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Evagination of the Thyroid Primordium Involves Novel Cell Behaviors

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EVAGINATION OF THE THYROID PRIMORDIUM INVOLVES NOVEL CELL BEHAVIORS. ((G.M. Kinebrew and S.R. Hilfer)) Departments of Biology, John Carroll University, University Hts, OH and Temple University, Phila., PA

The thyroid forms as a pouch in the pharyngeal floor during early embryogenesis. Our previous work showed that evagination results in part from contractile activity within successive rings of cells which become annexed by the original endodermal placode. This study was designed to answer the question of how a sheet of cells shaped like a washer is converted into a cylinder having the diameter of its inner margin. Individual cells were tracked by video time lapse microscopy and image analysis. There was little evidence of convergent extension at the edge of the evagination, a process which would narrow the ring of cells. Instead, cells jostled to form clusters, separated by deep clefts, with a concomitant decrease in cell diameter and increase in the size of the apical protrusions. The clusters moved, changed shape and decreased in surface area as they first piled up at the rim of the evagination and then moved below the surface. Changes in contractile rings were monitored in time lapse images with low levels of BODIPY-phallicidin. Individual cells underwent cyclic changes in circumference of the apical actin bundles with a tendency towards narrower apices in clusters about to enter the pit. Treatment with dihydrocytochalasin B resulted in flattening of the pit margin, decrease in size of cell protrusions, increased diameter of contractile rings, and relaxation of clefts between clusters with little effect on the established central pit. The normal pattern was reestablished after washing. These results demonstrate that evagination of the thyroid occurs gradually by partitioning the newly formed circular domains into smaller units of cell clusters, which rearrange within the epithelial sheet. The decrease in diameter caused by compaction of the clusters deforms the sheet, causing it to rise above the pharyngeal surface and to form clefts along stress lines.

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Labeling of the interface between the collecting duct ampulla and mesenchyme during nephrogenesis ((R. Strehl, S. Kloth, P. Steiner, W. W. Minuth.)) University of Regensburg, Dep. of Anatomy, D-93053 Regensburg

In the neonatal kidney the ampullary collecting duct epithelium acts as an inducer and generates all of the nephron anlagen. By this mechanism it pilots the architecture of the whole kidney. A presupposition for a successful induction is a close tissue interaction between the basal aspect of the collecting duct epithelium and the surrounding mesenchyme. Up to date it is unknown which are the morphogenic mechanisms leading to new nephron anlagen. To gain new insights into the induction process, we investigated the contact sites between the ampullary collecting duct epithelium and the nephrogenic mesenchyme in neonatal rabbit kidneys. We raised monoclonal antibodies (mab), which should give us information about specific structures localized at the tissue interface during the induction process. With the newly developed mab CDamp 1 we analyzed by immunohistochemical methods the reaction pattern between the collecting duct ampulla and the nephrogenic mesenchyme. We found an intensive mab CDamp 1 reaction profile between the basal aspect of the collecting duct ampulla and the mesenchyme. The labeling was highly concentrated at the ampullar tip and continuously decreased down towards the neck and the shaft. In the maturing and adult collecting duct no reaction could be found. The binding pattern is different from known markers such as laminin or peanut lectin (PNA). At the moment we are elaborating the subcellular location and the molecular characteristics of the newly found antigen.

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DIFFERENTIATION OF EMBRYONIC RENAL COLLECTING DUCT EPITHELIUM IS INFLUENCED BY THE ELECTROLYTE ENVIRONMENT. ((P. Steiner, R. Strehl, S. Kloth, M. Jamous*, M. Tauc*, W. W. Minuth.)) University of Regensburg, Dep. of Anatomy, D-93053 Regensburg and *Dep. of Cellular and Molecular Physiology, University of Nice, France

During kidney development the collecting duct acts as an inducer, which generates all of the nephrons. As the development continues, one part of the collecting duct cells in the ampullar tip retains the induction capability, the other develops into the Principal (P) and Intercalated (IC) cells. The molecular mechanisms of individual P an IC cell features are unknown. We investigated the development of collecting duct cells. The embryonic epithelia were kept on a tissue carrier within a kidney-specific support. The apical urine and the basal serum compartments were simulated in a gradient culture container. The two sides of the epithelium were each constantly perfused with a different medium. At the apical side was standard Iscove's modified Dulbecco's Medium (IMDM) with 85 mMol/l Cl- and 112 mMol/l Na+. Influence on protein expression was obtained by adding NaCl and Na-gluconat. Final concentrations were 99 mMol/l Cl- and 126 respectively 137 mMol/l Na+ as it is observed in the serum of neonatal rabbits. The generated collecting duct cells were traced with morphological and immunohistochemical methods. Light microscopy revealed morphologically faultless epithelia following gradient perfusion culture in standard, NaCl and NaCl / Na-gluconat adapted IMDM. Immunohistochemistry with the monoclonal antibodies 703 and 503 revealed that addition of NaCl to the medium during the whole perfusion culture period upregulated individual P and IC cell proteins within the epithelium. Immunopositive cells increased from less than 5% cells in controls to more than 80% in adapted IMDM. In contrast, the expression of the collecting duct protein PCD9 or the development of PNA binding is not influenced by the extracellular environment.

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DISTRIBUTION OF UROKINASE-TYPE PLASMINOGEN ACTIVATOR DURING CARDIAC DEVELOPMENT ((S. Ghosh and P.R. Brauer.)) Dept. of Biomedical Sciences, Creighton University, Omaha, NE 68178.

Studies suggest that extracellular and cell-surface proteases including urokinase-type PA (uPA), are important mediators of cell migration and tissue remodeling. Previous work shows uPA is synthesized during early stages of cardiac development but little data is available regarding the spatial and temporal distribution of uPA before, during, and after cushion cell migration. In this study, we immunolocalized uPA during these stages of heart development in the chick. Prior to cushion tissue formation, immunostaining was only found in the myocardium and the cardiac ECM. Myocardial immunostaining was observed at all stages and was more intense in areas undergoing trabeculation. Some immunopositive endocardial cells were also observed in the atrioventricular and outflow tract. During cushion cell migration, uPA immunostaining was prevalent in endocardial cells undergoing epithelial-mesenchymal transformation and surrounded migrating cushion cells. B y stage 21, much of this immunostaining was lost including that in the ECM surrounding the cushion cells. These observations suggest that uPA plays an important role in cushion cell migration and in myocardial remodeling. Immunostaining in the ECM may represent pro-uPA since uPA is usually secreted as a pro-enzyme and can be activated by binding to specific cell-surface receptors. Therefore, if uPA is an important mediator of cushion cell migration, uPA synthesis by these cells may not be a pre-requisite for their migration, rather, it may be mediated by the timely expression of uPA receptors on transforming endocardial cell-surfaces and cushion cells. The loss of extracellular immunostaining in cushion tissue of older hearts is consistent with the idea that cushion cells utilize ECM-sequestered uPA for migration. Supported by NIH (HL50397).

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HYALURONAN AND CD44 MEDIATE DUCTAL BRANCHING MORPHOGENESIS IN THE DEVELOPING MOUSE PROSTATE. ((P. Gakunga, S. Shuster, G.R. Cunha,* and R. Stern)) Departments of Pathology and *Anatomy, UC San Francisco School of Medicine, San Francisco CA 94143-0506. (Spon. by D. Bainton)

Hyaluronan (HA), a macromolecular carbohydrate polymer of the extracellular matrix (ECM) is prominent early in embryogenesis. Increased levels of HA correlate with rapid tissue growth and cell movement. CD44, a transmembrane glycoprotein, and the predominant receptor for HA on vertebrate cells, is composed of 20 exons, 10 of which are variably expressed due to alternative splicing of the nuclear RNA. These variant isoforms have a more restricted distribution. Mouse anterior prostate (AP) glands were examined for the expression of HA and CD44 at various postnatal timepoints. A biotinylated HA-binding peptide derived from cartilage was used in the histolocalization of HA. Antimouse CD44 monoclonal antibodies were used in the immunolocalization of CD44. In addition, reverse transcriptase polymerase chain reaction (RT-PCR) analysis was used to to map the temporal expression of specific CD44 isoforms. At each timepoint, HA was localized exclusively in the stromal ECM, most prominently at the early timepoints. Early in development, CD44 expression was prominent in the mesenchyme. However, with the onset of ductal branching morphogenesis, CD44 expression became associated exclusively with epithelial cells. Development of the mouse prostate gland occurs postnatally. Using a serum-free organ culture system and computerized morphometrics, we examined the effect of the antimouse CD44 antibodies on ductal branching morphogenesis of the mouse AP from birth to six days. Balb/c mouse APs were cultured on floating Millicell membranes in basic medium containing insulin and transferrin. In the presence of 10^{-8} M testosterone, organs underwent ductal branching morphogenesis. Treatment with either neutralizing anti- CD44 antibodies or the enzyme hyaluronidase inhibited androgen-stimulated ductal branching morphogenesis. HA-CD44 interactions play a significant role mediating androgen-induced prostatic epithelial growth and ductal branching morphogenesis. An apparent "switch" of CD44 expression from stroma to epithelium is a key event in this process. (Supported by DHHS NIH grant GM46765)