Program/Abstract # 98
Cadherin-11 functions during mammary gland branching morphogenesis
Julie R. Hens, Ashish Kumar, John Nuttall, Neha Sanyal, Megan Vos
Dept. of Biol., St. Bonaventure Univ., St. Bonaventure, NY USA

Several signal transduction pathways are active during mammary gland development. Parathyroid related hormone (PTHrP) is necessary for the maintenance and formation of the mammary gland. Canonical Wnt signaling is necessary for the formation of the mammary placode, and BMP4 has been shown to be involved in both the outgrowth of the mammary bud, and nipple skin differentiation. Cadherin-11 (Cdh11) is a mesenchymal cadherin that is expressed in mesenchymal cells of synovial joints, bone, and mammary gland. During mammary gland development, Cdh11 is expressed in the mammary mesenchyme. To further examine the role of Cdh11 in the mammary gland, we used the Cdh11 knock-out (KO) mice and the mesenchymal cell line, CH10T1/2 cells. Cdh11 knock-out mice showed a decrease in branching morphogenesis, thicker ducts, and enlarged terminal end buds at both day 1 and 5 week old mice (n=5, p <.05). Since this phenotype in the Cdh11 knock-out mouse appears to be similar to the over-expression of Wnt5a in the mouse mammary gland, several Wnts (Wnt1, Wnt4, Wnt10b, and Wnt5a) were examined for changes in expression. Wnt 1, 4, and 5a had increased RNA expression in the Cdh11 KO mouse. C3H10T1/2 cells were treated with PTHrP, LiCl or BMP4 to determine if they regulate Cdh11. PTHrP stimulated Cdh11 expression while LiCl, which mimics canonical Wnt signaling by inhibiting GSK, decreased Cdh11 expression. BMP4 did not affect Cdh11 protein expression. Therefore a complex regulation of Wnt and PTHrP signaling is required for mammary gland formation and Cdh11 is a focal point during this regulation.

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Program/Abstract # 99
NHE1: A Novel Determinant in Branching Morphogenesis
Edmund C. Jenkins\textsuperscript{a,b}, Sajini Gundry\textsuperscript{a}, Jimmie E. Fata\textsuperscript{a,b}
\textsuperscript{a}Dept. of Biology, College of Staten Island, S.I., NY, USA
\textsuperscript{b}Biology Doctoral Program, Graduate Center, City University of New York, New York, NY, USA

Regulation of intracellular pH (pHi) is primarily a function of the ubiquitous plasma membrane Na+/H+ exchanger-1 (NHE1), which transfers cytosolic H+ across membranes in exchange for extracellular Na+. NHE1 protects cells against cytosolic acidification and its activation, which can occur via growth factor stimulation, has been shown to permit regulated cell adhesion, migration, and proliferation. Since the majority of these findings have been evaluated in immortalized cell lines, the function of NHE1 in regulating normal tissue morphogenesis has not been fully evaluated. Here we show that in a 4 day 3D tissue culture model of TGF-\textalpha-induced mammary branching morphogenesis, specific inhibition of NHE1 and the subsequent acidification of pHi with 10\muM N-Methyl-N-isobutyl Amiloride (MIA) dramatically disrupts development such that structures resemble unorganized large tissue masses. This phenotype is associated with unusual tissue protrusions and retractions (as determined by live video-microscopy), extensive proliferation, and prevalent ectopic expression of keratin-6. We had previously reported that noraml branching morphogenesis in our assay is dependent on TGF-\textalpha-induced ERK-1/2 activation. Here we report that NHE1 inhibition and subsequent acidification of pHi leads to more widespread and extensive activated ERK-1/2 three hours after TGF-\textalpha-stimulation. Moreover, inhibition of ERK-1/2 completely suppresses all MIA-associated phenotypes. These findings indicate that NHE1 regulation of pHi is essential for regulated ERK-1/2 signaling and ultimately normal tissue development.

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Program/Abstract # 100
Coordinate regulation of cell motility and intercellular adhesion during mammary branching morphogenesis
Andrew J. Ewald, Ryan S. Gray
Dept. of Cell Biology and Center for Cell Dynamics, Johns Hopkins University, Baltimore, MD USA

Epithelial morphogenesis requires coordinate interactions between epithelial cells, stromal cells, soluble molecular signals and the extracellular matrix (ECM). Mammary branching morphogenesis further involves coordination of motility between luminal epithelial and myoepithelial cells. We have previously shown that normal mammary branching morphogenesis proceeds through a novel form of collective epithelial migration, without leading cellular extensions. We are now focused on identifying the molecular signals that initiate epithelial motility, that sustain ductal elongation and that spatially restrict migratory epithelial cells and prevent their dispersal into the ECM. We take a combined imaging and molecular genetic approach to dissect the tissue level process of branching morphogenesis into a series of discrete changes in the properties and behaviors of individual epithelial cells. We have identified critical roles for microtubule dynamics in the restraining myoepithelial cells population and Rac signaling in the elongating luminal epithelial cells. We are now focused on dissecting the role of E-cadherin signaling in regulating the migratory behavior of mammary epithelial cells in different ECM microenvironments. We have identified conserved and microenvironment-specific roles for calcium based intercellular adhesion more generally and for the E-cadherin pathway specifically. Our current work focuses on identifying the critical molecular signals in the ECM that determine cellular invasion strategies and that regulate tissue integrity during morphogenesis.

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Program/Abstract # 101
The coupling mechanism to generate synchronized oscillation of segmentation clock in mouse
Yusuke Okubo\textsuperscript{a}, Akatsuki Kimura\textsuperscript{a}, Shigeru Chiba\textsuperscript{a}, Yumiko Saga\textsuperscript{a}
\textsuperscript{a}Division of Mammalian Development, National Institute of Genetics, Japan
\textsuperscript{b}Division of Cell Architecture, National Institute of Genetics, Japan

The periodicity of somites is established by a clock mechanism which regulates cycic gene expressions in the presomitic mesoderm (PSM). In zebrafish, the gene oscillations in individual cells are synchronized with neighboring cells via so-called coupling mechanism utilizing Notch signaling. However the synchronizing mechanism in mouse somitogenesis is not clarified yet. To address this problem, I employed mosaic analyses using chimera embryos consist of two types of cells, wild-type and mutant lacking the Notch signaling component. In Dll1 chimera embryos, cyclic gene expressions were detected as abnormal broad patterns throughout PSM, indicating that Notch signaling through Dll1 is required for synchronized oscillation. Next, I focused on Lunatic fringe (Lfg) that is a core regulator of the clock in mouse. Lfg had been shown as a cell autonomous negative regulator of Notch activity. In the chimera embryos consist of wild-