Organogenesis

Program/Abstract # 215
Foxg1 is necessary for thymic epithelial cell differentiation
Qiaozhi Wei, Brian G. Condie
Dept. of Genetics, Univ. of Georgia, Athens, GA, USA

Thymic epithelial cells (TECs) are an essential component of the thymic microenvironment and are necessary for the production of normal T cells. Cortical (cTEC) and medullary TEC (mTEC) subtypes have different functions and distinct gene expression profiles. However, the transcription regulatory network controlling the specification and differentiation of TECs is poorly understood. We have initiated a screen for additional transcription factors that are likely to be required for TEC development. This screen identified Foxg1 as a candidate regulator of thymic epithelial cell differentiation. We have found that Foxg1 is expressed in TECs before the onset of TEC differentiation and continues to be expressed in them throughout fetal development. Analysis of Foxg1 mutant mouse embryos revealed that TEC differentiation initiates normally but then becomes abnormal at E13.5–E14.5. Foxn1, which is required for the initiation of TEC differentiation, is expressed throughout fetal development in Foxg1 mutant TECs suggesting that Foxg1 activity is not necessary for Foxn1 expression. Our results suggest that Foxg1 may regulate TEC differentiation independently or in collaboration with Foxn1.

doi:10.1016/j.ydbio.2010.05.259

Program/Abstract # 216
Loss of Prox1 activity predisposes mice to pancreatitis
Joby J. Westmoreland, G. Klic, J. Blain, N. Harvey, G. Oliver, B. Sosa-Pineda
Dept. of Genetics and Tumor Cell Biology, St. Jude Children’s Research Hospital, Memphis, TN, USA

The homeodomain transcription factor Prox1 is widely expressed during mouse embryogenesis and in adult tissues, and is critical for proper development of several organs. We previously reported that Prox1 is broadly expressed in multipotent progenitors in the endocrine and ductal lineages, but not in differentiating or mature acinar cells. To investigate the role of Prox1 during pancreas development, we deleted this gene in pancreatic progenitors of Prox1 ΔPanc mice. Prox1 ΔPanc mice were viable and survived through adulthood. However, in their pancreatic tissues we uncovered several abnormalities, most notably in the developing and postnatal ducts and in the postnatal exocrine tissue. Our analysis also revealed various hallmarks of pancreatitis in the pancreas of older Prox1 ΔPanc mice including exocrine cell death, intrapancreatic activation of CPA, accumulation of stromal cells, mild fibrosis and the presence of immune infiltrates. Using a combination of microarray and immunohistochemical analyses, we uncovered increased expression of the tight junction protein Claudin2 in the pancreatic ducts of Prox1 ΔPanc mice. Therefore, we hypothesize that impaired ductal function due to increased Claudin2 expression is a major factor contributing to the pancreatitis observed in Prox1 ΔPanc mice.

doi:10.1016/j.ydbio.2010.05.260

Program/Abstract # 217
The mechanisms of the boundary formation between the stomach and intestine endoderm in chicken embryo
Kenta Watanabe, Kimiko Fukuda
Dept. of Biol. Sci., Tokyo Metropol. Univ., Tokyo, Japan

There is a morphological and functional boundary between the stomach and the intestine endoderm. It is reported that Sox2 is expressed in the epithelium of stomachs, proventriculus and gizzard, while CdxA is expressed in those of duodenum, small and large intestines in the chicken embryo. On day 4, Sox2 is expressed complementarily to CdxA at the stomach/intestine boundary. While our previous work revealed that stomach and intestine epithelium are specified at Hamburger and Hamilton stages 8–10 and 5–8, respectively, there is no report showing the timing or molecular mechanisms for establishment of the stomach/intestine boundary. To reveal mechanisms involved in the boundary formation between stomach and intestine, we first analyzed Sox2 and CdxA expression in the endoderm of chicken somite stage embryos. CdxA starts to be expressed at the caudal endoderm with some distance from the Sox2-positive rostral endoderm. As Sox2 expression expands caudally, small region expressing both Sox2 and CdxA weakly are found between Sox2- and CdxA-positive regions before their expression boundary becomes evident. Then Sox2 and CdxA were overexpressed in the presumptive intestine and presumptive stomach endoderm, respectively, to examine the interactions between these transcriptional factors. We found that Sox2 in the caudal endoderm suppresses CdxA, and also that CdxA inhibits Sox2 expression in the rostral endoderm. These results suggest that the reciprocal suppression between Sox2 and CdxA makes their boundary defined and will generate a clear border between stomach and intestine.

doi:10.1016/j.ydbio.2010.05.259

Program/Abstract # 218
hnRNP I is required for the digestive organ development and intestinal homeostasis
Wenyan Mei, Chin-Yee Chan, Jing Yang
The Research Institute at Nationwide Children’s Hospital, The Ohio State University, USA
E-mail address: Wenyan.Mei@nationwidechildrens.org.

hnRNP I is an RNA-binding protein that regulates tissue specific mRNA alternative splicing, mRNA stability, localization, and transla-
tion. Interfering with hnRNP I results in developmental defects in Drosophila and amphibian. We characterized the adult phenotype of brom bones, a zebrafish mutant deficient in hnRNP I, and found that hnRNP I plays a novel role in regulating intestinal homeostasis. Brom bones display a number of defects in the intestinal epithelium, including abnormal cell lineage development, uncontrolled intestinal cell growth, and a markedly increased Notch signaling activity. Our biochemical analysis demonstrates that hnRNP I inhibits Notch signaling by controlling the stability of the intracellular domain of Notch (NICD). In addition to its role in the adult intestine, we found that hnRNP I is expressed in the developing digestive system in the zebrafish embryo. Morpholino knockdown of hnRNP I in zebrafish embryos impairs the development of multiple digestive organs, including the liver, pancreas and intestine. Our results demonstrate that hnRNP I plays important roles in the digestive organ development and adult intestinal homeostasis.

doi:10.1016/j.ydbio.2010.05.262

Program/Abstract # 219
Tubular extension and cell epithelialization are coordinately regulated and influenced by adjacent tissues
Yuji Atsuta, Emi Ohata, Ryosuke Todokoro, Daisuke Saitou, Yoshiko Takahashi
Grad. Sch. of Biosci., Nara Inst. of Sci. and Tech., Nara, Japan

Epithelial tubules are essential functional components in major organs of the body. When a tubular structure forms during development, cells undergo epithelialization and robust extension. We have asked how these morphological events are coordinated in three-dimensional environment. To address these questions, we have recently developed a novel model using Wolffian duct (WD, also called a nephric duct), the earliest basis for kidney formation. WD is a simple structure, and extends in an anterior-to-posterior direction as a straight cord. Time-lapse imaging analyses revealed that cells located at the extending-front (tip region) are actively motile with numerous filopodia whereas cells residing in the rear region are epithelial in shape with less motility. Remarkably, when replaced into the front region, the rear cells can be converted to the front cell-like and restarted to extend posteriorly. These observations suggest that tissues surrounding the front region play instructive roles in the tubular extension. To further elucidate the molecular mechanisms underlying the tubular extension, we investigated the role of the chemokine SDF-1, SDF-1 (ligand) is expressed in tissues adjacent to the front cells of WD, which express the receptor CXCR4. When ectopically administered, SDF-1 attracted WD cells, suggesting that the WD extension is controlled by interactions between the neighboring tissues where SDF-1/CXCR4 signals instruct the front cells.

doi:10.1016/j.ydbio.2010.05.263

Program/Abstract # 220
Wnt4 induces tubule formation in metanephric mesenchyme by a non-canonical mechanism
Shunsuke Tanigawa, Honghe Wang, Yili Yang, Nirmala Sharma, Terry Yamauchi, Alan Perantoni
Cancer Dev. Biol. Lab., NCI, Frederick, MD, USA

Wnt4 and β-catenin are both required for nephrogenesis, but studies of TCF-reporter mice suggest that canonical activation does not occur in metanephric mesenchyme (MM) during its conversion to nephric epithelia. To study the mechanism, we developed a model that permits progenitor propagation in primary explant culture. Using this, we found that recombinant Wnt4 protein induces tubule formation and differentiation markers Lim1 and E-cadherin in MM cells but does not activate a TCF reporter or expression of canonical Wnt target gene axin2 and minimally affects stabilization of β-catenin, which remains phosphorylated. Furthermore, Wnt4 caused localized of ZO1 and occludin to tight junctions. On the other hand, canonical activation with a TCF-β-catenin fusion construct, stabilization of β-catenin with a proteasomal inhibitor, or treatment of cells with a Wnt agonist, all of which activated a TCF reporter, were unable to induce tubule formation, and canonical Wnt inhibitor dkk1 could not block differentiation. Since a canonical mechanism is not operative in tubule formation, we assessed the role of non-canonical mechanisms with small molecule inhibitors. Both CaMKII and JNK inhibitors blocked tubule formation, and treatment of MM cells with Wnt4 caused a rapid activation of CaMKII and JNK. These results demonstrate that the canonical Wnt pathway is not responsible for mesenchymal–epithelial transition in nephron formation and suggest that both the non-canonical calcium–Wnt and the JNK-mediated Wnt/PCP pathways are involved in Wnt4-induced tubulogenesis in the kidney.

doi:10.1016/j.ydbio.2010.05.264

Program/Abstract # 221
Tissue interactions during formation of the pronephric duct in Xenopus laevis
Ian Hakkonen, Amanda Pinto, Julie Drawbridge
Dept. of Biology, Rider University, Lawrenceville, NJ, USA

In Xenopus laevis embryos, formation of the excretory system occurs in three temporally distinct phases: From stages 22 to 26 the pronephros and pronephric duct (PD) rudiments can be observed segregating from intermediate mesoderm directly ventral to somites IV–IX; from stage 29–33 cell migration extends the PD posteriorly from the level of somite IX to somite XIV; from stages 30 to 38 rectal diverticulae (RD) extend anteriorly from the cloaca to meet and fuse with the PD ventral to somite XIV. We have performed a set of tissue extirpation and tissue marking experiments to 1) investigate the tissue interactions required for maintenance and elongation of PD and RD tissue, and 2) determine whether cells from tissues other than the PD primordium contribute to the elongating PD. Tissue extirpation studies show that the epidermis is essential for maintenance of the PD and that removal of the PD does not prevent anterior extension of the RD, but is required for subsequent RD maintenance. Tissue marking studies indicate that neural crest does not contribute cells to the PD during PD elongation; removal of trunk neural crest results in foreshortening of the embryo but does not interfere with PD elongation. In addition, the width of the PD does not decrease as the PD elongates, indicating that the embryo can regulate PD size and may recruit cells from surrounding tissues.

doi:10.1016/j.ydbio.2010.05.265

Program/Abstract # 222
A role for GDNF in pronephric duct cell migration in Xenopus laevis
Erin McCafferty, Vanessa Gerrard, Nicole Revere, Julie Drawbridge
Dept. of Biology, Rider University, Lawrenceville, NJ, USA

Anterior to posterior extension of the Xenopus pronephric duct (PD) is complex, consisting of three distinct temporal phases: During the first phase, pronephric and PD tissue segregates from flank mesoderm directly ventral to somites IV–VIII; during the second phase, cells migrate throughout the duct extending it to the axial level of somite XIV; finally, anterior extension of rectal diverticulae (RD) from the cloaca to the posterior tip of the PD is required to