Program/Abstract # 435
Expression of the putative microRNA processing protein, Ars2, in the developing and adult mouse retina
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There is growing evidence that microRNAs play an important role in regulating transcriptional programs that control both the maintenance of stem and progenitor cells, and their differentiation into tissue-specific lineages during development. A recently identified gene, Arsenate resistance gene 2 (Ars2), is thought to play a role in microRNA processing and RNA metabolism, and is essential for at least two major processes of embryonic development: cellular differentiation and survival. We sought to determine whether Ars2 is expressed in the developing and adult retina. Immunocytochemical analysis was performed using four antibody clones generated against either the N or C terminus of human Ars2. Multi-labeling using retinal cell class-specific and cell cycle-related antibodies was performed on mice ranging from P0 to P90 in age. We describe dynamic patterns of Ars2 expression that include: (i) the upregulation of Ars2 in a subset of putative newborn retinal neurons, and (ii) cell-class-specific changes in Ars2 subcellular localization that persist in the adult retina. Ars2 expression appears to be specific to neuronal subtypes, and is not detected in Müller glia. The timing and cellular distribution of these changes in Ars2 expression is consistent with its playing a role in cell cycle exit and initiation of cellular differentiation. These results suggest a role for Ars2 in central nervous system development and adult retinal homeostasis.

doi:10.1016/j.ydbio.2009.05.461

Program/Abstract # 436
Building the eye: Semaphorin3A/neuropilin-1 signaling regulates periorocular neural crest migration
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Vertebrate eye development is an intricate process involving the merger of the optic cup, lens, ectoderm and neural crest cells. The neural crest, a highly migratory multipotent cell population, contributes to numerous ocular tissues including the cornea, ciliary body, and stroma of the iris. However, little is known about the molecular mechanisms underlying neural crest migration during ocular development. In exploring the spatiotemporal behavior of neural crest cells during eye development, we find that semaphorin3A (Sema3A) is expressed in the lens placode and epithelium continuously throughout eye development. Interestingly, neuropilin-1 (Npn-1) is expressed by periorocular neural crest but downregulated, in a manner independent of the lens, by the subpopulation that migrates into the eye and gives rise to the cornea endothelium and stroma. In contrast, Npn-1 expressing neural crest remain in the periorcular region and contribute to the anterior uvea and ocular blood vessels. Introduction of a Sema3A inhibitor results in the premature entry of neural crest cells over the lens that phenocopies lens ablation. Furthermore, Sema3A inhibits periorcular neural crest migration in vitro. Taken together, our data reveal a novel and essential role of Sema3A/Npn-1 signaling in coordinating periorcular neural crest migration that is vital for proper ocular development.

doi:10.1016/j.ydbio.2009.05.463

Program/Abstract # 437
The roles of FGFs, Wnts and BMPs in the induction of neural crest during early embryonic chicken development
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Neural crest cells (NCCs) make up a multipotent cell population that plays a key role in vertebrate development, and their dysregulation has been implicated in a number of human diseases. NCCs contribute to a wide variety of cell types, many of which are unique to vertebrates, most notably the bone, cartilage and connective tissue of the cranium. Traditional models of NC induction considered interactions of the ectoderm with underlying mesoderm or interactions between neural and non-neural ectoderm. Contrary to these models, recent evidence suggests that NCCs are specified even before the formation of definitive mesoderm or neural territory. Here the induction of neural, mesoderm and NCCs is analyzed through the application of beads loaded with combinatorial mixes of signaling molecules placed in the area opaca border of stage 3 chick embryos. After different incubation times, treated embryos were analyzed by in situ hybridization to determine the expression of NC (Pax7), neural (Sox2) and/or mesodermal (Brachyury) markers. Preliminary experiments identified specific cocktails able to induce NCC markers alone. The appearance of Pax7 in the absence of either Brachyury or Sox2 are in agreement with recent studies pointing towards an early induction of NCCs. Furthermore, these studies provide a controlled experimental model that offers the possibility to identify signaling pathways previously unrecognized for NC induction.

doi:10.1016/j.ydbio.2009.05.464

Program/Abstract # 438
Neural crest cells develop from pre-gastrulating chick embryo explants
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Abstract #438 will be presented as scheduled, but will not be published due to lack of license agreement between authors and publisher.

doi:10.1016/j.ydbio.2009.05.465

Program/Abstract # 439
Epigenetic control of neural crest cell development by histone demethylases JmjD2A
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The neural crest (NC) is comprised of multipotent stem-cell-like precursor that migrates and gives rise to a diverse set of derivatives. Many studies have been focused on transcriptional regulation underlying NC formation, but less is known about epigenetic influences. JmjD2 were recently described as important epigenetic genes, able to demethylate H3K9me3 and H3K36me3, implicated in
development and cell differentiation. Here we characterize for first time in vivo the functional role of JmjD2A in chick NC cell development. Analysis of its expression during early development by QPCR and ISH reveals that JmjD2A is present during early NC specification and migration. In contrast, JmjD2B and JmjD2C were not detected. To determine the effects of the loss of JmjD2A function, a morpholinon (mo-JmjD2A) was electroporated unilaterally at St4 embryos. The results show a clear diminution in the expression of several NC specifier genes, such as Sox10, FoxD3, Sox9 and Slug, on the electroporated compared with the contralateral side as well as with the mo-control embryos. In contrast, no changes were noted in Pax7 and Sox2 genes. Finally, gain of function experiments were performed by over-expressing vector containing the JmjD2A gene in ovo electroporated at St8; the dorsal neural tube was dissected and cultured to allow NC migration. Using specific antibodies against H3K9/K36me3 we observed that all the migrating NC H2GFP-JmjD2A-positives showed a pronounced loss of H3K9/K36me3 staining. Taken together, these results provide for the first time an important in vivo role for JmjD2A in epigenetic regulation of the H3K9/K36 methylation state in modulating NC development.

doi:10.1016/j.ydbio.2009.05.466

Program/Abstract # 440
The role of Hox genes in neural crest specification and migration
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The neural crest (NC) is a population of multipotent cells that originate from the dorsal neural tube during embryonic development and gives rise to various derivatives, including the enteric nervous system, dorsal root ganglia, cartilage/bone, and melanocytes. It is generally accepted that NC fate specification is based upon the environmental cues they encounter along their path of migration, versus intrinsic cues that predetermine their cell fates. To revisit this issue, microarray/qRT-PCR analyses were performed on cells derived from distinct NC derivatives: the enteric nervous system, dorsal root ganglia, cartilage/bone, and melanocytes. It is little is known about the role Hox genes play in NC derived from other regions of the neural tube. To test whether the Hox gene expression is sufficient to drive a posterior/trunk NC fate, Hoxb7 or Hoxc8 was misexpressed in anterior/cardiac NC cells. These experiments resulted in the formation of ectopic sensory neurons and/or aberrant migration. To test whether Hox genes are required for posterior/trunk NC fate, the expression of Pax1, a Hox co-factor that binds to multiple Hox genes, was silenced using shRNAs. Interestingly, migration was severely disorganized. Taken together, these data propose a novel role for Hox genes and their co-factors in the specification and migration of NC.

doi:10.1016/j.ydbio.2009.05.467

Program/Abstract # 441
Skeletogenic potential of trunk neural crest cells
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Neural crest cells (NCCs) appear early in vertebrate development, and generate migratory derivatives that colonize the entire body, like the neurons, and glia, of the peripheral nervous system, and skin melanocytes. They also generate endocrine, and mesodermal derivatives (cartilage, bone, muscle, dermis, and adipose tissue). Experimentally transplanted NCCs largely differentiate according to new environments, and their differentiation potential is bigger than their in vivo repertoire. However, the skeletogenic potential of NCCs is restricted in vivo to the cranial region of higher vertebrates, and does not arise in trunk NCCs grafted to cranial territories. Yet, in co-culture and long-term in vitro experiments, trunk NCCs generate skeletal elements. Alternative approaches offer conflicting results: direct placement into the first branchial arch, of a suspension of trunk NCCs obtained after overnight culture yielded skeletal elements, but direct placement without the overnight culture failed to do so. Past studies assumed that instructions for NCC differentiation were provided during or after migration. Here we re-addressed the skeletogenic potential of trunk NCCs using the quail-chick chimera system with younger hosts. We monitored the expression of Hox genes in these heterochronic and heterotypic grafting experiments along with the expression of chondrogenic markers. Our results demonstrate a clear contribution of trunk NCCs to various skeletal elements in the cranial region in the absence of in vitro steps, and expose a plasticity of Hox expression, which is responsive to the developmental progression of the early host embryo.

doi:10.1016/j.ydbio.2009.05.468

Program/Abstract # 442
Specific ablation of Tpbpa positive cells reveals functional significance of spongiotrophoblasts and its derivative cells in the placenta
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Tpbpa marks a subgroup of ectoplacental cone cells since embryonic day E7.5 and later the spongiotrophoblast layer of the mature placenta. Tpbpa positive cells are progenitors to many subtypes of trophoblast cells including spiral artery trophoblast giant cells (TGCs), parietal TGCs, canal TGCs and glycogen trophoblast cells. To better characterize the development and function of Tpbpa positive cells, we use the cell type specific ablation system (Cre recombinase activated diphertheria toxin/DTA transgene) to specifically delete Tpbpa positive cells in mice. In the double transgenic mice in which Tpbpa positive cells are deleted, all the embryos die during midgestation between E10.5 and E12.5, indicating Tpbpa positive cells are functionally essential for normal development. Characterization of the placenta phenotype in double transgenic mice showed that not only Tpbpa positive spongiotrophoblast cells are dramatically reduced at E10.5, Prl2c (Pl1) positive spiral artery TGCs and canal TGCs are also decreased. However, Prl3d2 (Plf) positive parietal TGCs are not significantly reduced, suggesting there might be a compensatory growth of Tpbpa negative cell population. In short, Tpbpa positive cells and their derivative cell types are essential for embryonic survival and normal development since midgestation and more detailed characterization of the placenta will clarify their role in maternal vasculature remodelling and labyrinth formation.

doi:10.1016/j.ydbio.2009.05.469