Program/Abstract # 397

**Heterotaxin: A novel TGF-beta signaling inhibitor identified in a multi-phenotype profiling screen in Xenopus embryos**

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Abstracts

Disruptions of anatomical left–right asymmetry result in life-threatening heterotaxic birth defects in vital organs. We performed a small molecule screen for left–right asymmetry phenotypes in Xenopus embryos and discovered a novel pyridine analog, heterotaxin, which disrupts both cardiovascular and digestive organ laterality and inhibits TGF-β-dependent left–right asymmetric gene expression. Heterotaxin analogs also perturb vascular development, melanogenesis, cell migration and adhesion, and inhibit the phosphorylation of Smad2, an intracellular mediator of TGF-β receptor activation. This combined phenotypic profile identifies these compounds as a novel class of TGF-β signaling inhibitors. Notably, heterotaxin analogs also inhibit angiogenesis in human cell culture, revealing their broad applicability. As TGF-β inhibitors are excellent candidates for anti-fibrotic or anti-metastatic drugs, our discovery of a new class of inhibitors with demonstrated in vivo efficacy may lead to important new therapeutics. Our results also illustrate that embryonic phenotypic profiling, in which multiple organ, tissue, cellular and molecular parameters are assessed in a whole organism context, is a valuable strategy for identifying the mechanism of action of novel compounds. Finally, this study reveals the utility of frog embryos as an emerging model for chemical genomics and in vivo drug discovery.

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**Transmembrane voltage gradient in GlyR-expressing niche cells controls behavior of neural crest derivatives in vivo**

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Understanding the mechanisms that guide stem cell behavior during complex morphogenesis is a high priority for developmental biology, regenerative medicine, and oncology. Like chemical cues, endogenous bioelectric signals are important regulators of morphogenesis. We exploited the native glycine receptor chloride channel (GlyR) to investigate the role of niche cells’ transmembrane potential in controlling embryonic stem cell function in *Xenopus laevis*. The neural crest gives rise to a number of tissue types including melanocytes, the pigmented cells of the epidermis. Molecular-genetic or pharmacological depolarization of a sparse, widely-distributed set of GlyR-expressing cells confers a neoplastic-like phenotype on distant melanocytes: they overproliferate, acquire an arborized cell shape, and migrate inappropriately, invading numerous tissues in a metaproteome-dependent fashion. A similar effect is observed in human melanomas in culture. The pathway linking depolarization of GlyR-expressing cells to metastatic behavior in melanocytes involves increase of extracellular serotonin levels by SERT and the up-regulation of *Slug* and *Sox10*. These data identify GlyR as a unique marker of cells with the ability to instruct neural crest, reveal a novel non-cell-autonomous aspect of the stem cell-cancer cell transition based on voltage gradients, identify a new role for non-neural serotonin, and suggest new strategies for manipulating embryonic stem cell behavior.

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Program/Abstract # 399

**Effects of methyl mercury (MeHg) on neural development in *X. laevis* and in regenerating planaria**

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Environmental mercury is known to have severe neurological effects during development. In humans, fetal exposure can occur through the mother’s consumption of seafood, and very small concentrations of methyl mercury are sufficient to cause subtle developmental changes. However, the effects of chronic low levels of mercury exposure on neural development have not been adequately characterized. Here we use *Xenopus laevis* as well as planarian species to analyze in more detail the effects of MeHg on development and regeneration, in particular of neurotransmitter pathways. Work from other laboratories has demonstrated that certain pathways essential to *X. laevis* neural development, including Notch signaling, as well as dopaminergic and GABAergic pathways, are functionally disrupted following MeHg exposure. Preliminary results indicate that *X. laevis* embryos that have been exposed to increasing concentrations of MeHg-containing media develop almost (morphologically) normally below 0.1 ppm, but experience an almost complete arrest of development at MeHg concentrations even slightly higher than this. Exposure at levels intermediate between 0.1 ppm and 0.01 ppm in conjunction with analysis of neural markers will demonstrate which neurotransmitter phenotypes and developmental pathways are being affected by MeHg exposure. Additionally, the use of regeneration experiments in planarian species, and subsequent neural expression analysis, will offer an important comparative perspective.

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Program/Abstract # 400

**Using zebrafish to understand the neurodevelopment role of susceptibility genes for autism spectrum disorder**

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Several studies in the last three years have revealed that members of a synaptic cell adhesion network are candidate susceptibility genes for autism spectrum disorder (ASD). ASD is increasingly attributed to a disorder of brain function rather than brain anatomy. We have begun to address the role of gene–gene interactions within the synaptic cell adhesion pathway involved in neural circuits associated with simple behaviours using the zebrafish animal model. We are focusing on interactions between identified susceptibility genes NLGN-1, NLGN-4, NRXN-1α, Shank3 and CNTNAP2 as well as on interactions of these genes with other known synaptic cell adhesion pathway genes (LRRTM2, PS-D95 and CASK) in order to begin to understand the function of gene networks underlying the emergence of early behaviours. Knock down of either NRXN-1αx, NRXN1βx or CNTNAP2 significantly reduced the touch response at 30 hpf to a similar extent. The high penetrance of these phenotypes (71–84%) suggests that these genes are playing a major role in the development of the underlying neural circuitry responsible for this behaviour. In contrast, at 45 hpf knock down of NRXN-1αx had no effect on the escape response, knock down of NRXN1βx either extinguished or reduced the response, while knock down of CNTNAP2 produced an abnormal response. These very different phenotypes suggest very different roles of these synaptic adhesion network genes in the underlying neural circuitry. Our analyses are beginning to reveal the
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Program/Abstract # 402
Regulatory elements controlling the expression of short-stature (Shox) genes during development
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The murine Shox (mShox2) gene is required for development of the proximal limbs, palate, jaws and heart. Deficiencies of the closely related human SHOX (hSHOX) gene cause the limb abnormalities associated with Turner, Léri–Weill and Langer syndromes. Some Léri–Weill and Langer patients have an intact hSHOX coding region, but deletions far downstream of the gene, suggesting that long-range enhancer elements are required for hSHOX expression in limbs. To begin to understand the regulation of Shox genes, we used transgenic mouse embryos to analyze the regulation of mShox2 and hSHOX. First we analyzed the regulatory potential of sequences near each gene by inserting a LacZ reporter at the start codon of each coding sequence on an appropriate bacterial artificial chromosome (BAC). Transgenic embryos produced from the mShox2 construct revealed the presence of regulatory elements driving expression in proximal limbs, sensory neurons and the hindbrain. Further analysis revealed that sensory neuron expression is associated with sequences close to the Shox2 gene, whereas limb and hindbrain expression requires an evolutionarily conserved region approximately 35 kb downstream. In contrast transgenic embryos containing the hSHOX-LacZ BAC showed expression only in the first pharyngeal arch, a known expression domain of hSHOX. Therefore we are now searching for hSHOX limb enhancers on BACs mapping further downstream of the gene. One prominent candidate is a conserved sequence with homology to the mShox2 limb/hindbrain enhancer region.

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