Program/Abstract # 295

Wnt/β signaling regulates expansion but not survival of mammary stem cells

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Wnt/β-catenin signaling is necessary for initiation of mammary gland development. Postnatally, Wnt signaling has been implicated in the expansion or survival of mammary stem cells. To distinguish between these roles, we used a temporally controllable doxycycline-inducible bi-transgenic system to ectopically and reversibly express the potent secreted Wnt inhibitor Dickkopf1 (Dkk1) in mammary basal cells during puberty. Dkk1 expression was induced from the onset of puberty, and produced a striking block in ductal elongation and branching that was associated with decreased mitotic activity in the stem cell-rich terminal end buds. Analysis of the (CD29/CD49f) hiCD24+ mammary stem cell population revealed that the percentage of stem cells within the mammary epithelium was unchanged by Dkk1 expression. As the total number of epithelial cells was greatly decreased in Dkk1-expressing mammary glands, these data indicate that expansion/self renewal of mammary stem cells is blocked concomitant with inhibition of ductal expansion, but that a core population of mammary stem cells is maintained. Consistent with this hypothesis, withdrawal of doxycycline mid-way through puberty or during pregnancy produced partial reversal of the block to ductal outgrowth and branching. Our results indicate that Wnt signaling is required at multiple stages of mammary gland development, and regulates expansion, but not survival of mammary stem cells.

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Program/Abstract # 296

Dicer is required for maintenance of hair follicle stem cells in adult skin

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Multiple miRNAs are expressed in developing and postnatal skin and hair follicles, and constitutive deletion of the miRNA processing enzyme Dicer in embryonic mouse skin causes failure of hair follicle morphogenesis and subsequent follicular degradation. To determine whether Dicer and miRNA functions are required in established hair follicles and epidermis in the adult mouse we generated Krt5-rtTA tetO-Cre Dicerfl/fl mice in which the Dicer gene can be inducibly deleted in the basal epidermis and hair follicle outer root sheath, including hair follicle stem cells, by dosage with oral doxycycline. Inducible Dicer deletion starting at postnatal day (P) 20, when hair follicles are just entering the first postnatal growth cycle, resulted in loss of external hair starting within 10days of doxycycline treatment. Induction of mutation during the resting stage of the hair follicle growth cycle did not immediately result in histological abnormalities or loss of hair. However, expression of the hair follicle bulge stem cell markers K15 and CD34 was lost soon after induction. Subsequent hair plucking stimulated a new cycle of hair follicle growth in control littermate, but not induced Dicer mutant skin. These results demonstrate a requirement for Dicer in maintaining the ability of adult hair follicles to grow and regenerate, and indicate that Dicer is required for maintenance of hair follicle epithelial stem cells.

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Program/Abstract # 297

Hedgehog-responding stem cells regenerate the anagen hair follicle

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The hair follicle cycles through phases of growth (anagen), apoptotic regression (catagen), and quiescence (telogen). Each anagen is accompanied by regeneration of the proximal follicle using stem cells from the follicular bulge. The Hedgehog (Hh) protein, Sonic Hedgehog (Shh) is necessary for cycle progression into anagen. While Shh is required for follicle regeneration, it is not clear if Shh signals directly to bulge stem cells, or if it drives proliferation of transient amplifying cells during regrowth. To investigate Hh signaling to stem cells, we examined the expression of the zinc-finger transcription factors Gli1 and Gli2, since in cells receiving a Hh signal activated Gli2 induces transcription of Gli1, a sensitive reporter of Hh signaling. Expression analysis of *Gli1-lacZ* and *Gli2-lacZ* indicates the telogen bulge contains a population of Hh-responding cells. We also used Genetic Inducible Fate Mapping with a *Gli1-CreER* allele engineered to mark Hh-responding cells and their progeny after the administration of tamoxifen. In *Gli1-CreER;R26R* mice, we found that Gli1-expressing cells labeled in telogen expand markedly during anagen, with staining seen in the entire follicle below the bulge. Furthermore, stained cells are maintained over many hair cycles. Thus, Hh-responding cells in the quiescent telogen bulge regenerate the proximal follicle — a function of bulge stem cells. These findings suggest that Hh-responding bulge stem cells participate in hair follicle homeostasis.

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Program/Abstract # 298

The dermal papilla regulates the activity of pluri-potent follicular stem cells and the differentiation of their progeny to direct morphogenesis and regeneration of the hair

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Summary: The hair follicle is a model system for studying tissue morphogenesis in the embryo and regeneration from epithelial stem cells in the adult. In the work reported here, lineage analysis identifies keratinocytes in direct contact with the specialized mesenchymal component of the follicle, the dermal papilla, as pluripotent stem cells. We have developed mouse strains that express cre recombinase specifically in the dermal papilla. In conjunction with the appropriately modified alleles, these allow genetic manipulation in the dermal papilla within the context of fully formed hair follicles. Single gene alterations within this population demonstrate that the activities of discrete genetic pathways in the dermal papilla regulate the activation,

proliferation and maintenance of adjacent follicular stem cells and the differentiation of their progeny to specify the size, morphology and pigmentation of the hair shaft. Furthermore, signaling to the DP from surrounding keratinocytes coordinates the activities of follicular stem cells and the dermal papilla to orchestrate morphogenesis. These studies complement analysis of genetic manipulations in follicular stem cells to provide detailed insight into the interactions between epithelial stem cells and their mesenchymal niche in an adult mammal.

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Program/Abstract # 299

Pax6 is required for neuronal progenitor cell proliferation during cone cell regeneration

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Pax6 is required for proper development of the vertebrate eye. Unlike mammals, the adult zebrafish retina possesses a robust regenerative response. We tested whether Pax6 was required for rod and cone photoreceptor regeneration in the light-damaged adult zebrafish retina. In this model, rod precursor and Müller glial cell divisions precede regeneration of the photoreceptors. Pax6-positive neuronal progenitors arise from the Müller glia, continue to divide, and migrate along the Müller glial processes to the outer nuclear layer where they differentiate into the lost photoreceptors. Zebrafish contain two *pax6* genes, *pax6a* and *pax6b*, that are functionally redundant in retinal development. To study the role of Pax6 proteins during retinal regeneration, we developed a method to conditionally inhibit protein expression by *in vivo* electroporation of a cocktail of anti-*pax6a* and anti-*pax6b* morpholinos into the regenerating retina. The *pax6a/6b* morphant retina exhibited reduced Pax6a and Pax6b protein expression. While the *pax6a/6b* morphant did not exhibit inhibition of rod precursor or Müller glial cell division, it did exhibit inhibition of Müller glial-derived neuronal progenitor proliferation. This subsequently inhibited cone photoreceptor regeneration. However, increased levels of rod precursor cell proliferation lead to a complete regeneration of rod photoreceptors, which is independent of Müller glial-derived neuronal progenitors. Thus, Pax6 is required for neuronal progenitor cell amplification during cone cell regeneration in the light-damaged adult retina.

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Program/Abstract # 300

Live cell imaging of the zebrafish dermomyotome

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The zebrafish dermomyotome contains a population of myogenic progenitors that contributes to growth of the myotome and possibly to the adult muscle stem cell population. We have characterized a unique line of transgenic zebrafish expressing GFP in the dermomyotome. Shortly before 24 h we find distinct expression of GFP in a pattern strongly reminiscent of the expression of Pax7 in myogenic progenitors. We have confirmed the identity of these cells as myogenic progenitors by colocalization of GFP and Pax7. We have also found changes in expression of GFP that mimic changes in Pax7 expression when altering Hedgehog signaling. We are now using this transgenic line as a tool to study novel aspects of dermomyotome development. The longevity of GFP has given us insight into the descendants of these myogenic progenitors. We have found GFP positive, small diameter muscle fibers, which express slow myosin, suggesting that the dermomyotome may give rise to slow fibers as well as fast fibers. We have also found that the sparse cells of the dermomyotome dispersed on the lateral surface of the myotome extend projections and make connections with one another. This morphology suggests possibilities for direct communication between cells within the dermomyotome. Finally this line allows us to follow myogenic cell behaviors in real time using time lapse microscopy. So far we have been able to confirm that this population is actively dividing. We believe this transgenic line provides a distinctive tool in studies of myogenesis.

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Program/Abstract # 301

Culture of primary myogenic cells derived from adult muscle and electric organ of the gymnotiform *S. macrurus*

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We have established primary myogenic precursor cell cultures from adult skeletal muscle and the muscle derived electric organ (EO) of the gymnotiform *S. macrurus*. Our protocol was modified from those of Fauconneau et. al., (2000) for the isolation of rainbow trout myoblasts. Briefly, skeletal muscle and EO were dissected from adult fish, cut into 5 mm^3 chunks and incubated overnight in growth medium (GM: 10% FBS/1% penicillin and streptomycin in L-15 medium). Tissue was then minced to 1 mm^3 pieces and treated with collagenase and trypsin for enzymatic dissociation. Following centrifugation, cells were plated on collagen-coated wells and maintained to confluence in GM. Myogenic cell differentiation was induced by switching GM to differentiation medium (DM: 2% FBS/1% penicillin and streptomycin in L-15 medium) for 5 days. We detected both mono and multinucleated cells that were immunolabeled with antibodies against mature muscle markers desmin, titin, and sarcomeric MHC. This differentiation capacity was observed even after several passages. These data represent the first known isolation of myogenic precursor cells from any electric fish muscle, as well as their differentiation in vitro. Establishment of a *S. macrurus* myogenic precursor cell line will permit investigations of molecular and cellular mechanisms involved in the regeneration of muscle and its derived tissues. Further, a myogenic precursor cell line will facilitate our studies on the developmental origin of EO.

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Program/Abstract # 302

Identifying the precursor zone of muscle satellite cells in *Xenopus laevis* embryos

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