Stem Cells

Oral Presentation **Monday November 7** **9.00am – 9.15am**

O-22

INSERTIONAL MUTAGENESIS IDENTIFIES GENES THAT PROMOTE THE IMMORTALIZATION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Retroviruses induce hematopoietic disease via insertional mutagenesis of cancer genes and provide valuable molecular tags for cancer gene discovery. Recently, we found that insertional mutagenesis can also identify genes that promote the immortalization of hematopoietic stem as well as hematopoietic progenitor cells; cells which normally have only limited self-renewal. Transduction of primary mouse bone marrow cells with replication-incompetent murine stem cell virus (MSCV) expressing only a *neo* marker gene, followed by serial passage of the infected cells in liquid culture containing SCF and IL-3, selects for immortalized immature myeloid cells with neutrophil and macrophage differentiation potential. In contrast, immortalized cells that are blocked at the early hematopoietic /stem cell stage can be selected by growing the infected cells in SCF plus FLT3L. Cloning and sequence analysis of the MSCV integration sites in the immortalized lines showed that in the majority of cases, immortalization resulted from the insertional mutagenesis of known leukemia genes or genes that are thought to regulate them. Thus, these immortalization genes by their nature could also be involved in the immortalization of the leukemic stem cell and represent attractive drug targets for treating cancer. Since our genetic screen employs a replication defective virus, it is not limited to murine cells. The MSCV LTR is also active in human cells. By using amphotropic packaging cells it may therefore also be possible to perform these screens directly in human cells. Finally, this screen could also theoretically be used to identify immortalization genes for many other types of stem/progenitor cells.

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A SENSITIZED MOUSE MUTAGENESIS SCREEN FOR NOVEL LOCI REGULATING MAMMALIAN NEURAL CREST DEVELOPMENT

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A mouse mutagenesis program is in progress to screen for mutations disrupting mammalian neural crest cell development and to develop an archived resource of mutagenized mice. The focused phenotype screen has been designed to detect mutations specifically affecting melanocyte and peripheral nervous system development. Progeny of N-ethyl-N-nitrosourea (ENU) treated mice are being bred to *Sox10*^{LacZ}/+ mice carrying a disruption in a transcription factor important for neural crest cell development. These mice manifest subclinical neural crest defects due to haploinsufficiency for SOX10. The sensitized screen uncovers mutations that act synergistically with *Sox10*^{LacZ}/+ resulting in clinically visible phenotypes such as white coat color spotting. Additionally, third generation embryos are being generated in a backcross to screen for embryonic phenotypes that alter expression of the LacZ reporter gene in Sox10 expressing cells. Our 3-generation breeding strategy utilizes two different mouse strains, BALB/cJ and C57BL/6J, to facilitate mapping of both dominant and recessive phenotypes and allows for subsequent recovery of lethal phenotypes. To date, we have identified two heritable phenotypes from the dominant screen of 100 pedigrees and five heritable mutations from a recessive embryonic screen of 60 pedigrees. The phenotypes observed in the embryonic screen include ectopic expression of Sox10-LacZ, abnormal glial cell patterning and migration, and loss of Sox10-LacZ expression in subsets of peripheral nervous system cell lineages. None of the loci localize to genes for the major mouse spotting mutants, demonstrating the feasibility of this approach for identifying novel loci regulating neural crest development.

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Oral Presentation **Monday November 7** **9.30am – 9.45am**

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A MUTATION IN STRATIFIN (14-3-3 SIGMA) IS PRESENT IN REPEATED EPILATION MICE

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The mouse repeated epilation (*Er*) mutation causes a semidominant phenotype that results in the cyclic loss of hair and an increased incidence of skin cancers. Homozygous *Er/Er* embryos have profound defects in epidermal development and keratinocyte differentiation that result in neonatal lethality. We have generated a high-resolution genetic map that localized the *Er* mutation to an 800 kilobase interval on mouse Chromosome 4. This interval included Stratifin (*sfn*), a member of the 14-3-3 protein family. Stratifin is repressed in several human cancers and is highly expressed in differentiating epidermis. We identified an insertion mutation in *sfn* that produces a truncated sfn protein. This truncated sfn is expressed in Er/+ mice and may act as a dominant negative molecule. Confirming the identity of Er as sfn, we were able to rescue the Er/+ phenotype with a BAC carrying the normal sfn protein. Ongoing work will address the functional nature of this mutation, and elaborate the molecular mechanisms that cause the Er phenotype.

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Oral Presentation **Monday November 7** **9.45am – 10.00am**

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MECHANISMS OF PHENOTYPIC VARIABILITY IN ADRENOCORTICAL DYSPLASIA (*ACD*) MICE

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Adrenocortical dysplasia (*acd*) is a spontaneous recessive mouse mutation that originated on the DW/J strain. *acd* mutant mice exhibit a strain-dependent pleiotropic phenotype; on the DW/J strain, the phenotype is lethal shortly following birth and includes caudal truncation, vertebral segmentation, and limb patterning defects. In contrast, *acd* mutant mice on a mixed DW/J X CAST/Ei background have developmental defects in urogenital ridge structures and can survive into adulthood, suggesting that one or more modifier genes influences the *acd* phenotype. We have previously characterized the *acd* mutation as a splicing defect in a gene (*Acd*) that encodes a novel component of the complex of telomere binding proteins that functions to maintain telomere integrity. Here, we report widespread expression of *Acd* mRNA in mouse embryos. We observed increased expression in the limb buds and developing tail, which are regions of highly proliferative cells that correspond to the structural defects observed in *acd* mutant embryos. In addition, using a highly sensitive fluorescent RT-PCR assay in *acd* mutant mouse embryonic fibroblasts, we can detect a very small percentage of appropriately spliced *Acd* mRNA transcripts from the mutant allele. To identify candidate modifier loci, we genotyped 11 DW/J^{acd} X CAST/Ei F_2 mutant mice with a panel of 402 SNPs. The SNP genotyping data so far are inconclusive. Our studies suggest the possibility that *acd* is a hypomorphic allele and indicate that further studies will be required to localize modifier genes.