Program/Abstract # 227

Etsrp/Etv2 initiates endothelial/endocardial and inhibits myocardial differentiation by two distinct mechanisms in zebrafish embryos

Sharina Palencia-Desai\textsuperscript{a}, Vikram Kohli\textsuperscript{b}, Jione Kang\textsuperscript{c}, Neil C. Chi\textsuperscript{d}, Brian L. Black\textsuperscript{c}, Saulius Sumanas\textsuperscript{b}  
\textsuperscript{a}Cincinnati Children’s Hospital Medical Center, University of Cincinnati, Cincinnati, OH, USA  
\textsuperscript{b}Cincinnati, OH, USA  
\textsuperscript{c}San Francisco, CA, USA  
\textsuperscript{d}La Jolla, CA

Previous studies have suggested that embryonic vascular endothelial, endocardial and myocardial lineages originate from multipotential cardiovascular progenitors. However, their existence in vivo has been debated and molecular mechanisms that regulate specification of different cardiovascular lineages are poorly understood. An ETS domain transcription factor Etv2/Etsrp has been recently established as a critical regulator of vascular endothelial differentiation in zebrafish and mouse embryos. In this study, we show that Etsrp functions as a critical factor in the choice of endocardial/endothelial versus myocardial cell fates during zebrafish embryonic development. Expression of multiple endocardial specific markers is absent or greatly reduced in eutsp knockdown or mutant embryos. We show that Etsrp regulates endocardial differentiation by directly inducing endocardial nfatc1 expression. In addition, Etsrp function is required to inhibit myocardial differentiation. These results argue that Etsrp initiates endothelial/endocardial and inhibits myocardial differentiation by two distinct mechanisms. Our findings are important for the understanding of genetic pathways controlling cardiovascular differentiation during normal vertebrate development and will also greatly contribute to the stem cell research aimed at regenerating heart tissues.

doi:10.1016/j.ydbio.2011.05.641

Program/Abstract # 228

Zebrafish mutant in Alpha-Cardiac Actin serves as a model for dilated cardiomyopathy

Nikki O. Glenn\textsuperscript{a}, Vikram Kohli\textsuperscript{b}, Thomas Bartman\textsuperscript{b}, Saulius Sumanas\textsuperscript{b}  
\textsuperscript{a}Cincinnati Children’s Hospital, Cincinnati, OH, USA  
\textsuperscript{b}Cincinnati Children’s Hospital, Cincinnati, OH, USA

Heart failure is the primary manifestation of dilated cardiomyopathies, half of which are characterized as idiopathic (IDC), of unknown etiology, which affect 5 to 8 in every 100,000 individuals (Abraham and Bristow, 1997). A subset of patients with hereditary IDC were found to have missense mutations in ACTC (Olson, 1998). Forward genetic screens in zebrafish have led to the unbiased identification of genes involved in important developmental processes. In a chemical mutagenesis screen (Beis, 2005), a cardiovascular-specific mutant was identified that lacks blood circulation, displays blood regurgitation in a dilated heart between the atrium and ventricle and lacks endocardial cushion formation. Through positional cloning techniques, we have identified the mutation as a single nucleotide change in Alpha-Cardiac Actin (actc1a), resulting in an amino acid substitution at residue 169. This Y169C change in actc1a results in an impaired ability of cardiac monomeric actin to polymerize into F-actin. Physiological analysis reveals that actc1a mutant embryos show a defect in contractility, measured by ventricular shortening fraction (VSF) and atrial stroke volume increase. Molecular markers for endocardial cushions including Notch1b, NFATc1 and klf2a are downregulated or mislocalized in actc1a mutants. Our results support a model that hemodynamics plays an important role in the cardiac valve formation. In actc1a mutants, abnormal cardiac blood flow patterns result in endocardial cushion-specific gene misexpression, and the absence of valvular development. Therefore actc1a zebrafish mutant will be an important tool to study the consequence of actc1 mutations in human congenital disorders that are also commonly associated with septal defects.

doi:10.1016/j.ydbio.2011.05.642

Program/Abstract # 229

Impaired heart function in embryos depleted for the voltage-gated calcium channel beta 2 subunit [CACNB2] is due to reduced cardiomyocyte proliferation and adhesion

Deborah M. Garrity\textsuperscript{a}, Yelena Chernyavskaya\textsuperscript{b}, Alicia Ebert\textsuperscript{b}, Emily Milligan\textsuperscript{b}  
\textsuperscript{a}Colorado State Univ Biol, Fort Collins, CO, USA  
\textsuperscript{b}Colorado State University, Fort Collins, CO, USA

Voltage-gated calcium channels (VGCCs) are oligomeric complexes composed of pore-forming CACNA subunits and several auxiliary proteins. Auxiliary CACNB subunits regulate VGCC electrophysiology and chaperone CACNA subunits to the cell membrane. To determine the contributions of CACNB2 to cardiac development, we depleted zebrafish embryos of CACNB2 transcripts using morpholinos. Although heart fields were of normal size initially, by the time of cardiac cone formation, fewer cells expressed cardiac markers. From 24 to 48 hours post-fertilization (hpf), hearts of CACNB2-depleted embryos (morphants) contained up to 30% fewer cells. Hearts of morphants demonstrated significantly fewer BrDU-positive cells, but no change from wildtype in the number of TUNEL-positive cells. Morphant heart tubes had increased expression of BMP4 (an inhibitor of cell proliferation) at 25 and 48 hpf. Therefore, CACNB2 plays an important role in cardiac cell proliferation. In addition, morphant heart tubes fragmented easily when placed under pressure, suggesting that adhesion among cardiomyocytes was weakened. Consistent with this hypothesis, Immunohistochemistry showed cadherins at cardiomyocyte membranes were depleted in morphant hearts. Since heart rhythm was normal in CACNB2 morphants, other CACN proteins expressed in the heart may enable overtly normal VGCC contractile activity. We are currently assessing whether CACNB2 phenotypes are mediated by loss of VGCC function per se, or by loss of other CACNB2:partner interactions. The latter possibility is intriguing in light of recent data suggesting that CACNBs, as MAGUK-family proteins, may interact with multiple protein partners via their SH3 or guanylate kinase domains.

doi:10.1016/j.ydbio.2011.05.643

Program/Abstract # 230

Zebrafish as a model to study cardiomyopathy

Nathalia S. Glickman Holtzmana, Corinna Singlemanb  
\textsuperscript{a}Queens College, CUNY Biology, New York, NY, USA  
\textsuperscript{b}Queens College, Flushing, NY, USA

Proper development of the heart is crucial to embryonic and adult survival. Cardiac maturation, both morphologically and physiologi-
cally, begins in the embryo and continues throughout development to adulthood. Cardiac development in zebrafish embryos has been studied extensively, and recent studies looking at adult hearts indicate significant maturation, however, little is known about how these changes take place. We hope to elucidate the mechanisms driving cardiac maturation and develop zebrafish as a model organism for cardiology. It has been established that form and function of organs effect their development. In the heart, contraction and blood flow are some of the factors effecting the development of the structure of the heart. Thus far we have determined that the lack of contraction of the atrium in weak atrium mutant, which has reduced or absent contraction of the atrium, has a clear effect on function and the resulting structure of the ventricle. In humans cardiomyopathy is attributed to both environmental and genetic factors, though specific causes are often unknown. The zebrafish mutant, weak atrium, through the reduced function of the atrium, develops a cardiomyopathy-like phenotype in the ventricle. We have examined the maturation of heart structure and this disease state in weak atrium mutant in order to gain insight into the mechanisms regulating cardiomyopathy.

do:10.1016/j.ydbio.2011.05.644

Program/Abstract # 231
The chromatin remodeling complex subunit Baf60c regulates essential gene expression programs in heart development
Xin Suna, John Wylieb, Yuqing Zhouc, Danos Christodouloud, Christine E. Seidmana, Jonathan G. Seidmana, Mark Henkelmana, Janet Rossantesa, Benoit Bruneaua

1Hospital for Sick Children, Toronto, ON, Canada
2Gladstone Institute of Cardiovascular Disease, San Francisco, CA, USA
3Mouse Imaging Centre The Hospital for Sick Children Toronto Centre for Phenogenomics, Toronto, ON, Canada
4Harvard Medical School, Boston, MA, USA
5Department of Genetics Harvard Medical School, Boston, MA, USA

SWI/SNF complexes are ATP-dependent chromatin remodeling complexes widely existing from yeast to mammals. In mammals they are also called BAF (Brm/BRG1 Associating Factor) complexes. Combinations of constitutive BAF members and multiple or cell type-specific BAF members can generate hundreds of different BAF complexes. Several have been reported to play critical roles in development processes including neuron differentiation and ES cell differentiation. Baf60c is one of the three Baf60 subunits in the mouse. Baf60c is highly expressed in the mouse heart from the cardiac crescent stage. Baf60c can associate with cardiac transcription factors including Tbx5, Nkx2-5, and myocardin. To understand the role of Baf60c in mouse heart development, a Baf60c conditional knockout mouse line was established and Baf60c knockout embryo phenotypes were characterized. Constitutive loss of Baf60c results in hypoplastic myocardium and embryonic death by E14.5. RNA-seq of E12.5 Baf60c knockout embryo hearts shows a broad down-regulation of mitochondria encoded or related genes, as well as cardiac myocyte sarcomere and contraction apparatus genes, indicating mitochondrial biogenesis defects and cardiomyocyte sarcomere assembly or function defects. High frequency echocardiography showed that Baf60c knockout embryo ventricle walls are hypocontractile. Transmission electron microscopy showed that Baf60c knockout cardiomyocytes have disarrayed myofilaments. Deletion of Baf60c with Myh6::Cre in cardiomyocytes also causes abnormal heart growth. Many of Myh6::Cre, Baf60cflx/flox—/—pups showed retarded growth and only some of them can survive to adulthood. Histology and echocardiography showed that these mice have thinner ventricle walls and dilated chambers. In summary, Baf60c is a cardiac-specific chromatin-remodeling factor that has critical functions in both embryonic and postnatal heart growth. The mechanism of Baf60c function in regulating cardiac growth in conjunction with transcription factors and other chromatin remodeling complexes remains to be elucidated.

do:10.1016/j.ydbio.2011.05.645

Program/Abstract # 232
Cardiac valve malformations: New insights from Pdlim7, an unexpected suspect in heart development
Jennifer Krcmerya, Rudyard Sadlerb, Rajesh Guptaa, Chrissy Kamidec, Sol Misener, Douglas Losordo, Hans-Georg Simonb

aNorwestern Univ., Chicago, IL, USA
bChicag, USA

PDZ-LIM proteins contain multiple binding domains, facilitating interactions with the actin cytoskeleton, nuclear factors, and signaling molecules, thereby allowing the proteins to carry out diverse biological functions. Here, we characterize a new family member, Pdlim7, which in zebrafish revealed important functions during cardiac and limb development. In order to determine the role of Pdlim7 in a mammalian four-chambered heart from early valve formation all the way to fully functioning adult valves, we generated a Pdlim7 knock-out mouse. Pdlim7 null embryos demonstrate misregulation of molecular and cellular processes necessary during early valve formation. These developmental problems translate into aberrant shaped heart valves in adult mice as demonstrated by quantitative measurements obtained from 3-dimensional reconstructions of serial heart sections. Utilizing echocardiography, we are able to visualize the physiological consequences of these structural abnormalities, which include increased mitral valve annulus dimension and subtle left ventricular dysfunction. This work identifies the actin-associated protein Pdlim7 as an unexpected and novel factor critical for mammalian cardiac valve development and maturation. Thus, the Pdlim7 knock-out mouse may provide a new model for studying cardiac valve disease progression from the embryo to the adult.

do:10.1016/j.ydbio.2011.05.646

Program/Abstract # 233
Proteomic analysis of cardiovascular development in the Ts65Dn Down Syndrome mouse model
Clara S. Moore, Erik Kelly, Arianna Franca

aFranklin and Marshall College Biology, Lancaster, PA, USA
bFranklin & Marshall College, Lancaster, PA, USA

down Syndrome (DS) due to triplication of human chromosome 21 (Hsa21) results in congenital heart defects in 50% of newborns with DS. The Mus musculus Ts65Dn model, with triplication of approximately 132 genetic orthologs to Hsa 21, has multiple DS phenotypes such as neonatal lethality and cardiovascular defects. Dosage imbalance of Ts65Dn embryos may cause misexpression of not only triplicated genes, but many other genes contributing to the observed cardiovascular abnormalities. We utilized proteomic methods to examine the array of proteins that are expressed in trisomic vs. euploid embryonic day (E)14.5 hearts via of two-dimensional protein gel electrophoresis, Delta 2D software analysis, mass spectrometry and Mascot data analysis. Modifications of proteomic methods — minimizing volumes, eliminating protein quantification steps, and optimizing staining methods — were critical. Comparison of proteins from single E14.5 hearts allowed identification of nine protein spots