Session 2: Developmental Processes in Toxicology and Disease

Development of a Rapid *In Vivo* Chemical Screening Method for the Identification of Antimetastatic Compounds

Shawn Burgess, PhD
Developmental Genomics Branch, National Human Genome Research Institute, U.S. National Institutes of Health

To date, most high-throughput screening models for cell migration, an integral component of cancer metastasis, were based on *in vitro* studies of cells. These studies generated many 'hits', however, they lacked relevant whole organism physiology to further validate the findings, and many of the assay positives do not replicate when tested *in vivo*. Thus, an *in vivo*, phenotype-driven screen will present better novel targets for therapeutic intervention. We developed a robust *in vivo* assay to identify new cell migration inhibitors and conducted a high-throughput screen using transgenic zebrafish and the migrating posterior lateral line primordium as a readout for migratory inhibition. We screened FDA-approved drugs and other bioactive compounds, as well as a collection of natural products and a set of kinase inhibitors to identify compounds that blocked migration. Demonstrating the utility of this approach, we confirmed that inhibition of the Src pathway prevented normal lateral line migration and decreases tumor metastasis *in vivo*. We also identified that inhibition by novel flavonoid-derived molecules and a cluster of structurally related kinase inhibitors disrupted primordium migration. Thus, this approach demonstrates that zebrafish can be used for large-scale, high-throughput screening for drugs that impact cancer metastasis.

Diversity As Opportunity: Using Fish Models to Understand the Role of Conditional Transcription Factors in Mechanisms of Developmental Toxicity

Mark E. Hahn, PhD
Department of Biology, Woods Hole Oceanographic Institution

The sensitivity of animals to environmental chemicals varies dramatically among developmental stages, individuals, populations, and species. Chemical effects are determined in part by changes in gene expression that are controlled by conditional (ligand-activated) transcription factors such as the aryl hydrocarbon receptor (AHR; a bHLH-PAS protein) and NF-E2-related factor 2 (NFE2L2 or NRF2; a CNC-bZIP protein). AHR and NRF proteins have multiple functions including poorly understood endogenous roles in embryonic development. Our research uses a comparative approach involving zebrafish (*Danio rerio*), Atlantic killifish (*Fundulus heteroclitus*), and other aquatic animal models to exploit the phylogenetic diversity that has evolved over millions of years to better understand the fundamental biology of these proteins and their roles in mechanisms of development, developmental toxicology, and differential susceptibility to chemical exposure. For example, studies in zebrafish provide evidence that multiple AHR and NRF2 paralogs in fish have partitioned the multiple functions of the single mammalian AHR and NRF2 proteins. Multiple allelic variants at AHR loci in killifish and population-specific differences in allelic diversity suggest mechanisms of differential susceptibility in populations with evolved resistance to dioxin-like compounds. Developmental exposure to oxidant chemicals causes stage-specific effects and overlapping but distinct patterns of altered gene expression in zebrafish embryos. Developmental changes in glutathione redox dynamics and transcription factor expression provide possible mechanisms for the stage-specific sensitivity to oxidant and pro-oxidant chemicals. Loss-of-function approaches involving transient knockdown (morpholino oligonucleotides) or permanent gene loss (targeting by ZFN or CRISPR-Cas) are being used to determine paralog-specific roles of AHR, AHRR, and NRF genes and crosstalk among signaling pathways during development. Recent studies are using zebrafish to explore mechanisms by which developmental exposure to marine toxins and toxicants may lead to adult disease.

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Growth of the Mosquitofish Anal Fin in Response to Androgens and Progestins

Nancy Denslow, PhD
Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida

Female eastern mosquitofish (*Gambusia holbrooki*) collected downstream of paper mills in Florida have an elongated anal fin (the gonopodium) plus hooks and serrae that are male secondary sexual characteristics. These male characteristics have not been documented in female fish from unimpaired aquatic ecosystems. Preliminary data we have collected with mosquitofish collected downstream from the Buckeye paper mill on the Fenholloway River suggest that exposure to androgens may be directly responsible for the phenotype in females. In addition, paper mill effluent (PME) has been linked to abnormal steroid levels, skewed sex ratios, and decreased reproductive output in aquatic animals collected downstream of effluent-receiving areas. It is believed that the causative chemical is released from the paper mill, as part of the pulp production process, but despite many efforts, the causative chemical has not been identified. The mechanisms by which androgens induce fin growth of the anal fin in poeciliidae species are still a mystery. Our preliminary data suggests that the gene network that regulates fin outgrowth, including *sonic hedgehog* (shh), *fgf8*, *hoxd13* and other genes, is activated by the androgen exposure. Activation of the shh pathway is likely to be an indirect effect, as there are no androgen response elements in promoters for sonic hedgehog. Although androgens promote fin elongation, they may be inhibitory to fin regeneration, which involves some of the same molecular mechanisms as elongation of the gonopodium.