Developmental Biology 331 (2009) 394–396

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Abstracts

Concurrent session 4: Neural and tissue specific stem cells

Program/Abstract # 29
Foxd3 regulates neural crest multipotency and self-renewal

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The neural crest (NC) is a specialized group of progenitor cells that arise from the developing spinal cord. At the onset of migration, NC is a heterogeneous pool of multipotent and fate-restricted progenitors that follow regionally defined pathways to sites of differentiation, giving rise to a variety of cell types including neurons, glia, melanocytes, smooth muscle, and cartilage. The forkhead transcription factor Foxd3 is required for self-renewal and maintenance of a multipotent state in two other progenitor cell types: embryonic stem cells and trophoblast stem cells. Foxd3 is also one of the earliest molecular markers of the NC. NC deletion of *Foxd3* in the mouse embryo results in severe defects including craniofacial defects, and complete loss of the enteric nervous system. The progenitor pool is depleted and much of the NC is lost by apoptosis in mutant embryos. Lineage labeling of *Foxd3* mutant embryos demonstrates that vagal NC fails to migrate into the foregut, and is greatly reduced in the outflow tract of the heart. Surprisingly, reduced cardiac NC mediates cardiovascular remodeling but not parasympathetic innervation of the heart. *In vitro* clonal analysis of multipotency demonstrates that *Foxd3* mutant NC has reduced potency to differentiate into multiple lineages. Serial neurosphere culture experiments indicate that mutant NC does not maintain progenitor self-renewal. These results demonstrate a global role for Foxd3 in NC maintenance along the anteriorposterior axis, and establish the requirement of Foxd3 in maintenance of NC stem cell subpopulations.

doi:10.1016/j.ydbio.2009.05.038

Program/Abstract # 30
The role of Gli3 in the neurogenesis of the forebrain

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The Gli3 is the major negative transducer of the Hedgehog (Hh) pathway as a processed form. Although its role in embryonic patterning has been extensively studied, later role of *Gli3* in cortical development, especially on the regulation of neural stem/progenitor cells, is largely unknown. In this study, we conditionally removed *Gli3* pan-neuronaly starting at E11 using *Nestin-Cre* (*NC*) mice. Compared to the controls (*NC; Gli3*$^{c/+}$), the mutants (*NC; Gli3*$^{c/-}$) exhibit enlarged lateral ventricles and the thinner cortex without a dramatic change in cortical lamination. The proliferation in the ventricular zone (VZ) is reduced at E16 and the intermediate progenitors (IPs) are mostly gone by E18 in mutants. Although the activation of canonical Wnt pathway has been shown to suppress IP formation, there was no change in the *Axin2* expression despite an up-regulation of β-catenin protein level throughout the developing cortex in *Gli3* mutants. Interestingly, more GFAP-positive cells with long radial processes were observed along the ventricular walls in the postnatal mutants. Taken together with the defect in the integrity of ependymal cells along the lateral wall of the lateral ventricle, this suggests that the cellular composition of the neurogenic subventricular zone (SVZ) has changed due to the earlier accumulation of radial glia at E18 that resulted in increased number of astrocytes and/or neural stem cells in the postnatal *Gli3* mutants. In summary, we show that *Gli3* plays an essential role in regulating neurogenic IP formation independent of Wnt pathway during development and in the maintenance of the postnatal SVZ.

doi:10.1016/j.ydbio.2009.05.039

Program/Abstract # 31
The role of cyclical Ph-Snail1 expression during stem cell migration

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Cell migration plays a crucial role in development and disease. The transcriptional repressor *snail* drives cell migration in many developmental systems as well as in some metastazing cancers. *In vivo* studies, where single cells can easily be traced and manipulated, are crucial for our understanding of the role of *snail* in cell migration. We are using the mesoteloblasts, the mesodermal stem cells of the crustacean *Parhyale hawaiensis*, as a model for cell migration. The mesoteloblasts are located under just one layer of cells, making them amenable to *in vivo* tracking. Moreover, their two precursor cells are amenable to injections, allowing lineage tracers and gene targeting reagents to be delivered specifically to these cells. Previous research in our lab has found that both *Ph-snail1* mRNA and protein are expressed in the mesoteloblasts when they are migrating, but not

before and during cell division, when they are stationary. We are testing the hypothesis that the cycling of Ph-Snail plays an important role in the migratory behavior of the mesoteloblasts by performing *Ph-snail1* misexpression and knockdown experiments.

doi:10.1016/j.ydbio.2009.05.040

Program/Abstract # 32
Blood stem cells: Regulation by the niche
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Hematopoietic stem cells (HSC) are responsible for the lifelong generation of all blood cell lineages and for rapid regeneration of the blood system after injury. To ensure their appropriate participation in these processes, and limit their possible exhaustion or transformation, HSC number and function must be tightly regulated. Regulation of HSCs appears to be achieved through a delicate balance of stem cell developmental decisions, including quiescence, self-renewal, differentiation, survival and migration. These in turn are governed by both HSC-intrinsic signaling pathways and extrinsic signals provided by the stem cell microenvironment, or niche. Our studies employing direct prospective isolation indicate that bone-lining osteoblasts are sufficient to regulate HSC number and function and therefore act as stem cell-regulatory niche cells. Osteolineage niche cells can directly affect the normal proliferative and migratory activities of HSCs, and recapitulate in vitro functional changes in the regulatory interactions of niche cells with HSCs. Direct isolation of osteoblastic niche cells thus provides a novel method for interrogating environmental signals that modulate stem cell self-renewal. Using this system, we have found that deregulation of extrinsic signals provided by the niche contributes directly to stem cell dysfunction in the contexts of aging and leukemic progression. These ongoing studies are revealing discrete cellular and molecular players that are critical for normal HSC activity, and ultimately will clarify the mechanisms by which HSC function is perturbed through aging and disease.

doi:10.1016/j.ydbio.2009.05.041

Program/Abstract # 33
Wnt16 is required for specification of hematopoietic stem cells
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Hematopoietic stem cells (HSCs) are self-renewing progenitor cells with the ability to provide all mature hematoimmune cells over the life of an individual. Understanding the endogenous mechanisms guiding HSC specification would be of clinical value for regenerative medicine. We have focused on the role of Wnt signaling in specifying HSCs during development, using zebrafish as a model organism. Wnt signaling regulates many patterning events during embryonic development, and, in adults, has been shown to be important in regulating stem cell homeostasis. Dysregulation of Wnt signaling is associated with disease, especially cancer. We have identified a single zebrafish Wnt ligand, Wnt16, that is required for HSC specification during embryonic development. In embryos where *wnt16* mRNA levels have been depleted by injection of a splice-blocking antisense morpholino oligonucleotide, HSCs are absent or greatly reduced based on the absence of HSC-specific transcripts and reporter transgenes. Although HSCs are absent in morphant animals, most other tissue types, including primitive blood, vasculature, somites, pronephros, notochord, and neural tube are present and animals appear grossly normal. Wnt16 is normally expressed in the dorsorostral somite, and we have identified somitic patterning defects that may be associated with the observed hematopoietic phenotypes. Wnt16 does not work through β-catenin/Tcf-dependent signaling at any of the timepoints during which its activity is required for HSC specification. Thus, we have identified a single noncanonical Wnt ligand that is required for emergence of HSCs during embryonic development.

doi:10.1016/j.ydbio.2009.05.042

Program/Abstract # 34
Dynamic binding of transcription factors and chromatin modifiers during endoderm formation in hESCs
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Endoderm is one of the first cell types to form in the vertebrate embryo. While the specification of endoderm is known to be mediated by Smad2 and FoxH1, components of the Nodal signaling pathway, their downstream targets and their ability to regulate chromatin has largely remained elusive. We have developed Human Embryonic Stem Cells (hESCs) as a model system in which to explore the biochemistry and genomics of endoderm. This is an important model as the endogenous tissues in both mouse and human are not accessible to study at this level of detail. To this end, we have performed ChIPSeq to elucidate the targets for Smad2/3, FoxH1, H3K4me3, and H3K27me3 within both undifferentiated hESCs and in endoderm derived from hESCs. While we find more than 400 targets for Smad2/3 in both undifferentiated hESCs and derived endoderm, less than 10 of these targets are the same at both timepoints, suggesting that Smad2/3 complexes are highly dynamic during early embryonic cell specification. Furthermore, we show that the presence of Smad2/3 binding, but not FoxH1 or the active mark H3K4me3, is highly correlated with gene expression, especially within the endoderm. Overall, we will discuss our findings of how these transcription factors and chromatin marks are predictive of transcriptional output and subsequent early embryonic fates.

doi:10.1016/j.ydbio.2009.05.043

Program/Abstract # 35
Interplay between Nodal and BMP signaling in the maintenance of the zebrafish mesodermal precursor pool
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Stem cells play an essential role in pattern formation and tissue growth during embryogenesis, primarily due to the ability of a stem cell pool to produce differentiated progeny while at the same time undergoing self-renewal. We have previously shown that Bmp4 signaling plays a critical role in establishing both the proliferative state and maturity of the zebrafish chordoneural hinge, a stem cell pool that supplies the tail with mesodermal precursors during segmentation. While we have shown that BMP signaling is critical to balancing stem cell self-renewal and maturation, these cells receive input from other signaling families. In order to better understand how these factors interact to influence fate within mesodermal precursors, we have begun to analyze genes known to play roles in stem cell maturation. Members of the *inhibitor of differentiation* (*id*) family of genes, involved in regulating stem cell maturation, are ex-