loop may result from deficiencies in cardiomyocyte shape, size or differentiation. We provide an update on ongoing work to determine the earliest developmental timepoints at which tbx5 is necessary for normal cardiac function, as well as the functional relevancy of graded tbx5 expression, by using the Tg(hsp70:tbx5-GFP) and Tg(cmlc2:tbx5-GFP) lines of zebrafish.

doi:10.1016/j.ydbio.2009.05.403

Program/Abstract # 374
FGF signaling regulates a secondary phase of cell addition to the initial heart tube in zebrafish
Xin-Xin I. Zeng, Deborah Yelon
Developmental Genetics, NYU School of Medicine, New York, NY, USA

Developing organs are assembled from multiple populations of progenitor cells that originate from distinct locations at different developmental stages. During heart development, the initial heart tube forms from cardiomyocytes arising from a portion of the anterior lateral plate mesoderm referred to as the first heart field (FHF). Prior studies in amniote have shown that new cardiomyocytes originating from a second heart field (SHF) are later added to the poles of the heart tube. Many congenital heart diseases affect portions of the heart derived from the SHF; however, we still do not understand the mechanisms that regulate the specification, migration, and differentiation of SHF cells. Recent studies from our laboratory have provided the first evidence that there are two phases of cardiomyocyte differentiation in zebrafish, strongly suggesting the existence of a zebrafish SHF. Treatment of zebrafish embryos with SU5402 inhibitor from 24 to 48hpf, covering the window when cells are added to the arterial pole, significantly reduces the number of cells added to the arterial pole. Thus, after differentiation of the FHF is complete, FGF signaling is still important for the addition of new cardiomyocytes from the SHF. Continuous observation of a transgenic reporter of FGF signaling indicates FGF-responsive cells scattered in a region adjacent to the arterial pole, followed by congeration of FGF-responsive cells at the arterial pole. These findings, together with the expression of fgf8 in the ventricle, suggest a model in which Fgf8 functions as an attractive cue regulating the migration of new cardiomyocytes to the arterial pole.

doi:10.1016/j.ydbio.2009.05.404

Program/Abstract # 375
Fgf3 and Fgf10 are required redundantly for neural crest migration and cardiovascular development
Lisa D. Urness, Tracy J. Wright, Suzanne L. Mansour
Department of Human Genetics, Univ. of Utah, Salt Lake City, UT, USA

Heart development requires contributions from, and interactions between, discrete cell populations including primary and secondary heart fields (SHF), cardiac neural crest (CNC), and the proepicardial organ (PEO). Birth defects caused by abnormal CNC and SHF development include DiGeorge and CHARGE syndromes. Aspects of these syndromes are phenocopied in fibroblast growth factor (Fgf)8 or Fgf15 null mutant mice. Fgf3 and Fgf10 are expressed in sites relevant to early heart development, but single null mutants do not have heart defects. Fgf3−/−:Fgf10−/− double mutants, however, die at E11.011.5. They lack NC-derived proximal 9th cranial ganglia, exhibit pericardial edema, hypoplastic ventricles and outflow tract cushions, and lack 4th pharyngeal arch arteries, showing that Fgf3 and Fgf10 are required redundantly for normal CNC and cardiovascular development. To test the hypothesis that Fgf3 and Fgf10 are required for correct migration and/or survival of CNC, and for development or morphogenesis of the heart, we assessed expression of NC and cardiac markers. We find that specification and early migration of NC are normal, but NC migration is reduced by E9.510.5. Expression of Nkx2.5 and Islet1 is markedly reduced in the double mutant; whereas Fgf8 and Fgf15 are unaffected. In contrast to the exclusively anterior pole defects of Fgf8 or Fgf15 mutants, Fgf3−/−;Fgf10−/− embryos also show posterior pole defects, including reduced investment of epicardial cells from the PEO. Studies are underway to define the expression sites of Fgf3 and Fgf10 required for normal CNC and cardiovascular development.

doi:10.1016/j.ydbio.2009.05.405

Program/Abstract # 376
BMP signaling regulates progenitors of the mammalian heart
John Klingensmith, Murim Choi, Chandra Davenport, Jianwen Que
Department of Cell Biology, Duke University Medical Center, USA

Development of the right ventricle and outflow tract of the mammalian heart involves cell populations within the primary heart tube, as well as extracardiac contributions, as cells from outside the primary heart tube progressively add to it. Proper morphogenesis of these tissues is critical for cardiac function. The anterior heart field (AHF) is a secondary cell lineage of the myocardium that contributes substantially to the outflow tract and right ventricle. Here we present evidence that extracardiac BMP signaling is essential for the addition of progenitor cells to the heart. Several tissue-specific genetic ablations and explant culture experiments demonstrate a direct requirement for BMP signaling in regulating myocardial differentiation and proliferation in the AHF. Embryos lacking BMP receptor 1A (BMPR1A) in the AHF invariably display severely hypomorphic outflow tract and right ventricle structures. In contrast, Bmpr1a in the primary heart tube is dispensable for development of these tissues, but is necessary for later cardiac gene expression and cardiomyocyte proliferation. We further find that BMP antagonism by Noggin is necessary to keep myocardial proliferation in check. Surprisingly, although BMPR1A signal transduction requires the canonical signal transducer Smad4 in the primary heart tube, BMPR1A signaling in the developing AHF is independent of Smad4. Thus, BMP signaling and its antagonism balance myocardial proliferation in the ventricles. Earlier, BMP signaling acts via a Smad4-independent pathway to regulate addition of myocardial progenitors to the outflow tract and right ventricle.

doi:10.1016/j.ydbio.2009.05.406

Program/Abstract # 377
Manta ray a novel ENU mutant with brain and craniofacial defects
Konstantinos Zarbalis,a,b, Youngshik Choi,e, Roy L. Maute4, Andrew S. Peterson5, Samuel J. Pleasure6
aDepartment of Pathology and Laboratory Medicine, UC Davis, USA
bInstitute of Pediatric Regenerative Medicine, Shriners Hospitals, USA
cDepartment of Neurology, UCSF, USA
dGerstner Sloan-Kettering, USA
eResearch Department, Genentech, USA

In a forward genetic screen in mice we identified a novel mutant line with a multitude of severe abnormalities and fetal lethality. We named this line manta ray (mray) in reference to its craniofacial abnormalities, which include orofacial clefting. In addition to craniofacial defects, homozygous mutants are defective in brain, heart, skin and vascular development. The brain defects in particular, include a smaller sized forebrain partly resulting from cortical thinning. The craniofacial phenotype points to an abnormal neural
We have ascertained seven mutations affecting CNS development and screen in the mouse. Using this unbiased, forward genetic approach, forebrain development, we have conducted an ENU mutagenesis reasoning and memory. To identify genes required for mammalian responsibility for many higher order cognitive functions including regulators of cellular morphology during development. The cranial nerves V, VII/VIII, IX and X. Aberrant aberrantly expressed in the region of the developing epibranchial placodes and neural crest-specific conditional Spry1/2 double knockout (Wnt1cre; Spry1;2flox/flox) are normal, suggesting that Spry expression within neural crest cells is not required for normal development. Alternatively Spry may have a role in placodal formation and differentiation as the expression of placodal and early neuronal markers is altered at E9.5 in Spry1, 2 double knockouts. Together this data indicates that Spry gene function is required for the development of the sensory cranial ganglia.

doi:10.1016/j.ydbio.2009.05.407

Program/Abstract # 378
An ENU screen reveals novel genes in mammalian forebrain development
Rolf W. Stottmann, Annick Turbe-Doan, Haiyan Qui, David Beier
Div. of Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

The forebrain is the largest portion of the human brain and is responsible for many higher order cognitive functions including reasoning and memory. To identify genes required for mammalian forebrain development, we have conducted an ENU mutagenesis screen in the mouse. Using this unbiased, forward genetic approach, we have ascertained seven mutations affecting CNS development and have thus far identified four by positional cloning. The most remarkable phenotype uncovered to date is the rudolph mutation with severe developmental defects in both the CNS and appendicular skeleton (smaller long bones). The organization of the neocortex is profoundly disrupted and contains clustered cell bodies, which appear to be neurogenic foci. The causal gene is known to play a role in cholesterol biosynthesis, which is notable given the recent implication of a role for oxysterols in mediating intracellular components of Hedgehog signaling. We see decreased induction of known Sonic hedgehog (Shh) target genes in the cortex, retina and skeleton. In vitro, this mutation results in decreased cellular response to Shh, revealing a requirement for embryonic cholesterol metabolism in both CNS development and normal Shh signaling. Other mutations in our screen show phenotypes such as cortical hypopcellularity, hydrocephaly, anterior encephalocele, and craniarachischisis. Thus, we have demonstrated the utility of a forward genetic approach in studying neurodevelopment. We will also describe our efforts to enrich our screen for mutations affecting forebrain development.

doi:10.1016/j.ydbio.2009.05.408

Program/Abstract # 379
Sprouty gene function is required for normal sensory cranial nerve morphology
Subreena L. Simrick, Mohi A. Ahmed, Michiel A. Basson
Department of Craniofacial Development, KCL, London, UK

The sensory cranial ganglia are derived from late migrating neural crest cells and regions of ectodermal thickening called placodes. Fibroblast growth factors (Fgfs) have been implicated in olfactory, epibranchial and otic placode development, as well as neural crest migration. The Sprouty (Spry) gene family encodes feedback antagonists of Fgf signalling. Between E8.5 and E9.5 Spry1 and 2 are transiently expressed in the region of the developing epibranchial placodes and late migrating neural crest cells. Embryos lacking both Spry1 and Spry2 exhibit abnormal morphology in the proximal and/or distal regions of the cranial nerves V, VII/VIII, IX and X. Aberrant Sox 10 expression at E9E9.5 implies that Spry is required for the development of late migrating neural crest cells. However, the sensory cranial ganglia in the neural crest-specific conditional Spry1/2 double knockout (Wnt1cre; Spry1;2flox/flox) are normal, suggesting that Spry expression within neural crest cells is not required for normal development. Alternatively Spry may have a role in placodal formation and differentiation as the expression of placodal and early neuronal markers is altered at E9.5 in Spry1, 2 double knockouts. Together this data indicates that Spry gene function is required for the development of the sensory cranial ganglia.

Folate supplementation has been used to prevent neural tube defects (NTDs) during pregnancy for many years. NTD and NTD related defects span a wide range from minor spinal malformations through occulta (mild), to anencephaly (severe). Although folate appears to prevent most NTDs some still occur whether from genetic abnormalities or for environmental factors. For our research group two important questions are: is there one level of folate that should be sustained during pregnancy or should the recommended folate levels be stage specific and whether excess levels folate can alter embryonic development? Timed pregnant ICR mice were treated on the evening of E11 with sterile saline, 1X FA (12mg/kg folic acid) or 4X FA (48mg/kg folinic acid). On days E12 and E13 they were treated in the morning with either sterile or 20mg/kg Methotrexate (MTX) and 1X FA or 4X FA and in the evening with either sterile or 1X FA or 4X FA resulting in 4 experimental groups. We observed spinal deformities in mice that received higher dose of folinic acid. Defects observed included improper neural tube closure at the cervical and/or lumbar region of the spinal cord, improper fusion of the spine resulting in major defects. Defects were also noted in the fore and hindbrain. This leads us back to the key question: Do you need the same level of folate at all times during pregnancy? Or should folate levels be stage specific?

Funding: NEIU Committee on Research.

doi:10.1016/j.ydbio.2009.05.410

Program/Abstract # 381
Characterization of the ontogeny of the circadian clock in the embryonic eye of Xenopus laevis
Kristen L. Curran, Sarah Meyer, Joseph Dodge, Jessica Solis, Brittany Bronson
Department of Biological Sciences, UW-Whitewater, Whitewater, WI, USA

Circadian oscillators are endogenous time-keeping mechanisms that drive twenty four hour rhythmic changes in gene expression, metabolism, hormone levels, and physical activity. We have characterized the developmental expression of genes known to regulate circadian rhythms. Core circadian oscillator genes (xPeriod1 and 2, xBmal1, xClock, xCryochrome1, and 2) as well as genes acted upon by the oscillator (outputs; xNocturnin and xNat) are expressed in the developing nervous system and eye. These genes were differentially expressed in non-neural tissues such as the somites, heart, cement gland, and pronephros. The ontogeny of circadian rhythm in the embryonic eye was studied by isolating eyes at the appropriate developmental age every 4h in a 12hour lightdark cycle. xBmal1