

Sunday November 12
Student Satellite Symposium Sessions

Student Satellite Symposium Session 1 Sunday November, 12**1.00 – 1.15pm****S1/P70****CHARACTERISATION OF SHORTY, AN ENU DERIVED MUTANT MOUSE WITH DEFECTS IN RIB FORMATION**TL Harboe¹, DR Beier¹, BJ Herron²¹Brigham & Women's Hospital/Harvard Medical School, Boston MA, United States, ²Wadsworth Center, Albany NY, United States

Here we report the identification of a mutant mouse from an ENU mutagenesis screen for late embryonic developmental phenotypes, called *shorty* (*srt*). The defect is lethal and the affected pups are smaller in size than control littermates. The mutant has a severe vertebral malformation and is lacking several ribs. However, the development of both long bones and caudal vertebrae appears grossly normal. The phenotype is also similar to that of *pudgy* (*pu*), which is caused by a mutation in *Dll3*, a Notch ligand, although our mapping analysis has excluded the possibility that *srt* is an allele of *pu*. The mutation has been localised to a 1.06 Mb region on mouse chromosome 17. We then used microarrays to screen for genes in the interval differentially regulated in mutant tissue. We found a 7-fold decrease in the expression of the Tubulin beta 5 (*Tubb5*) gene. Sequencing *Tubb5* in *srt* mutant tissue identified an A → T sequence change introducing a new ATG upstream of the original start codon. This new ATG may lead to nonsense mediated decay or hinder translation from the original start codon. Also under investigation is the expression of various genes involved in somitogenesis, especially those in the Notch and Shh pathway. Several genes such as *Pax9*, *Tbx18*, *Uncx4.1* and *Lfng* seem to be expressed at a lower level in the mutant compared to wildtype. This novel mouse mutant results in abnormal rib and vertebrae development and could provide insight into the patterning of the ventrolateral sclerotome, somitogenesis and the role of *Tubb5* in these processes.

Student Satellite Symposium Session 1 Sunday November, 12**1.15pm – 1.30pm****S2/P57****NELL1-DEFICIENT MICE HAVE REDUCED EXPRESSION OF EXTRACELLULAR MATRIX PROTEINS CAUSING CRANIAL AND VERTEBRAL DEFECTS**JB Desai¹, ME Shannon², MD Johnson³, DW Ruff², LA Hughes⁴, MK Kerley⁴, DA Carpenter⁴,DK Johnson⁴, EM Rinchick⁴, CT Cuiat⁴University of Tennessee-Oak Ridge National Laboratory, Oak Ridge, TN, United States, ²Applied Biosystems, Foster City, CA, United States, ³The University of Tennessee Graduate School of Medicine, Knoxville, TN, United States, ⁴Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, United States

The mammalian *Nell1* gene encodes a PKC- β 1 binding protein that belongs to a new class of cell-signaling molecules controlling cell growth and differentiation. Overexpression of *Nell1* in the developing cranial sutures in both human and mouse induces craniosynostosis, the premature fusion of the growing cranial bone fronts. Here we report the generation, positional cloning and characterization of *Nell1*^{6R}, a recessive, neonatal-lethal point mutation in the mouse *Nell1* gene, induced by N-ethyl-N-nitrosourea. *Nell1*^{6R} has a T → A base change that converts a codon for cysteine into a premature stop codon, resulting in severe truncation of the predicted protein product and marked reduction in levels of the *Nell1* transcript. In addition to the expected alteration of cranial morphology, *Nell1*^{6R} mutants manifest skeletal defects in the vertebral column and ribcage, revealing a hitherto undefined role for *Nell1* in signal transduction in endochondral ossification. Real-time quantitative RT-PCR assays of 219 genes showed an association between the loss of *Nell1* function and reduced expression of genes for extracellular matrix proteins critical for chondrogenesis and osteogenesis. Several affected genes are involved in the human cartilage disorder Ehlers-Danlos Syndrome and other disorders associated with spinal curvature anomalies. *Nell1*^{6R} mutant mice are a new tool for elucidating basic mechanisms in osteoblast and chondrocyte differentiation in the developing skull and vertebral column and understanding how perturbations in the production of extracellular matrix proteins can lead to anomalies in these structures. [Research sponsored by the Office of Biological and Environmental Research, U.S. Department of Energy under contract DE-AC05-00OR22725 with UT-Batelle, LLC.]

Student Satellite Symposium Session 1 Sunday November, 12

1.30pm – 1.45pm

S3/P63

AN ENU MUTANT MOUSE MODEL OF OCULODENTODIGITAL DYSPLASIA IS EXPLOITED TO UNDERSTAND THE ROLE OF CONNEXIN 43 IN BLOOD AND BONE DEVELOPMENTNM Anderson¹, R Zirngibi¹, C Owen², F Chen¹, L Moreno¹, M Grympas¹, J Henderson³, J Aubin¹, W Stanford¹¹University of Toronto, Toronto ON, Canada, ²Mount Sinai Hospital, Toronto ON, Canada, ³McGill University, Montreal Quebec, Canada

Oculodentodigital dysplasia (ODDD) is an autosomal dominant disorder characterized by a variety of developmental abnormalities including syndactyly, enamel hypoplasia, craniofacial abnormalities and cardiac dysfunction. Recently, ODDD has been shown to be caused by mutations in gap junction protein alpha 1 (a.k.a., Connexin 43). Gja-1^{JRT} is an N-ethyl-N-nitrosurea (ENU) induced-strain that was identified in a dysmorphology screen by its presentation of syndactyly. Gja-1^{JRT} displays many of the classical symptoms ODDD. Gja-1^{JRT} encodes a mutation in a highly conserved amino acid G60S and has been shown to act in a dominant negative manner disrupting gap junction assembly and function. During the course of phenotyping the Gja-1^{JRT} mutant, additional phenotypes yet to be reported in ODDD patients were found and include decreased bone mass and mechanical strength as well as alterations in hematopoietic progenitor frequencies. Gap junctions are intercellular channels that allow the exchange of small molecules between cells. Gap junctions are known to play an important role in the communication between bone marrow stroma and hematopoietic tissues during embryogenesis and during cytoblastic treatments in the adult. Gja-1 homozygous null mice are neonatally lethal and heterozygous null mice show no steady state hematopoietic defects. Therefore Gja-1^{JRT} provides an opportunity to investigate the role of gap junctions in the development of two tissues that are closely connected. We will present a comprehensive longitudinal study to look at the relationship of Gja-1 in the development of blood and bone. Notably, Gja-1^{JRT} presents distinct blood and bone phenotypes at that change as the animal ages.

Student Satellite Symposium Session 1 Sunday November, 12

1.45pm – 2.00pm

S4/P69

CHARACTERIZATION OF A NOVEL GENE TRAP DERIVED CHD7 ALLELE DEMONSTRATES EMBRYONIC LETHALITY, INNER EAR DEFECTS, AND TISSUE SPECIFIC CELL SURVIVALEA Hurd, HK Poucher, PL Capers, DM Martin
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CHD7 is a novel chromodomain gene mutated in 60-80% of humans with CHARGE syndrome, a multiple congenital anomaly condition characterized by ocular Coloboma, Hear defects, Atresia of the choanae, Retarded growth and development, Genital hypoplasia, and characteristic Ear abnormalities including deafness. CHARGE phenotypes are highly variable and incompletely penetrant. The single unifying clinical feature in children with CHARGE is semicircular canal dysgenesis, indicating that the developing ear has a unique requirement for normal *CHD7* function and is sensitive to changes in *CHD7* dosage. We hypothesized that *CHD7* is necessary for normal cell proliferation and survival in the ear and other CHARGE tissues. To explore developmental roles of *CHD7*, we generated a novel *Chd7* deficient, gene trapped *lacZ* reporter allele, *Chd7*^{Gt1Dmm}. RT-PCR of embryo RNA demonstrated significantly reduced levels of wildtype transcript in *Chd7*^{Gt1Dmm/Gt1Dmm} embryos. *Chd7*^{Gt1Dmm/Gt1Dmm} embryos survive only up to E10.5, indicating prenatal lethality. *Chd7*^{Gt1Dmm/+} male and female mice are viable, growth delayed, and exhibit variable degrees of head-bobbing and circling, consistent with vestibular dysfunction. Paint-filling of E16.5 heterozygous inner ears revealed defects of the semicircular canals. The pattern of β-galactosidase activity in *Chd7*^{Gt1Dmm/+} embryos mimics *Chd7* mRNA expression in wildtype embryos, confirming the fidelity of the *lacZ* reporter. We observed tissue-specific β-galactosidase in the E12.5 and E14.5 *Chd7*^{Gt1Dmm/+} brain, pituitary, ear, heart, and craniofacial structures, indicating survival of *Chd7*^{Gt1Dmm/+} cells in CHARGE relevant organs. These studies demonstrate the utility of *Chd7*^{Gt1Dmm} as a reporter tagged loss of function allele for future studies exploring developmental mechanisms of *Chd7* deficiency.

Student Satellite Symposium Session 1 Sunday November, 12**2.00pm – 2.15pm****S5/P58****FILAMIN B REPRESSES RUNX2 THROUGH THE TGF- β /SMAD3 PATHWAY TO REGULATE CHONDROCYTE HYPERTROPHY**

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Mutations in *FILAMIN B*, which encodes a cytoplasmic actin binding protein, have been found in five human skeletal disorders^{1,2}. To further investigate the disease mechanism, we generated mice lacking the full-length Filamin B protein. Mutant mice display ectopic bone formation in numerous skeletal elements, phenotypic abnormalities similar to human skeletal diseases associated with nonsense mutations in *FILAMIN B*. Here we show that the aberrant bone formation is due to abnormal chondrocyte hypertrophy, which is a mandatory step of endochondral bone formation controlled by the transcription factor Runx2 and its repressor HDAC4^{3,4}. Removing one copy of *Runx2* rescues the *Filamin B* accelerated chondrocyte hypertrophy phenotype. Further, we show that Filamin B binds Smad3 and that the amount of phosphorylated Smad3 is increased in the mutant mice, suggesting that Filamin B inhibits Runx2 activity through the TGF- β /Smad3 pathway. Thus, our data reveal a new molecule in the Runx2 signaling cascade, providing insight into the mechanism of *FILAMIN B*-associated human diseases.

Student Satellite Symposium Session 1 Sunday November, 12**2.15pm – 2.30pm****S6/P68****IN VIVO AND MOLECULAR CHARACTERIZATION OF BONE RELATED PHENOTYPES**

F Thiele, T Lisse, GKH Przemeck, H Fuchs, M Hrabé de Angelis
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Due to the consequences of populace aging and changes of living standards, the incidence of skeletal diseases will rapidly increase. But despite vast efforts in bone research, most molecular mechanisms involved in skeletal diseases remain unclear. The mouse is probably one of the best model systems to study bone biology and the pathology of related diseases. To broaden the bone-related analyses offered by the German Mouse Clinic (GMC, www.mouseclinic.de), we have established a cell culture-based system for standardized analysis of osteoblast and osteoclast function in mice that have shown striking differences within the primary and secondary screens of the GMC (Gailus-Durner et al. 2005). We measure several parameters in order to obtain deeper cellular and molecular mechanistic insights that entail apoptotic and metabolic cell activity, proliferation, RT-PCR analysis of bone related genes, as well as differentiation and mineralization processes. Here we present the first results from the AGA2 mutant line, which is a new model for *osteogenesis imperfecta*.

Student Satellite Symposium Session 1 Sunday November, 12**2.30pm – 2.45pm****S7/P50****CHEMICAL MUTAGENESIS OF THE MOUSE GENOME TO UNVEIL HOST RESISTANCE GENES**E Richer¹, R Wilkinson⁴, D Albert⁴, I Angers⁴, ST Qureshi², S Vidal³, D Malo¹¹Department of Human Genetics, McGill University, Montréal, Québec, Canada, ²Department of Medicine, McGill University, Montréal, Québec, Canada, ³Department of Microbiology and Immunology, McGill University, Montréal, Québec, Canada, ⁴Centre for the Study of Host Resistance, McGill University, Montréal, Québec, Canada

At the beginning of 20th century infectious diseases were the primary cause of death in the western world. Following the Second World War, a rapid decline in the incidence of infectious diseases owing to better hygiene accompanied by the development of antimicrobial agents and vaccines generated great optimism. Unfortunately this view has recently been dampened by new emerging and re-emerging pathogens that show increasing resistance to established treatments.

In order to deepen our knowledge of the natural host response to pathogens our team undertook an interrogation of the mouse genome to define genes involved in pathogen recognition, viral defense, and bacterial defense. Mice were chemically mutagenized with ENU (N-ethyl-N-nitrosourea) and third generation recessive screens were initiated for the three phenotypes.

Splenocytes from mutagenized C57BL/6J were stimulated using purified microbial structures to identify mutations affecting pathogen recognition, an essential first step of the immune response. Complementary to this in vitro approach, an in vivo screen of mutagenized 129S1 mice with Coxsackie virus, a cause of myocarditis in humans, as well as Salmonella Typhimurium, a mouse model of typhoid fever, have been established. To date over 3000 mice have been screened, leading to the identification of several phenotypic deviants. Individual pedigrees are currently being mapped and homozygous lines established as new models for the study of human infectious diseases.

Student Satellite Symposium Session 1 Sunday November, 12**2.45pm – 3.00pm****S8/P72****A SENSITIZED ENU MUTAGENESIS SCREEN FOR DOMINANT GENETIC MODIFIERS OF THROMBOSIS IN THE FACTOR V LEIDEN MOUSE**RJ Westrick¹, SL Manning², ME Winn¹, SL Dobies¹, GM Stotz¹, DR Siemieniak², E Sanford², D Ginsburg²¹University of Michigan, Ann Arbor, MI, United States, ²Howard Hughes Medical Institute, Ann Arbor, MI, United States

Venous thrombosis affects ~300,000 individuals per year in the USA. A gain-of-function mutation in the factor V gene, Factor V Leiden, (FVL) is the most common known inherited risk factor for venous thrombosis. Penetrance is incomplete, with only ~10% of FVL individuals experiencing clinically significant thrombosis. We are performing a whole genome mouse mutagenesis screen to identify modifier gene candidates contributing to the penetrance of FVL in humans. Previously, we demonstrated synthetic lethality between FVL and genetic deficiency of a key coagulation component, tissue factor pathway inhibitor (TFPI). Complete TFPI deficiency in mice is embryonic lethal, whereas heterozygosity is compatible with normal survival. However, homozygosity for FVL (*FvQ/Q*) in the context of heterozygosity for TFPI (*Tfpi+/-*) is uniformly lethal due to disseminated perinatal thrombosis. This synthetic lethal interaction was utilized as a phenotyping tool for a sensitized ENU mutagenesis screen. We aim to uncover novel dominant mutations that improve hemostatic balance leading to survival of *FvQ/Q Tfpi+/-* mice. We have proven our approach by rescuing *FvQ/Q Tfpi+/-* with tissue factor (*Tf+/-*) heterozygosity. Male *FvQ/Q* mice were ENU mutagenized and bred to *FvQ/+ Tfpi+/-* double heterozygous females. Surviving G1 offspring were analyzed to identify rescued mice with the *FvQ/Q Tfpi+/-* genotype. Analysis of 4600 G1 offspring thus far has identified 52 mice that survived to weaning. Of the mutants progeny tested to date, 5 are heritable. A number of progeny have been produced for genetic mapping of these mutant lines. Our preliminary findings demonstrate the feasibility of our sensitized approach in the identification of dominant suppressors of the *FvQ/Q Tfpi+/-* lethal phenotype.

Student Satellite Symposium Session 2**Sunday November 12****3.30pm – 3.45pm****S9/P56****MAMMALIAN NURF IS AN ESSENTIAL COMPONENT OF TGF β SIGNALING IN THE PRE-GASTRULATING EMBRYO**

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Emerging research has shown that mutations in chromatin remodeling complexes frequently lead to defects in development and disease, stressing the importance of characterizing their biological functions. In this work we characterize the biological functions of the mammalian NURF remodeling complex through the mutation of the gene encoding its largest and unique subunit BPTF. We first show that *BPTF* is expressed ubiquitously in the embryo proper from 5.5 dpc to 13.5 dpc. Its expression is essential for development because mutants are early embryonic lethal from 7.5 to 8.5 dpc. An analysis of mutant embryos in section and an expression analysis by *in situ* RNA hybridization show that the mutant embryos fail to expand ectoderm, establish the AVE, form a primitive streak and localize mesoderm. Delocalization of *Nodal*, *Cripto*, *T* and *FGF8* and reduced or lack of expression of *Otx2*, *Lhx1*, *Gsc*, *Cer1*, and *Hex1* all phenocopy mutants of the TGF β signaling pathway. Using P19 cells depleted of BPTF by siRNA interference we show defects in the regulation of the TGF β responsive gene *JunB*. This in combination with *in vitro* pull down assays showing a direct interaction between the NURF complex and the Smad transcription factors strongly suggests that NURF is a co-regulator of TGF β regulated genes. We suggest that one effect of mutations in NURF are an inability of the embryonic tissues to respond to TGF β signals causing defects in the proliferation of the epiblast and establishment of the distal visceral endoderm resulting in an inability to establish anterior-posterior asymmetry.

Student Satellite Symposium Session 2**Sunday November 12****3.45pm – 4.00pm****S10/P59****FUNCTIONAL CHARACTERIZATION OF A NOVEL MINICHROMOSOME MAINTENANCE PROTEIN, MCM9**

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Minichromosome maintenance proteins ensure that the genome replicates only once per cell cycle. The 6 proteins MCM2,3,4,5,6 & 7 comprise the replicative helicase involved in initiation and elongation of the replication fork. These proteins have a highly conserved domain, the MCM domain, which is conserved throughout eukaryotes. This domain is 200 amino acids long and contains Walker A and Walker B domains, which are involved in ATP binding and ATPase activity. Recently an additional MCM domain-containing protein was described in higher eukaryotes, MCM9. Mcm9 is widely expressed and the Walker B domain contains non-conserved changes from the canonical sequence. There is a shorter splice isoform of the protein that has its own 3'-UTR, this contains only the Walker A domain. In order to elucidate the function of MCM9, we imported a collection of mouse ES cell lines containing gene-trap insertions within this gene, and were able to create mice from these lines. The 5' gene-trap that inserted before the MCM domain causes lethality. A second gene-trap that inserted after the Walker A domain, which truncates the full-length protein to the shorter isoform, is viable yet has fertility defects. These data provide the first evidence for an MCM gene in mammalian gametogenesis, in addition to a presumed essential role in cell proliferation.

Student Satellite Symposium Session 2**Sunday November 12****4.00pm – 4.15pm****S11/P2****EVALUATING THE DISEASE RELEVANCE OF NON-CODING CONSERVED SEQUENCES AT RET**EA Grice, A Chakravarti, AS McCallion

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Variation within non-coding sequences is predicted to play a significant role in human genetic disease. However, the nature and identity of such disease-causing mutations remain largely unknown. Using computational tools, *in vitro* prescreens, and transgenic analyses in mouse and zebrafish, we previously reported the functional evaluation of non-coding conserved sequences at *RET*, a crucial developmental gene in which regulatory mutations contribute to susceptibility to Hirschsprung disease (HSCR; aganglionic megacolon). Human genetic evidence suggests that variation in a *RET* intron one enhancer (MCS+9.7) underlies HSCR susceptibility; our recent published work has now demonstrated the biological relevance of this sequence. Although the above strategies are highly informative, they are not sufficient to evaluate the pathological relevance of implicated non-coding sequences. We have developed the ideal *in vivo* reagent to address this question. We engineered a GFP reporter BAC (156 kb) that encompasses 100 kb 5' and 13 kb 3' of the mouse *Ret* gene. Mice transgenic for the reporter BAC recapitulate all aspects of endogenous *Ret* expression. Furthermore, our data suggests the BAC contains all regulatory elements necessary for appropriate *Ret* expression. We will report progress on our ongoing efforts to determine if the BAC is capable of rescuing the pleiotropic phenotype observed in *Ret* null mice. Most importantly, our *Ret* BAC transgenic mice are an ideal platform to determine the pathological relevance of MCS+9.7. We have deleted MCS+9.7 from the BAC and ultimately will compare the capacity of the wild-type and mutant BAC transgenes to complement the *Ret* null mouse phenotype.

Student Satellite Symposium Session 2**Sunday November 12****4.15pm – 4.30pm****S12/P61****TRANSCRIPTIONAL PROFILING OF MOUSE EMBRYONIC STEM CELL DERIVED CARDIOMYOCYTES TO IDENTIFY NOVEL CARDIAC GENES**RA Miller, AS McCallion, JD Gearhart

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Cardiac disease (CD) accounts for the largest proportion of adult mortality in the industrialized world. A common characteristic of CD is the loss of cardiomyocytes as a result of ischemic damage. Embryonic stem cell based therapies represent a potentially exciting approach to repair this damage through the transplantation and engraftment of cells that can generate viable new myocardium. However, the establishment of authentic cardiomyocytes precursor cells *in vitro* is hampered by an incomplete understanding of the regulatory programs that control early cardiogenesis. To address this problem, we set out to determine the transcriptional profile of mouse embryonic stem cells (mESCs) as they differentiate along a cardiac lineage. Using an Nkx2.5 cardiac specific promoter driving GFP, we marked and isolated differentiating cardiomyocytes (DCMs) at specific time points during the differentiation. By comparing the profile of DCMs with time-matched nonDCMs and undifferentiated mESCs we have identified genes whose expression is enriched in DCMs compared with non-DCM populations. Approximately 50% of these genes already have established roles in cardiac function and development. We will describe our efforts to evaluate the biological relevance of the remainder to cardiac development. To this end we are completing RNA *in situ* hybridization of all novel candidates identified in this screen, determining the embryonic expression patterns at key points during cardiogenesis (E7.5, E8.5, E9.5). We will present this data as well as on-going experiments to evaluate the pathological relevance of selected candidates

Student Satellite Symposium Session 2**Sunday November 12****4.30pm – 4.45pm****S13/P54****THE GENETIC ARCHITECTURE OF THE DDK SYNDROME**

FY Ideraabdullah, K Kim, F Pardo-Manuel de Villena
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The DDK syndrome is a parent of origin dependent phenotype characterized by the early embryonic lethality observed when females of the DDK inbred mouse strain are mated to non-DDK males. This phenotype is due to an incompatibility between a maternal DDK factor, present in the oocyte, and a non-DDK paternal gene, both of which map to the *Om* locus on chromosome 11. Multiple studies demonstrate the presence of recessive modifiers of the gene encoding the maternal DDK factor that are unlinked to *Om* and increase the severity of the lethal phenotype. Here, we demonstrate the presence of modifiers in two *Mus musculus domesticus* wild-derived inbred strains, PERA/Ei and PERC/Ei, which completely rescue the DDK syndrome lethality. Using whole genome linkage analysis we have mapped a major modifier locus, *Rmod1*, Rescue modifier of the DDK syndrome 1, to a 9 Mb region on proximal chromosome 13. Furthermore, we show that rescue of the lethal phenotype depends on the parent-of-origin of *Rmod1* alleles. DDK syndrome associated embryonic lethality is only rescued when a PERA or PERC allele at *Rmod1* is inherited from the dam. Inheritance of a PERA or PERC allele at *Rmod1* from the sire has no effect on embryo survival. Overall, these findings further our understanding of the complex genetic architecture of the DDK syndrome and provide significant insight into interactions between the ooplasm and the parental genomes that are essential for normal mammalian development.

Student Satellite Symposium Session 2**Sunday November 12****4.45pm – 5.00pm****S14/P100****USE OF CHROMOSOME 17 POLYMORPHISMS AND RECOMBINANTS TO MAP THE LIVER TUMOR SUSCEPTIBILITY LOCUS HCF1 IN C57BR/CDJ MICE**

SEM Peychal, N Drinkwater
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Male mice are more susceptible than females to spontaneous and chemical carcinogen-mediated liver tumor induction. This sexual dimorphism is due to testosterone promotion of liver tumor development in males, while ovarian hormones in females suppress hepatocarcinogenesis. C57BR/cdJ (BR) females are extremely susceptible to liver tumor development relative to other strains and this sensitivity is due in part to a lack of protection by ovarian hormones, possibly mediated by loci on Chromosomes 1 and 17. The *Hepatocarcinogenesis in females 1 (Hcf1)* locus on Chromosome 17 is responsible for a majority of the increase in susceptibility, and the polymorphism of this chromosome in the susceptible BR and resistant C57BL/6J (B6) strains was evaluated using simple sequence length polymorphisms and single nucleotide polymorphisms. The polymorphisms were taken into consideration when breeding a complete set of sixteen recombinant lines between the BR and B6 strains that sufficiently span Chromosome 17. The susceptibility of these recombinants to chemically-induced liver tumors was evaluated by injection at 12 days of age with *N,N*-diethylnitrosamine, allowing the *Hcf1* locus to be mapped to less than 3 cM.

Student Satellite Symposium Session 2

Sunday November 12

5.00pm – 5.15pm

S15/P101

CANDIDATE TESTICULAR GERM CELL TUMOR GENES FROM THE CONSOMIC, 129.MOLF-CHR 19, MOUSE STRAIN

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Testicular germ cell tumor (TGCT) development is a multigenic disease in both humans and mice involving several susceptibility genes. The molecular mechanism of TGCT development is still unclear. The progression of tumor development during embryonic stages can be monitored in mouse strains. One mouse strain, the chromosome substitution strain (CSS), was generated by replacing chromosome (Chr) 19 of the 129 with its homolog from the MOLF strain. The 129 inbred strain of mice develops TGCTs at a low frequency of 6%, whereas the CSS strain develops spontaneous TGCTs at a much higher frequency of 75%. By utilizing those two mouse strains, we have mapped two 2.5 Mb regions on mid-Chr 19 (Locus 1) and proximal-Chr 19 (Locus 2). Locus 1 is responsible for a tumor incidence of 30%. Locus 2 is associated with increased incidence of small testes (30% incidence) in which spermatogenesis is impacted. Gene expression profiling revealed 3 genes that are consistently down-regulated in CSS mice compared to 129. One of them, named *D19Bwg1357e*, resides in Locus 1. A non-synonymous single nucleotide polymorphism (SNP) is also found in the carboxyl-terminal domain of D19Bwg1357e protein from the CSS strain. Another gene, *Zfp162*, was located to Locus 2. Our findings suggest that *D19Bwg1357e* and *Zfp162* may be behaving as functional hypomorphs in the CSS strain. To test this, I am generating mouse lines carrying deletions of *D19Bwg1357e* or *Zfp162* genes to determine if that results in a more severe phenotype with regard to tumor development and germ cell defects.

Student Satellite Symposium Session 2

Sunday November 12

5.15pm – 5.30pm

S16/P103

IDENTIFICATION OF EGFR-INDEPENDENT SIGNATURES IN INTESTINAL NEOPLASIA IN APC^{MIN} MICE: CORRELATION TO HUMAN COLORECTAL CANCER

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The epidermal growth factor receptor (EGFR) has been intensely pursued as a therapeutic target for colorectal cancer (CRC) due to its aberrant activity in tumor tissues. However, large-scale clinical trials have achieved limited success, suggesting greater complexity of EGFR biology than previously anticipated. Using the *Apc^{Min}* mouse model of CRC, we previously showed that a subset of intestinal polyps arise on a background with reduced EGFR activity; the size, expansion, and pathological progression of these polyps appear EGFR-independent. Therefore, we hypothesize that although normal EGFR signaling is critical for establishment of most intestinal tumors, tumors can grow independent of EGFR activity. To test this hypothesis, we generated mice with cre-mediated intestinal epithelia-specific *Egfr* deletion. We observed a 57.5% reduction in total polyp number in 3-month-old *Egfr^{tm1Dwt/tm1Dwt}*, *Vil-Cre*, *Apc^{Min}* mice compared to wild-type *Egfr* littermates (46.2 ± 25.7 , versus 108.8 ± 61.2 ; $p_{(one-sided)} = 0.006$). Interestingly, polyps forming in *Egfr^{tm1Dwt/tm1Dwt}*, *Vil-Cre* mice were slightly larger than those forming in the controls (1.08 ± 0.57 mm versus 1.04 ± 0.56 mm; $p_{(one-sided)} = 0.04$), suggesting that absence of EGFR signaling does not alter the growth of tumors. Microarray gene expression profiles of these EGFR-independent tumors were analyzed by Significance Analysis of Microarray software and revealed distinctive molecular features that partition tumors based on EGFR status. These signatures generated from mouse models will be applied to genomic analysis of human CRC samples and generate potential EGFR-independent signatures in CRC, which will elucidate compensatory mechanisms used by CRC cells when exposed to EGFR inhibitors.

Student Satellite Symposium Session 2

Sunday November 12

5.30pm – 5.45pm

S17/P111

CHARACTERIZATION OF THE MECHANISM OF THE MODIFIER OF MIN 2 (MOM2) LOCUS ON INTESTINAL POLYPOSISAA Baran¹, KA Silverman², AM Buchberg¹, LD Siracusa¹¹Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, United States, ²Children's Hospital of Philadelphia, Department of Oncology, Philadelphia, PA, United States

Colorectal cancer is the second leading cause of cancer-related mortality in the United States. In addition to the many genomic alterations that occur in a typical carcinoma, modifier genes have a role in enhancing or suppressing the initiation, growth and progression of tumors. The *Apc*^{Min} mouse has proven useful in dissecting factors responsible for tumor development. We identified a Modifier of Min 2 (*Mom2*) locus that arose as a spontaneous mutation in a C57BL/6 (B6) *Apc*^{Min/+} mouse. One *Mom2*^R allele significantly decreases polyp number in *Apc*^{Min/+} mice. Our goal is to identify *Mom2* candidate genes; exclusion mapping strategies narrowed the location to distal chromosome 18. Sequence analysis revealed a duplication in one candidate gene in this region. Previous reports show that polyps from B6 *Apc*^{Min/+} mice tend to arise from loss of the entire wildtype chromosome 18. We microdissected intestinal polyps from B6 *Apc*^{Min/+} mice and *Apc*^{Min/+} *Mom2*^{R/+} congenic mice to analyze their histological phenotype and allelic genotype. Our data show that B6 mice possessing one *Mom2*^R allele retain the *Apc*⁺ allele in polyps more so than *Mom2*^S mice, suggesting that these polyps arise from a method of inactivation of the *Apc*⁺ allele other than whole chromosome loss. These studies will help define the biology behind the disruption of homeostatic mechanisms that occur during intestinal tumorigenesis and provide an understanding of genetic pathways. The results provide a foundation for the prevention, diagnosis, and treatment of human cancer. Research supported by NCI PO1 CA72027 and NIH T32-CA09678.

Student Satellite Symposium Session 2

Sunday November 12

5.45pm – 6.00pm

S18/P18

CANDIDATE GENE ASSOCIATION STUDIES FOR ANXIETY AND ACTIVITY IN HETEROGENOUS STOCK AND RECOMBINANT INBRED (BXD) MICEMJ Parsons, L Liu, C Fernandes, JL Paya-Cano, LC Schalkwyk

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The genetic components influencing individual differences in anxiety and activity are likely to be complex. We recently conducted association studies for over 170 SNPs both in house and from public databases. The association studies were conducted in an outbred population of 680 Heterogeneous Stock (HS) mice for numerous anxiety and activity-related measures taken from an extensive behaviour battery including the following behavioural tasks: spontaneous activity in the home cage, open field, elevated plus maze, light/dark box, and SHIRPA primary screen. Numerous SNPs yielded significant associations with both anxiety and activity measures. Using the WebQTL tool (www.genenetwork.org), we further investigated these genes by correlating their hippocampal mRNA expression levels with these behaviour traits in BXD recombinant inbred mice (n=254, 24 lines). We additionally conducted *in silico* expression QTL mapping for some of the genes of interest using the WebQTL tool to look for eQTLs that may additionally influence these behavioural measures. We were able to successfully validate these associations, using a converging method, for a number of genes including two glutamatergic genes (*Grid1* and *Grik4*) and a serine/threonine kinase gene (*Stk31*). Further studies are necessary to elucidate the specific role that these genes play in anxiety and activity.