Program/Abstract # 216

A Wnt receptor, Frizzled 7, is essential for foregut organ formation
Zheng Zhang4, Scott Rankin3, Aaron Zorn2
1Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA
2Cincinnati Children’s Hospital Medical Center Neonatology, Cincinnati, OH, USA

Recent work suggests a model that Wnt antagonist sfrp5 coordinates foregut specification and morphogenesis in Xenopus by repressing canonical and non-canonical Wnt signaling. However, Wnt11 and receptor Frizzled 7 (Fz 7) are co-expressed with Sfrp5 in the foregut endoderm, suggesting that the current model of Wnt-Off in the foregut may be too simplistic. Here I test the hypothesis that low levels of Wnt/Fz 7 signaling are essential for foregut development. Depletion of Fz 7 in the foregut by targeted injection of antisense Fz7 MO causes severe morphological defects of foregut organs such as in liver, pancreas and stomach. Moreover, Fz 7 is required at earlier stage for foregut patterning and foregut organ initiation. Loss of Fz 7 causes loss of foregut organ markers, early foregut epithelium disruption and cell morphology defects, including the change of cell division plane, cell size, orientation and shape, which is not accompanied by enhanced apoptosis, but by reduced cell proliferation. Further analysis showed that both canonical and non-canonical Wnt signaling are down-regulated by Fz 7 knockdown, suggesting that Fz 7 mediate a low level of Wnt activity that is required for foregut organ formation.

doi:10.1016/j.ydbio.2011.05.630

Program/Abstract # 217

Wnt/β-catenin signaling in the early mammalian anterior foregut endoderm
Latasha Redmond, Jason Spence, Aaron Zorn, James Wells
Cincinnati Children’s Hospital, Cincinnati, OH, USA

The Wnt/β-catenin signaling pathway is important for multiple developmental processes that include proliferation, differentiation, migration, and stem cell maintenance. In early gastrula and somite Xenopus embryos, repression of Wnt/β-catenin activity in the anterior endoderm is necessary for foregut identity to allow liver and pancreas development. In contrast, high Wnt/β-catenin activity in the posterior endoderm inhibits the anterior endoderm and promotes intestinal identity (McLin et al., 2007). However, it is still unclear if Wnt/β-catenin plays a role in foregut development in mammals. We hypothesized that Wnt/β-catenin downregulation is required for proper anterior endoderm development. To test this hypothesis, we used a genetic gain-of-function (GOF) approach to increase Wnt/β-catenin in the mouse endoderm. Two different mouse endoderm specific transgenic mouse Cre lines (Foxa3Cre and Foxa2CreEr) were used to recombine floxed β-catenin in the foregut endoderm. Using Foxa3 Cre, β-catenin is constitutively active in the anterior foregut endoderm around early embryonic day (E8.5). For the Foxa2CreEr line, tamoxifen was administered between E6.0—E6.5 and β-catenin is active in the endoderm shortly after gastrulation (E7.5). Using these two different Cre lines that are active at different time points allows us to obtain a broad overview of anterior endoderm patterning and development. Embryos were collected at different time points to analyze any consequential changes for activating β-catenin early in the mouse foregut endoderm. Activating β-catenin early in the presumptive foregut endoderm in mouse results in higher cytosolic levels of β-catenin in the foregut compared to their control littermates. β-Catenin activation in the endoderm affects several processes that include: patterning, foregut induction, foregut morphogenesis, and organ bud formation. In addition, β-catenin GOF mutant embryos display liver agenesis and defective ventral foregut morphogenesis. Severely mutant β-catenin GOF embryos also ectopically express posterior markers in the anterior foregut region, suggesting that β-catenin is required for posterior identity. Our data supports the hypothesis that Wnt/β-catenin regulation of endoderm patterning is conserved in the mouse anterior endoderm and low levels of β-catenin activity are required for foregut identity.

doi:10.1016/j.ydbio.2011.05.631

Program/Abstract # 218

Szzled functions as an essential BMP feedback inhibitor that preserves foregut progenitor survival
Scott Rankin2, Allison Prewitt4, Andrew Albee3, Zheng Zhang6, Alan P. Kenny6, Emily Shifley5, Aaron Zorn2
1Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA
2Cincinnati Children’s Hospital Medical Ctr Neonatology, Cincinnati, OH, USA
3Children’s Hospital Boston, Boston, MA, USA
4Sanford Burnham Medical Research Institute, La Jolla, CA, USA
5Nagoya University, Nagoya, Japan
6University of Wisconsin-Madison Dept of Anatomy, Madison, WI, USA

Background and aims: Lung, liver, and pancreas are induced from the ventral foregut endoderm by FGF and BMP signals from adjacent mesoderm. Despite significant gains in our understanding of this process, a fundamentally important question remains: what molecular mechanism(s) establish and maintain foregut progenitor cells. The aims of this research are to: (1) identify the genetic program activated in foregut endoderm progenitor cells by mesoderm signals and (2) determine the essential role of identified extracellular BMP modulator sizzled in foregut progenitor development. Methods: (1) We separated the foregut endoderm containing the organ progenitor cells from its apposed mesoderm at 0 somite stage (ss) and cultured it to 12 ss. We performed microarray analysis on this endoderm and compared its gene expression with foregut endoderm that had received mesoderm signal during this interval. (2) We performed foregut-specific loss-of-function studies on one identified gene, szzled, looking specifically on the loss-of-function effect on foregut in situ marker expression. We also examined by immunohistochemistry the effects of szzl loss-of-function on BMP signaling and cell death in foregut progenitor cells. Results: Microarray experiments identified 82 genes in the foregut endoderm territory specifically induced by mesoderm including ones functioning through the FGF, Notch, and BMP signaling pathways. Three are BMP pathway inhibitors, including sizzled (szzl). In situ hybridization reveals expression in foregut progenitor endoderm. Morpholino knockdown of szzl specifically in the foregut area produced a decrease in the expression of the foregut organ progenitor cell marker hhx5 and loss of heart, lung, liver, and pancreas anlage marker expression. Furthermore cell death and BMP signaling were increased in the context of szzl foregut loss of function. Conclusions: Together, these results suggest a mesodermally-induced BMP antagonism in the foregut progenitor is essential to maintain progenitor survival for lung, liver, and pancreas specification.

doi:10.1016/j.ydbio.2011.05.632

Program/Abstract # 219

Molecular regulation of the formation of the Phalanx Forming Region (PFR) at autopod stages of limb development
Joseph J. Lancman1, Takayuki Suzuki3, Sean M. Hassou4, Yina Li5, Chin Chiang6, John F. Fallon7
1Sanford Burnham Medical Research Institute, La Jolla, CA, USA
2Nagoya University, Nagoya, Japan
3Children’s Hospital Boston, Boston, MA, USA
4Massachusetts General Hospital, Charlestown, MA, USA
5Vanderbilt University Medical Center, Nashville, TN, USA
6Univ of Wisconsin-Madison Dept of Anatomy, Madison, WI, USA

The aims of this research are to: (1) identify the genetic program activated in foregut endoderm progenitor cells by mesoderm signals and (2) determine the essential role of identified extracellular BMP modulator sizzled in foregut progenitor development. Methods: (1) We separated the foregut endoderm containing the organ progenitor cells from its apposed mesoderm at 0 somite stage (ss) and cultured it to 12 ss. We performed microarray analysis on this endoderm and compared its gene expression with foregut endoderm that had received mesoderm signal during this interval. (2) We performed foregut-specific loss-of-function studies on one identified gene, szzled, looking specifically on the loss-of-function effect on foregut in situ marker expression. We also examined by immunohistochemistry the effects of szzl loss-of-function on BMP signaling and cell death in foregut progenitor cells. Results: Microarray experiments identified 82 genes in the foregut endoderm territory specifically induced by mesoderm including ones functioning through the FGF, Notch, and BMP signaling pathways. Three are BMP pathway inhibitors, including sizzled (szzl). In situ hybridization reveals expression in foregut progenitor endoderm. Morpholino knockdown of szzl specifically in the foregut area produced a decrease in the expression of the foregut organ progenitor cell marker hhx5 and loss of heart, lung, liver, and pancreas anlage marker expression. Furthermore cell death and BMP signaling were increased in the context of szzl foregut loss of function. Conclusions: Together, these results suggest a mesodermally-induced BMP antagonism in the foregut progenitor is essential to maintain progenitor survival for lung, liver, and pancreas specification.

doi:10.1016/j.ydbio.2011.05.632