

Biochemical basis of the tumour suppressor function of CSK-homologous kinase

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Since aberrant activation of Src-family of protein tyrosine kinases (SFKs) contributes to pathogenesis and disease progression of many types of cancer, they are the targets of therapeutic intervention for cancer treatment. Despite the effort made by many researchers, specific small-molecule SFK inhibitors suitable for therapeutic use are still unavailable.

SFKs are rarely mutated in human cancers. Abnormalities in their regulation account for their constitutive activation in cancer cells. SFKs in normal cells are tightly controlled by two endogenous inhibitors – the C-terminal Src kinase (CSK) and its homologous kinase, CSK-homologous kinase (CHK). My colleagues and I have been studying how abnormal regulation of these two inhibitors allows aberrant activation of SFKs in colon carcinoma and chronic myelogenous leukaemia (CML). In this seminar, I will present evidence demonstrating CHK as a tumour suppressor in these two types of cancer – its expression is suppressed in colon cancer and CML cells; expression of recombinant CHK inhibits SFKs and in turn induces growth arrest or apoptosis of these cancer cells. Biochemical analysis revealed two mechanisms employed by CHK to inhibit SFKs: (i) specific phosphorylation of the conserved C-terminal regulatory tyrosine of SFKs, and (ii) a unique non-catalytic mechanism involving directing binding of CHK to SFKs to form stable CHK-SFK complexes. More importantly, CHK can employ this non-catalytic inhibitory mechanism to inhibit all active forms of SFKs.

Since CHK is a specific endogenous inhibitor of SFKs, small-molecule compounds mimicking its non-catalytic mechanism to inhibit SFKs are potential therapeutics for the treatment of cancer. In this seminar, I will discuss how our ongoing attempt to define the structural basis of this non-catalytic inhibitory mechanism of CHK can benefit development of these compounds.

Calcium signaling in vascular smooth muscle

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$\text{Ca}(2^+)$ is a ubiquitous messenger for a wide variety of physiological functions. In pulmonary arterial smooth muscle cells, we have examined $\text{Ca}(2^+)$ release pathways, including ryanodine receptor and $\text{IP}(3)$ gated pathways and their involvement in Local $\text{Ca}(2^+)$ events, called $\text{Ca}(2^+)$ sparks, as well as $\text{Ca}(2^+)$ influx pathways including the transient receptor potential (TRP) gene superfamily. Recent studies have identified multiple TRP channel subtypes, belonging to the canonical (TRPC), melastatin-related (TRPM), and vanilloid-related (TRPV) subfamilies, in pulmonary arterial smooth muscle cells (PASMCs). They operate as specific $\text{Ca}(2^+)$ pathways responsive to stimuli, including $\text{Ca}(2^+)$ store depletion, receptor activation, and mechanical stress. Increasing evidence suggests that these channels play crucial roles in agonist-induced pulmonary vasoconstriction, vascular remodeling, and pulmonary arterial hypertension. In this talk, I will highlight and discuss these $\text{Ca}(2^+)$ pathways in pulmonary vasculatures. Since $\text{Ca}(2^+)$ ions regulate many cellular processes via specific $\text{Ca}(2^+)$ signals, investigations of these novel channels will likely uncover more important regulatory mechanisms of pulmonary vascular functions in health and in disease states.

Gene copy-number variations in health and disease

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Array comparative genomic hybridizations and molecular genetics experiments in the past decade have revealed an important phenomenon that escaped the attention of most human geneticists: many genes in our genomes exhibit an inborn, inter-individual variation in copy-numbers of specific DNA segments. Such common copy-number variation (CNV) loci often contain genes engaged in host-environment interaction including those with immune effector functions. DNA sequences within a CNV locus often share high degree of identities but beneficial and deleterious polymorphic variants are present. Thus, common gene CNVs can contribute both qualitatively and quantitatively to a spectrum of phenotypic variants. We have shown the vast phenotypic and genotypic diversities of complement C4 created by copy-number variations of RCCX modules consisting of four contiguous genes *RP-C4-CYP21-TNX* at the central region of the HLA on chromosome 6p21.3. A direct outcome of *C4* CNV is the generation of two classes of polymorphic proteins, C4A and C4B, with differential chemical reactivities towards substrates with amino groups and hydroxyl groups, plus a range of C4 plasma protein concentrations from 15-70 mg/dL among different human subjects. Complete deficiency of complement C4 is one of the strongest genetic risk factors associated with autoimmune disease systemic lupus erythematosus (SLE). The prevalence of SLE is three times higher in subjects of African and Asian ancestries than those of European ancestry. The primary objective of this study is to determine the role of complement *C4* CNV in SLE disease risk in different racial backgrounds. Our study populations include 233 European American SLE patients and 389 race-matched controls from Ohio, 197 African American SLE patients and 201 controls from South Carolina, and 176 Asian-Chinese SLE patients and 369 controls from Hong Kong. Copy-numbers of total *C4*, *C4A* and *C4B* and RCCX modules were determined by

Southern blot analyses of (1) large genomic DNA fragments generated by *PmeI* digestion and resolved by pulsed-field gel electrophoresis, and (2) regular agarose gel electrophoresis of *TaqI*, *PvuII-PshAI* digested genomic DNA. Further refinement and validations were achieved by quantitative real-time PCR assays. The total *C4* gene copy-number per diploid genome ranges from 2 to 8; each of these *C4* genes either code for *C4A* or *C4B*. Among healthy European Americans, 60.8 % have four copies of *C4* genes, 27.2 % have less than four copies, and 12% have more than four copies. In SLE, the frequency of individuals with less than four copies of *C4* is significantly increased (42.2%), while the frequency of those with more than four copies is decreased (6%) ($p=0.000016$). The mean gene copy-number of a CNV locus in a human population is defined as the gene copy-index (GCI). The GCI of total *C4* among healthy European American, African American and Asian-Chinese are 3.81 ± 0.75 (mean \pm SD), 4.02 ± 0.71 , and 4.22 ± 0.92 , respectively; while those for SLE patients are 3.56 ± 0.77 , 3.74 ± 0.72 , and 3.98 ± 0.81 , respectively. The GCI of total *C4* in SLE are consistently lower by ~ 0.25 compared to those of raced-matched controls with p-values of 0.0001 in both European Americans and African Americans, and 0.0028 in Asian Chinese. The decrease in total *C4* is mainly attributed to homozygous and heterozygous deficiencies of *C4A*. Further analyses revealed that *C4A* deficiency among Europeans and African Americans are characterized by the presence of a monomodular RCCX haplotype containing a single *C4B* gene that is linked to *HLA-DR3*. Among Asian-Chinese, haplotypes with *C4A*-deficiency are characterized by bimodular RCCX containing two different *C4B* genes with multiple remnant markers of genetic recombinations. In summary, low gene copy-numbers of complement *C4A* is a common risk factor for disease susceptibility of SLE among patients of European, Asian and African ancestries. We have extended our CNV studies to other autoimmune diseases including type 1 diabetes and rheumatoid arthritis; and to other immune effector genes coding for complement factor H-related proteins *CFHR3* and *CFHR1*, and for immunoglobulin Fc γ -receptors *FCGR3B* and *FCGR3A*.

Hox genes and transcriptional regulation of craniofacial development

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Craniofacial morphogenesis in mammals depends on proper migration of neural crest cells to the facial primodium, as well as signaling between the epithelium and neural crest derived mesenchyme. *Hox* genes provide positional information and have an important role in patterning the hindbrain, neural crest and branchial arches. However, cross-talks between *Hox* genes and signaling factors during craniofacial morphogenesis are not well understood.

By mouse mutant studies, we showed that alteration of the combinatorial *Hox* code in the *Hoxb3^{Tg}* transgenic mutant led to a series of craniofacial defects, including facial skeletal defects, abnormal facial nerve innervation and deafness. Interestingly, in r4 of the *Hoxb3^{Tg}* mutant where *Hoxb3* was ectopically expressed, the expression of *Hoxb1* was specifically abolished. We identified a novel *Hoxb3* binding site S3 on the *Hoxb1* locus and confirmed protein binding to this site by EMSA and in vivo ChIP analysis. We further showed that *Hoxb3* could suppress *Hoxb1* transcriptional activity by chick in ovo luciferase reporter assay. We demonstrated a novel negative regulatory mechanism by which *Hoxb3* as a posterior gene serves to restrict *Hoxb1* expression in r4 by direct transcriptional repression to maintain the rhombomere identity.

Branchial arches are transient structures that form the embryonic face. The second branchial arches of the *Hoxb3^{Tg}* mutants were reduced as a result of apoptosis of both migratory and post-migratory neural crest cells. Strikingly, the expression of genes involved in the endothelin-1 (ET1) signaling pathway including *Gsc*, *dHAND*, *Twist* and *Prx2* in the branchial arches were specifically affected. By genetic analysis of *Hoxb3^{Tg}* and *ET1* double mutants, we showed that heterozygous loss of *ET1* exacerbated the phenotype of *Hoxb3^{Tg}* mutant with an accompanied down-regulation of *dHAND*, a direct target of ET1 signaling. We have shown that combinatorial expression of *Hox* genes is specifically required in mediating endothelin-1 signaling for the maintenance of postmigratory neural crest cells and the morphogenesis of facial skeletal elements.

The structure of P protein complex in ribosome and its interaction with ribosome-inactivating protein

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Ribosome-inactivating proteins (RIPs) inhibit protein synthesis by enzymatically depurinating a specific adenine residue at the sacrin-ricin loop of the 28S rRNA. For the RIP to attack the 28S rRNA, we hypothesize that it needs first docking on the ribosomal protein(s). Using trichosanthin (TCS), an RIP isolated from a medicinal herb as model, we have shown that TCS interacts with human acidic ribosomal proteins P0, P1 and P2, which constitute the lateral stalk of eukaryotic ribosome. Deletion mutagenesis showed that TCS interacts with the C-terminal tail of P2, the sequence which is conserved in P0, P1 and P2. The P2-binding site on TCS was mapped to the C-terminal domain by chemical shift perturbation experiments. We subsequently solved the crystal structure of TCS complexed to a conserved 11-aa peptide at the C-terminal domain of the P protein. The interaction is through a hydrophilic interaction at the N-terminal and a hydrophobic interaction at the C-terminal of the peptide and the C-terminal domain of TCS. Docking of this peptide to selected plant and bacterial RIPs showed that similar interaction may also occur with other RIPs.

The lateral stalk of ribosome is responsible for binding of translation factors and activation of GTP hydrolysis for protein synthesis. In eukaryotes, the complex is a pentamer with P0, P1 and P2 in the ratio of 1:2:2. To understand the structure and function of this complex, we have determined the solution structures of the N-terminal dimerization domain of P2 and that of P1-P2. The structure of NTD-P2 shows that it forms a symmetric homodimer, with each monomer containing four helices. The dimeric interface is formed by helices 1, 2 and 4 packing with each other in an antiparallel fashion. The structure of NTD-P1/P2 is similar to that of NTD-P2, but NTD-P1/P2 is asymmetric and there exists a hydrophobic cavity that can accommodate a loop region of the P0 subunit. These discoveries shed light on the structure of the eukaryotic P complex.