this work, we have characterized the structural organization of the stalk complex. We have identified the stabilizing interactions within P1/P2 heterodimer and showed that P1/P2 heterodimer is preferred to P2 homodimer due to its higher conformational stability. We have also identified an exposed hydrophobic patch on helix-3 of P1 that is important for anchoring P1/P2 heterodimers to P0 and we have mapped two spine helices on P0 as the binding sites for P1/P2 heterodimer. Based on homology modelling and mutagenesis experiments, we have proposed a new model of the eukaryotic stalk complex where the two heterodimers display a P2/P1:P1/P2 topology on P0. Our model provides an explanation for the difference of GTPase activities contributed by each P-protein and the functional cooperativity between P1/P2 heterodimers on the two spine helices of P0. Our model represented the stalk complex in an orientation that is the most effective for recruiting translation factors to their binding sites.

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References

Roles of Hippo Signaling in Bone Development

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The Hippo pathway is an evolutionally conserved signaling pathway involved in regulating cell proliferation and organ size control. However, the role of Hippo signaling in endochondral bone formation remains to be elucidated. To investigate the function of Hippo signaling pathway in bone development in vivo, we generated transgenic mice that overexpress Yap under the control of the collagen type II alpha 1 promoter (Col2a1-Yap) and Mst1/2 conditional knockout mice using Dermo1-Cre. We found that the proliferation rate of chondrocytes in the Col2a1-Yap mice significantly increased compared with their WT littermates. In addition, ectopic expression of Yap promoted chondrocyte hypertrophy and ossification of growth plates. To further understand the function of Yap in chondrocyte differentiation process, we performed micromass cultures with cells from Col2a1-Yap embryonic limb bud mesenchyme to mimic the in vivo chondrogenesis in vitro, we found that Yap could promote cartilage nodule formation. Nevertheless, deletion of Mst1/2 impaired the embryo development with delayed angiogenesis and ossification. These findings revealed the primary functions of Hippo signaling in the regulation of skeletal development, and further studies need to be done to illustrate the molecular mechanism.
Optimization of novel anti-HIV compounds to overcome drug resistance of HIV-1

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Human immunodeficiency virus (HIV) is a retrovirus that causes acquired immunodeficiency syndrome (AIDS). Highly active antiretroviral therapy (HAART) is currently the most effective treatment for HIV-1 infection and AIDS, however, the reverse transcription process of viral RNA is highly error-prone, allowing HIV-1 to acquire resistance to all known inhibitors that target viral-specific proteins and develops multidrug-resistance. Therefore, novel anti-retroviral therapies are required.

The process of viral DNA generating mature mRNA is tightly regulated by the specific interactions between cis-regulatory elements on the HIV pre-mRNA and the trans-acting cellular splicing factors serine-arginine rich proteins (SR proteins) and hnRNPs. Cellular functions of SR proteins are tightly governed by the phosphorylation state of their RS domains. One main family of kinases that phosphorylates SR proteins is SR protein kinases (SRPKs), whose member has also been implicated in HIV-1 replication. It has been identified that a docking groove of SRPK1 can interacts strongly with a docking motif and the RS domain of ASF/SF2 (a prototypic SR protein). The interference of this interaction results in aberrant phosphorylation and subcellular localization of ASF/SF2, which influence alternative splicing of HIV-1 mRNA with small side effects because of its high specificity to SRPK1 compared with normal ATP competitive inhibitors.

Therefore, we propose to target the host cell’s splicing machinery in order to interfere the expression of integrated HIV-1 provirus. We have performed structure-based in silico screening to identify inhibitors that bind to the docking groove of SRPK1 and interfere the phosphorylation of ASF/SF2, which in turn inhibit the splicing of HIV-1 mRNA ex vivo. Several candidates were identified and splicing assay and detailed kinetic analysis are underway to determine the inhibiting effects of these new candidates.
The molecular mechanism of histone deacetylase inhibitor PCI-24781-induced cell death in SK-N-DZ neuroblastoma cells

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Neuroblastoma is the second most common solid tumor diagnosed during infancy. The survival rate among children with high-risk neuroblastoma is less than 40%, highlighting the urgent needs for new treatment strategies. PCI-24781 is a novel hydroxamic acid-based histone deacetylase (HDAC) inhibitor that has high efficacy and safety for cancer treatment. However, the underlying mechanisms of PCI-24781 are not clearly elucidated in neuroblastoma cells. In the present study, we demonstrated that PCI-24781 treatment significantly inhibited tumor growth at very low doses in neuroblastoma cells SK-N-DZ, not in normal cell line HS-68. However, PCI-24781 caused the accumulation of acetylated histone H3 both in SK-N-DZ and HS-68 cell line. Treatment of SK-N-DZ with PCI-24781 also induced cell cycle arrest in G2/M phase and activated apoptosis signaling pathways via the up-regulation of DR4, p21, p53 and caspase3. Further proteomic analysis revealed different protein expression profiles between non- treated and PCI-24781 treated SK-N-DZ cells. Total 43 differentially expressed proteins were identified by MALDI-TOF MS system. The functional classification revealed that PCI-24781 affected a series of cellular processes, including cytoskeleton organization, energy metabolism, DNA repair, cell cycle and transcriptional regulation. Western blotting confirmed the expression level of five candidate proteins including prohibitin, hHR23a, RuvBL2, TRAP1 and PDCD6IP. The present results provide a better understanding of the potential mechanism of PCI-24781 in SK-N-DZ cells.
The Genetic and Phenotypic Effects of Coliphage on *E. coli*

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Recent findings of *Escherichia coli* population persisting in the natural environment indicate that *E. coli* can expand its niche from animal hosts to natural environment. Although the mechanism is not clear, studies have shown that environmental *E. coli* populations are genetically distinct from those found in animal hosts, implying selection and evolution may occur in the environment. It is well known that prophages (i.e. DNA of phages residing in the genome of bacterial cells) play a significant role in the genome diversification and niche expansion of pathogenic *E. coli* strains from one host specie to another. However, whether prophages can also facilitate the niche expansion of *E. coli* from animal hosts to the natural environment remains unknown. In our study, a pair of pre- and post-lysogenic fecal *E. coli* strains was created to investigate the impacts of newly-acquired prophage(s) on the survivability of *E. coli* under nutrient-rich and -deprived conditions. Applying molecular methods, the transfer of a P2-like prophage from an environmental *E. coli* strain to the fecal strain was confirmed. Further studies demonstrated that in comparison with the pre-lysogenic one, the post-lysogenic fecal strain has a different nutrient utilization pattern and a better survivorship in seawater and sediment under certain conditions, which implies that the newly-acquired P2-like prophage may help the post-lysogenic strain to cope with environment stresses and affect its metabolic pattern. These findings suggested that the prophage may contribute to the niche expansion of *E. coli* from animal hosts to the natural environment.
Population Genetics of E. coli from Seawater

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Escherichia coli (E. coli) have been widely used as Fecal Indicator Bacteria (FIB) for many years, based on the assumption that it cannot survive in external environments (outside the guts). However, recent research found that E. coli may persist in external environments. In the past, research about persistence of E. coli in external environments mainly focused on its monitoring. In this project, we introduced Multi Locus Sequence Typing (MLST) to analyze population genetics of E. coli from the seawater. Totally 419 E. coli isolates were taken from Eel Pond (EP) and Stony Beach (SB) in Woods Hole, MA, U.S.A, two geographically close but separated sites. EP was surrounded by several septic tanks receiving and releasing waste from the nearby residential areas, while there was no observable fecal emission to the SB. Seven housekeeping genes (adk, fumC, gyrB, icd, mdh, purA, recA) of E. coli were sequenced and the results were processed to population genetics analyses. Preliminary results show different frequency and distribution of sequence type (ST) of E. coli. In both years, E. coli isolates from SB were assigned less STs, but those from EP were far more diverse. Our phylogenetic analysis also shows that ten isolates differed strongly from the main group of isolates, indicating that E. coli in the sampling sites may be more diverse than we have thought. In short, our result will provide knowledge about E. coli population genetics in the seawater, which serves as a fundament to study the mechanism of persistence of E. coli in external environments.
Transcriptional profiling of non-alcoholic steatohepatitis-associated hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is a devastating malignant tumor of global importance. It is the fifth most common cancer and the third leading cause of cancer-related death worldwide. In most cases, HCC is related to cirrhosis or advanced fibrosis resulting from chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections, alcohol abuse, non-alcoholic steatohepatitis (NASH), or congenital diseases like hemochromatosis. Occasionally, liver carcinogenesis can be directly promoted independent of cirrhosis and advanced fibrosis. The dismal outcome in HCC patients is largely due to our limited understanding of tumorigenesis, tumor progression, and metastatic process. In this study, differential expression analysis on genome-wide gene expression data (transcriptome) generated from tumor tissues of 3 patients, who suffered from NASH-associated HCC, with their matched adjacent non-cancerous tissues was performed. The sequence reads were analyzed at the gene level with Burrows-Wheeler Aligner (BWA) followed by ANOVA and also at the transcript isoform level with TopHat followed by Cufflinks. This study will provide a better understanding of the genetic events during tumorigenesis.
Parallel but non-redundant roles of PaxBP1 and Carm1 in promoting the interaction between Pax7 and the H3K4 histone methyltransferase complex

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Pax7 is a key transcription factor that regulates muscle satellite cells. Pax7 activates its downstream target genes via recruitment of the H3K4 histone methyltransferase (HMT) complex. H3K4 tri-methylation is an epigenetic modification that is normally associated with gene activation. Our laboratory previously identified a novel protein PaxBP1 that can directly bind to Pax7 and serve as an adapter between Pax7 and the H3K4 HMT complex. Interestingly, Carm1, a protein arginine methyltransferase, was recently shown to directly methylate Pax7 on specific Arg residues, which promotes the interaction between Pax7 and MLL1/2, the catalytic component of the H3K4 HMT complex. Thus, questions arise regarding the relationships between PaxBP1 and Carm1 in facilitating Pax7 to recruit the H3K4 HMT complex. Here, using both the HMT enzymatic assays and protein binding assays, we demonstrated that both PaxBP1 and Carm1 are required for the Pax7-mediated HMT recruitment. Knockdown of PaxBP1 did not affect the binding of Pax7 with CARM1, while knockdown of Carm1 did not affect the binding of Pax7 with PaxBP1. This suggests that PaxBP1 and Carm1 have parallel but non-redundant roles in facilitating the Pax7-mediated recruitment of HMT. We are conducting more functional assays to further test this model.

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Crosstalk between inorganic polyphosphate and interleukin-11 in osteoblasts

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Inorganic polyphosphate (polyP) is a linear polymer of orthophosphates (Pi) that varies in chain length from less than ten to several hundred residues. PolyP is a ubiquitous molecule in both prokaryotes and eukaryotes and has important physiological functions. Since its discovery in bacteria around 100 years ago, research has mainly been performed on prokaryotic functions with discoveries relating to polyphosphate function in survival and pathogen virulence. At present, the various functions of polyP in eukaryotes, especially in mammalian cells, are being elucidated. PolyP can play important roles in blood coagulation, inflammation, controlling mitochondrial calcium level thus affecting cell apoptosis and bone and cartilage formation. PolyP has been known to have relatively high concentrations in osteoblasts but the mechanisms for its action have been unknown. Here, we investigate the roles of polyP in signaling in cell culture studies of osteoblasts. We find polyP has significant influence on human-osteoblast like SaoS-2 cell proliferation and migration. Microarray studies showed a number of genes up and down-regulated in response to polyP. In particular, we investigated interleukin-11 in detail, and showed that interleukin-11 is specifically upregulated at both RNA and protein levels in response to polyP, but not in response to orthophosphate, pyrophosphate or triphosphate, indicating specificity for long-chain polyphosphate. Work is ongoing to unravel the crosstalk in signaling between inorganic polyphosphate and interleukin-11.
Neural-specific elimination of *Mab21l2* results in respiratory failure in mouse neonates

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The patterning of vertebrate central nervous system depends on specific cell fate determination and generation of specific neuronal subtypes. We focus in this study the role of an evolutionary conserved gene, *Mab21l2*, in this process. *mab-21* was first identified in *C. elegans* and orthologues, *Mab21l1* and *Mab21l2*, were isolated in vertebrates subsequently. *Mab21l2* depletion was shown to result in embryonic defects in eyes induction, ventral body wall closure and embryonic lethality. Over-expression of *Mab21l2* in P19 embryonic carcinoma cells potentiate neuronal differentiation upon retinoic acid induction. Extensive neurite outgrowths and increased expression of axonal marker *Mapt* was observed.

We have generated a nervous system-specific *Mab21l2* knock-out using the loxP-Cre technology. These conditional knock-out mice die shortly after birth, symptomatically due to failure of breath initiation. Postmortem lungs examination never revealed dilated lung alveoli in mutant mice despite a normal pattern of diaphragmatic innervation and neuromuscular junction formation. Hence, we believe that the “no breathing” phenotype is due to a defect in the central control for respiration. Many respiratory nuclei are derived from hindbrain where *Mab21l2* is also expressed. Detailed expression pattern of *Mab21l2* in hindbrain at different development stages are documented and follow-up work on the physiological function of respiratory center will be evaluated to establish the cause of this death phenotype. (The study is supported by the Research Grants Council, Hong Kong)
Protective effects of ursolic acid-enriched Herba Cynomorii extract against carbon tetrachloride hepatotoxicity and gentamicin nephrotoxicity in rats

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Herba Cynomorii, which is an herbal tonic for treating ‘kidney deficiency’ in Chinese medicine, serves as a popular health-promoting or invigorating food in China, particularly in Inner Mongolia Province. Recent studies showed that an ursolic acid-enriched fraction (HCY2) isolated from Herba Cynomorii ethanol extract protected against myocardial ischemia/reperfusion injury in rats. In the present study, we further investigated the effects of HCY2 on carbon tetrachloride (CCl4) hepatotoxicity and gentamicin nephrotoxicity in rats. The results indicated that HCY2 pretreatment protected against CCl4 hepatotoxicity and gentamicin nephrotoxicity, as evidenced by the significant inhibition of plasma aspartate aminotransferase and alanine aminotransferases activities as well as decreases in plasma creatinine and blood urea nitrogen levels, respectively. The hepatoprotection and nephroprotection were associated with the improvement in mitochondrial functional ability, as assessed by the measurement of ATP generation capacity, as well as the enhancement in glutathione redox status, possibly through the induction of mitochondrial uncoupling in rat liver and kidney tissues.
Characterization of effects of GULP1 on human amyloid precursor protein (APP) processing in a *Drosophila* AD model

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Alzheimer’s disease (AD) is the most common age-dependent neurodegenerative disorder, affecting 50% of the population aged over 85. It is characterized by accumulation of amyloid-β (Aβ), which is derived from amyloid precursor protein (APP), in senile plaques. Several phosphotyrosine binding (PTB) domain containing proteins have been found to interact with the NPTY-motif within APP intracellular domain (AICD) and affect Aβ production. Recently, we and others have identified that engulfment adaptor protein 1 (GULP1) is a novel AICD interacting protein. It has been demonstrated the PTB domain of GULP1 interacts with AICD, and such interaction alters APP processing. In this study, we generated *Drosophila* fly lines overexpressing GULP1, and crossed the lines with a transgenic *Drosophila* AD model. Biochemical analysis indicated that overexpression of GULP1 reduces APP processing in *Drosophila*. Pseudopupil assay revealed that GULP1 protects photoreceptor neurons from degeneration in the AD model. Additionally, the climbing ability and longevity of the flies are increased by GULP1. Thus, we demonstrate the *in vivo* protective effect of GULP1 in the *Drosophila* AD model.
Alzheimer’s disease (AD) is a progressive neurodegenerative disease affecting 36 million people worldwide. Genetic and biochemical research indicated that the excessive generation of amyloid-β peptide (Aβ) from its precursor protein, amyloid precursor protein (APP), is critical to AD pathology. APP is a type I transmembrane protein with a large extracellular domain. When processed sequentially by β- and γ-secretases, the toxic Aβ fragment would be liberated. To date, it is believed that a number of APP-interacting proteins are involved in the modulation of APP processing. Of these, FE65, a brain-enriched adaptor protein, is a well-known example. However, the mechanism by which APP-FE65 interaction is regulated remains unclear. In the study, we evaluated the effect of a reported phosphorylation site of FE65, Serine-610, on APP-FE65 interaction and APP processing. Here, we demonstrated that APP intracellular domain only binds to the FE65 dephosphomimetic mutant S610A but not the phosphomimetic mutant S610D. Consistent with this finding, immunofluorescence staining showed that only FE65 S610A colocalizes with APP in the perinuclear region, while FE65 S610D retains in the nucleus. We also examined the effect of FE65 S610 phosphorylation on APP processing. Our data indicates that both FE65 and FE65 S610A could enhance APP β- and γ-cleavage and Aβ generation, while FE65 S610D is unable to stimulate APP processing. The current findings suggest that phosphorylation of FE65 S610 regulates APP processing. Further investigation is necessary to unravel the molecular mechanism that governs the phosphorylation of FE65 S610.
The use of aptamer-conjugated nanoparticles in novel malaria diagnosis platforms

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The on-going battle against malaria not only requires novel therapies to combat emerging resistant strains, but also requires effective point of care diagnosis so that such therapies can be appropriately administered, especially in underdeveloped regions where the disease is most prevalent. Current point-of-care rapid diagnostics rely on antibody-based technologies which have drawbacks of cost and stability. Aptamers are oligonucleotide sequences which are developed to specifically bind desired targets, essentially DNA-based antibodies. The stability and cheap manufacture cost of short DNA sequences make aptamers a viable alternative to antibodies in malaria diagnosis (aptamers are usually < 50 bases in length). The common malaria antigens which are detected by most malaria diagnosis blood tests are histidine-rich protein 2 (HRP2) and \textit{Plasmodium falciparum} lactose dehydrogenase (PvLDH). Aptamers for these antigens have been developed, characterised and found to bind with nanomolar affinities. Conjugation of these aptamers to gold and silver nanoparticles has allowed for visual detection of HRP2 and PvLDH in solution (owing to the visual properties of metal nanoparticles) and the next step of the project is to incorporate such aptamer-functionalised nanoparticles into a paper-based lateral flow devices for future sensitive, cheap and easy-to-use malaria diagnostics.
Measles Virus defective interfering RNA of copy-back type as a potent activator of interferon production

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Measles virus (MV) in the family of Paramyxoviridae has a non-segmented negative-stranded RNA genome. MV is highly contagious, and brings about significant morbidity and mortality worldwide. Since the introduction of an effective vaccine derived from live attenuated MV in 1963, the disease has been nearly eliminated in developed countries. When compared to wild type strains, vaccine strains of MV enhance type I interferon production to a far greater extent in infected cells. Investigating immunogenic component of MV vaccine is of great interest, because it may derive insights to generation of novel and chemically defined adjuvants that can boost the immunogenicity of vaccine antigens. Here we identify copy-back type defective interfering RNA of MV (cb-DI-RNA-mv) to be a potent activator of type I interferon production. In particular, cb-DI-RNA-mv interacts with RIG-I and dsRNA binding protein PACT. Meanwhile, it was found that abundant production of cb-DI-RNA-mv can be seen when VERO-CD150 is challenged with Hu-191 vaccine strain of MV. Thus, the immunogenic character of attenuated MV is ascribed to cb-DI-RNA-mv. Our work not only suggests a mechanism by which live attenuated MV vaccines activate innate immunity, but may also bring insights on the rational design of novel and chemically defined adjuvants.
Herpes simplex virus type 1-encoded interferon-antagonizing protein Us11 suppresses PACT-dependent activation of RIG-I activity

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Herpes simplex virus type 1 (HSV-1) is a common human pathogen. Type I interferons (IFNs) which restrict viral replication are induced by HSV-1 infection. To ensure its successful replication and persistent infection, HSV-1 encodes several IFN-antagonizing proteins such as Us11. Us11 is a double-stranded RNA (dsRNA)-binding protein which is known to associate with multiple cellular proteins such as PKR, RIG-I and PACT. RIG-I is a cytosolic sensor of HSV-1. Optimal activity of RIG-I requires PACT. PACT is a cellular dsRNA-binding protein. Although Us11 is known to interact with PACT but the importance of this interaction in HSV-1 biology remains to be elucidated. Us11 is thought to suppress type I IFN production by directly interacting with RIG-I. However, it is unclear whether the interaction between Us11 and PACT might also be influential in the perturbation of RIG-I-dependent IFN production. In this study, we demonstrated the role of PACT in Us11-mediated suppression of IFN induction.

PACT is a novel target of HSV-1 IFN-antagonizing protein Us11. PACT-mediated RIG-I activation was blunted by Us11. In HSV-1 infected cells, PACT and Us11 exist in the same fractions of protein lysate. More importantly, the increased production of IFN-β during the infection of PACT-competent cells with Us11-deleted HSV-1 recombinant virus was not observed in infected PACT-compromised cells. Our findings reveal a new mechanism of IFN antagonism by HSV-1 and a new target of viral IFN-antagonizing proteins.

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The Genetics of SELEX

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SELEX is a robust technique for identifying nucleic acid aptamers with selectable characteristics. SELEX is a combinatorially tedious exhaustive search system, typically involving up to 1010 possible aptamers. Fully understanding functional relationship(s) between sequence and binding for each SELEX round would be prohibited by the sheer quantity of information. This leaves somewhat of an unknown as to the specific interactions that take place during a particular SELEX round, what the optimal conditions for aptamer evolution are, and additionally how SELEX could be improved upon. Here, using VB.net we have designed and scripted a computer program, “AptSim”, which simulates aptamer mutation, recombination and selection. AptSim demonstrates the value of an extended SELEX approach that exploits both mutation and recombination. It can also be used to find optimal strategies for maximising the rate of evolution, sometimes dramatically reducing the size, time span and/or cost of developing effective aptamers. Using AptSim, optimisation of SELEX experiments can be performed with increased speed and reduced cost when compared to a purely wet lab approach. In addition it may be possible to evaluate theoretical advancements to the SELEX technique which could lead to improvement of ligand evolution.
Cotranscriptional recruitment of yeast TRAMP complex to intronic sequences promotes optimal pre-mRNA splicing

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Yeast Trf4/5p-Air1/2p-Mtr4p polyadenylation (TRAMP) complex recognizes spliced-out introns and then targets them for nuclear-exosome-mediated degradation. However, it remains unclear that how and when these spliced-out introns are recognized by TRAMP. In this study, we demonstrated that both TRAMP and the nuclear exosome component Rrp6p are cotranscriptionally recruited to nascent transcripts, particularly to intronic sequences. Deletion of TRF4, both AIR1 and AIR2, or RRP6, resulted in accumulation of unspliced pre-mRNAs. Surprisingly, while such pre-mRNAs accumulated in rrp6 cells owing to defects in pre-mRNA degradation, the same phenotype in trf4 and air1 air2 cells involved splicing defects. We also uncovered genetic and physical interactions between Trf4p and several splicing factors, and that Trf4p is required for optimal recruitment of the splicing factor Ms15p to intronic regions. We conclude that in addition to its canonical role in spliced-out intron recognition, the cotranscriptional recruitment of TRAMP also directly stimulates pre-mRNA splicing by enhancing the recruitment of Ms15p, and probably other splicing factors. This may serve as a fail-safe mechanism to ensure that pre-mRNA splicing only proceeds at the optimal efficiency after the cotranscriptional recruitment of TRAMP to intronic sequences, such that the potentially harmful spliced-out introns can be recognized by TRAMP and designated to subsequent nuclear-exosome-mediated degradation immediately after their production.
Nervous system development is a complex process that involves precise control of the development and organization for various neuronal cell types. While different types of the transcription factors have been implicated in various roles in neurogenesis, the precise roles of different forkhead domain containing transcription factors during neuronal development are yet to be understood. Using zebrafish as a genetic model, here we have shown that foxk2, a new member of the “K” subfamily of the forkhead factors, regulates neuronal survival during early embryogenesis. foxk2 is a maternal transcript and it is expressed at all stages of zebrafish development. foxk2 is expressed strongly in the central nervous system (CNS), suggesting it may play a critical role in CNS development. foxk2 knockdown in zebrafish embryos by antisense morpholino resulted in severe apoptosis in CNS and defective neuronal development. The apoptotic phenotype of the foxk2 morphant can be partially rescued by simultaneous knockdown of the tumor suppressor p53, indicating that the loss of foxk2 could induce both p53 dependent and p53 independent apoptotic pathway. Our data suggest that foxk2 may play an important role in maintaining neuronal cell survival and inhibiting apoptosis in-vivo.
Investigating the Protective Role of FOXM1 against DNA Damage in Human Embryonic Stem Cells under Oxidative Stress

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Human embryonic stem cells (hESCs) possess two unique characteristics, indefinite self-renewal and the capacity to differentiate into cell types of all three germ layers. They are derived from the inner cell mass of blastocysts and hold immense potential for tissue engineering and regenerative medicine. For hESCs to function properly, it is hypothesized that they are endowed with stringent mechanisms to maintain genome integrity. The Forkhead box (FOX) transcription factor FOXM1, also known as TRIDENT, HFH-11, WIN or INS-1, is ubiquitously expressed in embryonic tissues that are highly proliferating and regenerative. Recent studies have clearly shown that FOXM1 plays important roles as mediator of the DNA damage response and to protect against cellular senescence. In human primary fibroblasts, FOXM1 regulates the expression of antioxidant enzymes, such as MnSOD and catalase, which defend against reactive oxygen species (ROS) intracellularly. Moreover, DNA repair genes like BRIP and RAD51 have recently been shown to be the transcriptional targets of FOXM1. We hypothesized that FOXM1 is a critical regulator in hESCs to protect against oxidative stress and DNA damage. In this study, FOXM1 was shown to be expressed at high levels in the hESC cell line Val3. We demonstrated that FOXM1 is stabilized when hESCs is subjected to treatment with hydrogen peroxide, suggesting that FOXM1 may play a role in the regulation of hESC genome stability. Further studies will be conducted to explore the transcriptional regulation by FOXM1 in hESCs after oxidative stress-induced DNA damage.
Application of OBOC Peptide Library in Screening Mimotopes for Treatment of Shellfish Allergy

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Shellfish is one of the “Big Eight” categories of food allergens. The incidence and prevalence of shellfish allergy have increased significantly over the last few decades. In Hong Kong, shellfish is the leading cause for food hypersensitivity in both adults and children.

Shellfish allergy is a classical type I hypersensitivity response mediated by IgE antibodies. The allergic reactions are initiated by the cross-linking of IgE on mast cells or basophils upon repeated exposure to the same allergen. To date, no permanent cure for shellfish allergy is available and strict avoidance is the only way to prevent allergic reactions. We hypothesize that IgE mediated hypersensitivity to shellfish can be prevented by mimotope-mediated immunotherapy. Mimotopes are short peptides resembling the structure of an epitope and have the ability to induce IgG as “blocking antibodies” against the allergens.

In this study, we have applied a combination of combinatorial peptide chemistry, immunology and recombinant DNA technology to construct mimotope vaccines against shellfish allergy. Mimotopes were obtained by screening one-bead-one-compound (OBOC) combinatorial peptide libraries using sera samples from shellfish allergy patients with a significant level of specific IgE. Mimotopes were cloned and expressed in expression plasmids and analyzed for their capacity to induce tropomyosin specific IgG antibodies in a well-established mouse model of shrimp hypersensitivity. Our pilot data showed that mice immunized with mimotope plasmids produced IgG recognizing tropomyosin. Current works are in progress to further evaluate the efficacy of mimotope-based DNA vaccines in animals and potential clinical applications in IgE mediated hypersensitivity.

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Tryptanthrin Inhibits Angiogenesis Both in vitro and in vivo by Inhibiting VEGFR2-mediated ERK1/2 Signaling Pathway

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Tumor angiogenesis is the key step involved in tumor growth and metastasis and anti-angiogenesis has been proposed as an important strategy for cancer therapy. Tryptanthrin (12-dihydro-6, 12-dioxoindolo-(2, 1-b)-quinazoline) is a weakly basic alkaloid isolated from the dried roots of medicinal indigo plants known as Banlangen and has been shown to possess anti-tumor activities. However, the anti-angiogenic activity of tryptanthrin and the molecular mechanisms have not been investigated. Therefore, the objective of this study is to investigate the anti-angiogenic effects of tryptanthrin and the underlying mechanisms. Our results showed that tryptanthrin dose-dependently inhibited human microvascular endothelial cells proliferation, migration, and tube formation in vitro. Moreover, tryptanthrin significantly suppressed angiogenesis in Matrigel plugs in mice. Mechanistic studies indicated that tryptanthrin reduced the expression of several angiogenic factors (Ang-1, PDGFB and MMP2). Tryptanthrin bond to the ATP binding site of VEGFR2 and suppressed VEGFR2-mediated ERK1/2 signaling pathway in human endothelial cells. Collectively, the current study demonstrated that tryptanthrin exhibited anti-angiogenic activity both in vitro and in vivo by specifically targeting VEGFR2-mediated ERK1/2 signaling pathway and might be exploited as a potential therapeutic candidate for the treatment of angiogenesis related diseases.
Study of *Espn* in dendritic development of Purkinje cells in mouse cerebellum

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Purkinje cell, an important cerebellar neuronal cell type, has extensively branched dendrites to receive signals from other cerebellar neurons. It helps integrating the signals and plays a role as the sole efferent neuron of cerebellar cortex. Due to its pivotal role, any defects in its dendritic development will impair the cerebellar function and lead to motor disability.

*Espn* encodes for espin, a calcium-insensitive F-actin bundling protein which is specifically expressed in Purkinje cells of cerebellum. From our lab previous study, we have found that *Espn* was downregulated in conditional *Lhx1/5*-null mutant mice which showed motor defects with abnormal dendritic development in Purkinje cell during adulthood. With the fact that F-actin is an essential cytoskeleton underlying the dendritic morphology, we expect that *Espn* can regulate dendritic development of Purkinje cells in postnatal mouse cerebellum.

In dissociation culture of Purkinje cells, we found that the expression level of espin, together with F-actin level increased significantly in Purkinje cells after entering later phase of dendritic development. To further investigate the function of *Espn* in Purkinje cells, we down-regulated *Espn* in organotypic cerebellar slice culture by siRNA-mediated gene-specific knockdown. The down-regulation of *Espn* disrupted the normal establishment of dendritic arborization in Purkinje cells, causing a reduction in branching in the distal compartment of dendritic tree. The results implied that *Espn* was essential for postnatal dendritogenesis of Purkinje cells. In the future, we will further investigate the cellular and molecular mechanism regulated by espin in the dendritic development of Purkinje cells.
The double-stranded RNA-binding protein PACT is a cellular activator of cytoplasmic viral sensor MDA5 in innate antiviral immune response

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Innate immunity is an evolutionarily conserved first-line defense mechanism against pathogen infection. Detection of pathogen-associated molecular patterns (PAMPs) by specific host pattern recognition receptors (PRRs) is required to elicit an efficient innate immune response. Among different types of PRRs, RIG-I-like receptors (RLRs) are a family of ubiquitously-expressed cytoplasmic viral sensors which detect the presence of viral double-stranded RNA (dsRNA) during the course of virus infection. RIG-I and MDA5 are members of this family. Although dsRNA is the prototypic ligand for RLRs, recent publications support the view that accessory host proteins may also serve as the co-activator of RLRs. Here we reported that a double-stranded RNA-binding protein PACT is a cellular activator of MDA5 in innate antiviral immune response. We demonstrated that endogenous PACT and MDA5 from the whole cell lysate can associate with dsRNA analog polyinosine-polycytidylic acid (poly(I:C)) and both proteins are physically associated with each other. Functional analysis also revealed that PACT augments MDA5-mediated interferon signaling pathway in response to poly(I:C) induction, whereas depletion of endogenous PACT by siRNA dampens interferon production in response to virus infection. Collectively, our results support the model that PACT functions as a cellular activator of RLRs, plausibly at the ligand recognition level and amplifies the activation signal triggered by dsRNA in innate antiviral immune response.

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The role of Sox10 in enteric neural crest cell migration

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Sox10 is a transcription factor required for enteric neural crest cells to colonize the gut during development. Sox10 functions to maintain enteric neural cell survival and multipotency. We have shown that Sox10 mutant enteric neural crest cells have abnormal migration trajectories with reduced migration speed and persistence in ex vivo gut culture, therefore we hypothesize that Sox10 is also required for enteric neural crest cell migration, possibly through regulating neural crest cell-neural crest cell or neural crest-extracellular matrix interactions. Here we aim to characterize how Sox10 influences the interaction between enteric neural crest cells and extracellular matrix molecules.

We examined the behavior of enteric neural crest cells on ECM molecules by 1-day ex vivo gut explant culture of E12.5 embryonic gut. Sox10NGFP/+ enteric neural crest cells were less dispersed and formed clusters on fibronectin and laminin substratum, indicating Sox10NGFP/+ enteric neural crest cells were unable to interact with extracellular matrix molecules in a normal manner. Analysis of time-lapse videos capturing cell movements for 6 hours showed a significant reduction of speed and displacement in Sox10NGFP/+ enteric neural crest cells. We also showed by western blot analysis that pFAK397 was reduced in Sox10NGFP/+ guts, implying a role of Sox10 in regulating enteric neural crest cell motility.

Altogether our findings point to a role of Sox10 in regulating enteric neural crest cell migration by modulating the interactions between enteric neural crest cells and extracellular matrix molecules.
Serine arginine rich protein (SR protein) is an essential family of splicing factors that involves in the regulation of both constitutive and alternative pre-mRNA splicing. Different phosphorylation states of the C-terminal serine/arginine rich domain (RS domains) of the SR proteins govern their subcellular localization as well as their recruitment to the site of splicing and functions during spliceosome assembly. The phosphorylation of SR proteins is predominantly regulated by two protein kinase families, the SR protein kinases (SRPKs) and the Cdc2-like kinase/Ser-Thr-Tyr (Clk/Sty) kinases.

Extensive investigations have been done on how ASF/SF2, a prototypic SR protein that contains two RNA recognition motifs (RRMs), is regulated by SRPK1. However, little information on the regulation of other SR proteins is available. Serine/arginine-rich splicing factor 3 (SRSF3), formerly known as SRp20, belongs to a subclass of serine/arginine rich proteins that contains only a single RRM followed by the C-terminal RS domain. The absence of the second RRM, which is important for the interaction between ASF/SF2 and SRPK1, suggests the mechanisms of recognition and binding of SRSF3 by SRPKs are likely to be different. In the present project, we aim to investigate the mechanisms of interaction as well as the phosphorylation of SRSF3 by SRPK2, another member of the SRPKs family which specificity and functions are distinct from SRPK1.

Our works demonstrated that a conserved docking groove in the C-lobe of SRPK2, as well as residues between Arg86 to Ser128 in the RS domain of SRSF3 are essential for the interaction between the two proteins. Localization studies reveal that the RS domain of SRSF3 regulates its subcellular localization in the nucleus. Furthermore, unlike ASF/SF2, which only the N-terminal region of RS domain can be phosphorylated by the SRPKs, the entire RS domain of SRSF3 can be phosphorylated by SRPK2 at 10-12 sites from N to C-terminal. We further showed that half of these sites are phosphorylated in a processive manner. These results reveal that SRPKs adopt different mechanisms in the phosphorylation of the two subclasses of SR proteins.
Characterization of B-cell and T-cell Epitopes of the Major Shrimp Allergen *Metapenaeus ensis* Tropomyosin Met e 1

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Shellfish allergy is a type I hypersensitivity disorder and is characterized by the presence of shellfish specific IgE antibodies. In Hong Kong, shellfish represents the top leading cause of adverse food reactions with a prevalence of 37.8% among children and adolescents. Although tropomyosin has long been identified as the major shellfish allergen and its cDNA cloned, the molecular understanding of the B and T cell epitopes of tropomyosin remains elusive.

To define the major IgE-binding epitopes of the shrimp tropomyosin Met e 1, serum samples from shrimp allergy subjects and Met e 1-sensitized BALB/c mice were analyzed against a panel of 18 overlapping synthetic peptides of Met e 1 in ELISA and dot-immunoblotting. Three online computational models were also employed for the prediction of IgE-binding regions. Major T cell epitopes were defined through T cell proliferation assay using splenocytes isolated from Met e 1-sensitized mice. Met e 1 was delivered through the intraperitoneal or intragastric route for the comparison of epitope profiles between different sensitization regimens.

The combination of data from ELISA, dot-immunoblotting and computational models defined nine regions on Met e 1 as the major IgE-binding epitopes in shrimp allergy patients. Similar IgE epitope profiles were shared between human and BALB/c mice, with greater epitope diversity exhibited in mice. Four dominant T cell epitopes of Met e 1 were identified in both intraperitoneally and intragastrically-sensitized mice. These data provide significant groundwork for designing allergen specific immunotherapy in shrimp allergy and holds potential applications in treatment modalities for shellfish allergy.

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Multiple myeloma (MM) is an incurable neoplasm of terminally differentiated B cells and accounts for 2% of all cancer deaths. Overexpression of cellular muscular aponeurotic fibrosarcoma (c-Maf) is found in 50% of myeloma cells, suggesting c-Maf plays an important role in multiple myeloma development. C-Maf belongs to the Maf transcription factor family. Genes regulated by c-Maf, including IL-4, cyclin D2, β-integrin 7 are tissue specific and important in the pathogenesis of many human cancers. Post-translational modification of c-Maf, such as phosphorylation, ubiquitination and SUMOylation can modulate the transcription activity of c-Maf. Recently, accumulating studies have demonstrated that SUMO conjugation to c-Maf at Lysine 33 contributes to immune deviation in nonobese diabetic mice by suppressing c-Maf transactivation of IL-4. However, the molecular basis and biological role of c-Maf SUMOylation in multiple myeloma and its association with the other post-translational modification are still unknown. In this study, we have purified c-Maf and c-Maf K33R mutant. In a combination of in vitro SUMOylation, immunoblotting and mass spectrometry analysis using the wild type and K33R c-Maf constructs, our results suggested that there is more than one SUMOylation site on c-Maf. Furthermore, modification of K33 may affect the attachment of SUMO on K283 and K350. While also, we have tried the crystallization of c-Maf and its various truncated fragments, with or without c-maf specific DNA oligonucleotides for structural study. Recently, HERC4 has been shown to be a potential ubiquitin E3 ligase targeting c-Maf. Interaction and structural study of c-Maf and HERC4 has been carried out.
The roles of *Irx3* and *Irx5* in mammalian inner ear development

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Iroquois genes encode a family of transcription factors containing TALE class homeodomain. They are regarded as prepatterning genes in Drosophila sensory organ development. There are six members (*Irxi~Irxs*) of *Iroquois* genes in mouse and human. *Irx3* and *Irx5* are linked genes on mouse chromosome 8, which are involved in many mammalian developmental processes. However, the roles of *Irx3* and *Irx5* in mammalian hearing loss are poorly understood. To identify the function of these two genes in inner ear development, we have investigated two reporter knock-in mouse mutants: *Irx3*<sup>lacZ</sup>, *Irx5*<sup>EGFP</sup>, and a double knock-out mutant: *Irx3/5<sup>−/−</sup>*. *Irx3* and *Irx5* have overlapping expression domains in the developing inner ear. Physiological tests indicated that the *Irx3*<sup>lacZ</sup> and *Irx5*<sup>EGFP</sup> mutant mice displayed hearing defect, while *Irx3/5<sup>−/−</sup>* mice were embryonic lethal. Although paint filling analysis showed the normal cochlea morphology of *Irx3*<sup>lacZ</sup> and *Irx5*<sup>EGFP</sup> mutant mice, ectopic inner hair cells have been discovered in the organ of Corti. Interestingly, the cochlear duct of *Irx3/5<sup>−/−</sup>* mice was enlarged and shortened, and the basal part of the cochlea was fused with the saccule. There were also numerous vestibular-like ectopic hair cells surrounded by ectopic Sox2-positive cells in the greater epithelial ridge of cochlea. The organ of Corti was malformed with neither hair cell differentiation nor supporting cell differentiation at E16.5. In summary, our results indicate that *Irx3* and *Irx5* cooperatively pattern the boundary between the vestibule and the cochlea and they are important for the cochlear sensory neural cell specification.
Structure of UreG/UreF/UreH complex reveals how urease accessory proteins facilitate maturation of Helicobacter pylori urease

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Urease is a metalloenzyme essential for the survival of Helicobacter pylori in acidic gastric environment. Maturation of urease involves carboxymethylation of lysine-219 and insertion of two nickel ions at its active site. This process requires GTP hydrolysis and the formation of a preactivation complex consisting of apo-urease and urease accessory proteins UreF, UreH, and UreG. UreF and UreH form a complex to recruit UreG, which is a SIMIBI class GTPase, to the preactivation complex. We have determined the crystal structure of the UreG/UreF/UreH complex, which illustrates how UreF and UreH facilitate dimerization of UreG, and assembles its nickel binding site by juxtaposing two invariant Cys66-Pro67-His68 nickel binding motif at the interface to form the (UreG/UreF/UreH)2 complex. Interaction studies revealed that addition of nickel and GTP to the UreG/UreF/UreH complex releases a UreG dimer that binds a nickel ion at the dimeric interface. Substitution of Cys66 and His68 with alanine abolishes the formation of the nickel-charged UreG dimer. This nickel-charged UreG dimer can activate urease in vitro in the presence of the UreF/UreH complex. Static light scattering and atomic absorption spectroscopy measurements demonstrated that the nickel-charged UreG dimer, upon GTP hydrolysis, reverts to its monomeric form and releases nickel to urease. Based on our results, we propose a mechanism on how urease accessory proteins facilitate maturation of urease.
Kinesin-1 is involved in chondrocytes adhesion to extracellular matrix and motility

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Intercalation movement of proliferative chondrocytes is crucial for their columnar organization which is essential for proper function of growth plate cartilage. The conventional motor protein kinesin-1 directionally transporting various cargos along microtubules might be involved in this polarized cell movement. Kinesin-1 is suggested to transport unknown cargo(s) modulating focal adhesion (FA) turnover which is a key step in cell movement. To investigate kinesin-1’s role in chondrocytes intercalation, we generate kinesin-1 heavy chain (Kif5b) knockout mouse. In the growth plate of KIF5B deficient mouse, we observed abnormal cell morphology and disrupted columnar structure. Isolated mutant chondrocytes show reduced motility and adhesion ability to ECM proteins. Vinculin, the key regulator of focal adhesions, is found as a potential protein associated with KIF5B in mouse chondrocytes. Further study will investigate whether KIF5B affects chondrocytes motility and adhesion via FAs modulation.
Transmembrane and Coiled-Coil Domain Family 1 Is a Novel Protein of the Endoplasmic Reticulum

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The endoplasmic reticulum (ER) is a continuous membrane network in eukaryotic cells composed of the nuclear envelope, the rough ER and the smooth ER. ER performs many important functions and has a characteristic structure. The putative protein transmembrane and coiled-coil domain family 1 (TMCC1) belongs to the TMCC family. In human, this family includes at least three members (TMCC1, 2 and 3), all of which containing both coiled-coil domains and transmembrane domains, and TMCC proteins share high similarity of protein sequences. TMCC1 protein is also conserved in different organisms from nematode to human. In this work, we find that TMCC1 is ubiquitously expressed in different kinds of human cells. We also show that TMCC1 localizes to the rough ER through the C-terminal transmembrane domains, and its N-terminal region resides in the cytoplasm. Through the large coiled-coil domain, TMCC1 may form oligomer with TMCC proteins. These results suggest that TMCC1 may play a role in protein recruitment to the ER membrane, regulation of local membrane motility, and intermembrane connection.
The role of Stat3 in muscle satellite cells in vivo

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Signal transducer and activator of transcription factor 3 (STAT3), a member of the STAT transcription factor family. Previous work in our laboratory showed that STAT3 is expressed in myoblasts and regulates myoblast proliferation and differentiation in an in vitro cell culture model. However, it remains unclear whether STAT3 regulates muscle satellite cells in vivo. In this study, we took advantage of the Cre/loxP system and deleted STAT3 specifically in Pax7-positive muscle precursor cells. By further interbreeding the STAT3 conditional knock-out mice (cKO) with the mdx mice (i.e., the mouse model for the Duchenne muscular dystrophy), we also obtained a Stat3-/-:mdx double knock-out (dKO) mouse line. Although the satellite cell number in the cKO mice did not seem to greatly differ from that of the wild type controls, the regeneration potential of cKO mice was impaired and the size of the regenerating muscle fibers was reduced in a cardiotoxin-induced muscle regeneration model. Moreover, the adult dKO mice displayed reduced body size and muscle weight, increased fibrosis, and increased infiltration of inflammatory cells. We also found that Stat3-/- myoblasts had accelerated differentiation potential. In addition, there was decreased Pax7 expression in Stat3-/- muscle satellite cells. So far, our work has demonstrated that STAT3 is involved in regulating muscle satellite cells in vivo in a cell-autonomous manner. We are now planning to perform both Stat3 ChIP-sequencing and RNA-sequencing experiments using Pax7-positive muscle satellite cells in order to understand the detailed molecular mechanisms by which STAT3 exert its functions in muscle satellite cells.