

A Microfluidic Gradient Generation Platform and Applications in Cell Migration

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Chemical gradients play an important role in mediating biological activity in vivo. Insight into the interplay between a chemical gradient treatment and the corresponding cellular response may help to determine the cues that trigger changes in gene expression that are responsible for regulating specific cellular activities. Understanding the importance of these chemical cues could help researchers develop controlled microenvironments where the desired cellular response is produced by combining the effects of exogenous controlled gradient treatments with ongoing endogenous cell-cell signaling.

We present a convection-free (static) microfluidic gradient generation platform that facilitates our ability to study the effect of cell-cell communication on cell behavior. We discuss a particular application using our static gradient generator that investigates the migratory characteristics (e.g. migration speed, slope dependence, and sensing threshold) of neutrophils in response to controlled (and predictable) chemotactic factor gradients. We describe future applications using our device where we can begin to answer questions about the importance of cell-cell communication and the degree to which this communication affects the chemotactic behavior of migratory cells.

A Rat Knock Out Model of Human Familial Adenomatous Polyposis

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Humans with mutations in the Adenomatous Polyposis Coli (APC) gene get hundreds to thousands of adenomas in the colon and develop colorectal cancer. We are pursuing the experimental analysis of human colon cancer in murine systems to develop an accurate model for studying tumor initiation, progression, and potential treatments. The laboratory of Michael Gould has recently developed the technology to knock out rat genes of interest through mutagenesis with N-ethyl-N-nitrosourea (ENU). The Dove and Gould labs have collaborated to identify a single mutant rat with an A to T transversion changing a lysine to a stop codon at amino acid position 1137 of the Apc gene. Over 23 Apc^{am1137} mutant carriers have been necropsied to date. The rats developed an average of over 5 adenomas in the colon with older male rats developing up to 25 colonic tumors at almost one year of age. We have named this rat Pirc for polyposis in the rat colon. Histological immunofluorescence staining of the tumors for the oncogene β -catenin confirmed the tumor-associated relocalization of β -catenin from the lateral membrane to the cytoplasm and nucleus. Tumor DNA was analyzed by quantitative allele specific sequencing; 8 out of 8 colon tumors analyzed showed loss of heterozygosity for the wild type allele. Genetic crosses of the Fisher 344 Pirc rat to the inbred strain Wistar Furth (WF) revealed that F1 Apc^{am1137} mutant carriers develop only one tumor on average at 180 days of age. This fivefold reduction in tumor number indicates that the WF background has a dominant suppressor of tumor formation. Additional F2 intercross animals will be analyzed for the presence and estimated number of modifying loci in the WF genome. To follow tumor progression over time, colonic adenomas have been visualized in vivo using dual modality CT/PET imaging. Rats were injected with either I¹²⁵ labeled NM404 or FDG for PET imaging. In a rat harboring 25 colonic tumors one was labeled with NM404 indicating a possible progression from adenoma to adenocarcinoma. The primary colonic phenotype and longer life span of the Pirc rat combined with the growing resources of rat genetics, genomics and in vivo imaging poise the rat as an excellent model for tumor progression and experimental treatment of human colon cancer.

Whole Genome Methyloomic Profiling via Single Molecule Analysis

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DNA methylation plays a crucial role in development, cancer progression and aging. Alterations to genomic methylation profiles have profound and long lasting effects that may mediate the fate of a given cell. Through the analysis of a massive number of single genomic DNA molecules, we profiled the “methyloome” of a mid passage human embryonic stem cell line (H1). Using a variation of our laboratory’s molecular “barcoding” approach, which places individual DNA molecules on to any sequence scaffold after restriction mapping, we created a dataset of “barcodes” using a bolus of two restriction enzymes-one to create the barcode (methylation insensitive) and another that is methylation-sensitive. On barcoded molecules, the methylation status of CpG islands was assessed by the absence of restriction cleavage at known cognate sites across the whole human genome. Finally, molecular barcodes also reveal sites of structural alterations, which may correlate with discerned methylation patterns.

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Haplotypes, Microarrays, and Recombinants in the Identification of Hcs7, a Potent Liver cancer Modifier

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The Hcs7 liver cancer modifier, which accounts for the majority of the high susceptibility of C3H mice relative to B6, maps to distal Chromosome 1. This region affects both spontaneous and chemically induced tumorigenesis and is orthologous to regions of human chromosome 1q that are amplified in approximately half of liver and breast cancers.

Beginning with a 6.4 Mb region defined by congenic recombinants, we have analyzed SSLP and SNP haplotypes, gene expression in normal and neoplastic tissue, and fine-structure recombinants to identify candidates for Hcs7. For the haplotype analysis we sequenced exons and introns of 83 of the 114 known and predicted genes in the region from C3H genomic DNA. We have also sequenced segments from over 50 of these genes from two additional strains, CBA and BR, whose sensitivity relative to B6 also maps to distal Chromosome 1. Haplotypes were derived from ~265 kb of sequence from 303 exons and flanking introns, yielding 1137 SNPs. SSLP markers were chosen at 120 kb intervals. The two maps are remarkably similar, revealing the near-identity of B6 and BR throughout the region, as well as large blocks where the closely related CBA and C3H strains resemble or differ from each other. We have combined these SNP and SSLP data with the publicly available SNP data for an additional 7 strains for which we have phenotypic and mapping data. By comparing haplotypes for these strains at 38 loci throughout the 6.4 Mb region, we have identified two smaller regions (1.3 and 1.6 Mb) that display the expected haplotype patterns. Fine-structure congenic recombinant data, combined with this haplotype analysis, limit the susceptibility region to approximately 1 Mb. Gene expression analysis supports the candidacy of a family of genes in this interval.

The Antioxidant Enzyme System and Energy Metabolism Form a Network That Prostate Cancer Cells Exploit as They Move From Niche to Niche

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Our laboratory used different oxygen levels as a selective agent during growth of the DU145 prostate cancer cell line to develop three cell lines that grow in normoxic (4%), hyperoxic (21%), or hypoxic (1%) O₂ conditions. These levels were selected to approximate O₂ levels measured in cancerous prostate glands or normal adjacent tissues in human patients. Growth characteristics differed significantly between these cell lines without alterations in ATP levels, mitochondrial mass, or antioxidant enzyme levels. The 4% O₂ line demonstrated a more invasive phenotype, which correlated with an overall shift in redox potential towards greater reduction in comparison to the other two cell lines. In our current studies, differences were revealed upon transient changes in the O₂ levels. The parental line consumed significantly more O₂ than the daughter cell lines, and these lines were unable to increase O₂ consumption when switched to higher O₂ levels. Furthermore, the mitochondria in 1% cells were uncoupled but became coupled upon switching to higher O₂ levels or when transduced with adenoviral constructs containing manganese superoxide dismutase. These results suggest that low O₂ levels selected phenotypes with altered energy metabolism at the level of O₂ consumptive capacity and mitochondrial coupling. Furthermore, these results demonstrate use of the antioxidant system to control reactive oxygen species during transient, but not long-term changes in O₂ levels as well as offer evidence for the existence of a network linking O₂ levels, metabolism, and the antioxidant system that is exploited by these DU145 cancer cells as they move from niche to niche.

The structure of the calcium-rich signature domain of human thrombospondin-2 reveals insight into the role of thrombospondins in homeostasis and disease

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The five vertebrate and one *Drosophila* thrombospondins are secreted glycoproteins that play key roles in interactions between cells and the extracellular matrix. Several diseases have been linked to mutations and polymorphisms within the signature domain, a region that is conserved with exact spacing in all thrombospondins (53-82% identity across the whole family). We determined the 2.6 Å resolution crystal structure of the signature domain of human thrombospondin-2, which includes three EGF-like modules, 13 aspartate-rich repeats, and a lectin-like module. These elements interact extensively to form three striking structural regions termed the stalk, wire, and globe, which are stabilized by 30 bound calcium ions and 18 disulfide bonds. This structure reveals how thrombospondins are related to one another, how thrombospondins interact with binding partners, and how genetic alterations of thrombospondins result in disease.

Molecular Alterations in Murine and Human Intestinal Adenomas Associated with the Epithelial-Mesenchymal Transition

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Background & Aims: The epithelial-mesenchymal transition (EMT) is an event normally occurring during carcinoma invasion and metastasis, but not in early adenomas. Preliminary microarray data showed that vimentin, a mesenchymal marker, is elevated in intestinal adenomas of Apc^{Min} (Min) mice. The possible involvement of EMT was therefore studied in murine and human intestinal adenomas. *Methods:* Vimentin expression was detected in murine and human intestinal adenomas at different stages. Expression of markers associated with EMT and EMT-related signaling pathway were detected and compared with vimentin in murine tumors with different backgrounds.

Results: Strongly elevated vimentin expression was localized within epithelial tumor cells in intestinal adenomas of Min mice and colonic tumors induced by azoxymethane. In human colonic lesions, ectopic vimentin production was also observed both in adenomas and in invasive adenocarcinomas, but not in the normal colonic epithelium. Ectopic vimentin could not be detected in hyperplastic polyps. E-cadherin expression varied inversely with vimentin. Fibronectin expression appeared to be elevated while E-cadherin was decreasing. Elevated vimentin expression in adenoma cells was not correlated with Ras signaling, but was strongly correlated with reduced proliferation indices, active Wnt signaling, and TGF- β signaling, as confirmed by its dependence on Smad3.

Conclusions: EMT-associated molecular alterations can be detected in some early murine and human intestinal adenomas. This surprising observation can be explained by either of two distinct hypotheses, one related to complete EMT, the other related to tissue remodeling.

MHC class II expression functionally enhances CD1d presentation of an exogenous glycolipid by accelerating CD1d recycling

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NKT cells are a specialized subset of T cells that may play an important role in immune responses to tumors. NKT cells recognize self and foreign lipids presented by CD1d glycoproteins that are expressed on antigen presenting cells. The loading of exogenous lipid antigens into CD1d molecules is thought to occur mainly in late endosomal intracellular compartments, but little is known about the mechanisms that control the intracellular trafficking of human CD1d molecules through the endosomal vesicular system. Previous studies have shown that a fraction of CD1d molecules co-traffick with MHC class II molecules and the invariant chain, but the functional impact of this observation remains unclear. Here we show that MHC class II expression markedly enhances the kinetics of presentation of an exogenous lipid antigen called alpha-galactosylceramide (α -GalCer) to human NKT cells. This effect is due to more rapid CD1d recycling between the cell surface and intracellular compartments in MHC class II⁺ cells than in MHC class II⁻ cells, which appears to allow more rapid antigen loading in MHC class II⁺ cells. In contrast, antigen presentation is more stable in MHC class II⁻ cells than in class II⁺ cells, consistent with the observation that CD1d molecules are not as rapidly internalized in these cells. These results suggest that the fraction of CD1d molecules that traffick with MHC class II molecules are specialized for rapid loading of exogenous antigens, whereas the duration of CD1d-mediated antigen presentation is improved in the absence MHC class II trafficking. These findings have important implications for CD1d-mediated presentation of exogenous antigens in human dendritic cells (DCs), as MHC class II molecules are known to traffick actively in immature dendritic cells and are nearly completely static in mature DCs.

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Positional Cloning of a Type 2 Diabetes Quantitative Trait Gene: *SorCS1*

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The C56BL/6 (B6) and BTBR mouse strains, when obese, differ in diabetes susceptibility. We previously mapped the *t2dm2* locus, that affects fasting plasma insulin levels, to distal chromosome 19 in obese (*Lep^{ob/ob}*) F2 mice. B6 alleles at this locus were associated with a ~30% reduction in fasting insulin. Congenic mice in which a 7 Mb segment of B6 chromosome 19 was introgressed into the BTBR background (1339A) have a 30% reduction in fasting insulin levels, impaired insulin secretion *in vivo*, and disrupted islet morphology, confirming a locus in this region. Using subcongenic strains derived from 1339A, and a statistical approach we developed to allow us to analyze data from all the strains simultaneously, we localized *t2dm2* to a 242 kb segment. This region comprises the promoter, first exon and most of the first intron of the *SorCS1* gene, identifying variation in the *SorCS1* gene as underlying *t2dm2*. *SorCS1* is the only gene in the 1339A region for which we have identified mutations affecting the coding sequence, and the only gene for which we have detected differences in expression levels (a 10-fold difference, specific to islets). We assessed the contribution of *SorCS1* to human diabetes-related traits and found association between SNPs within *SorCS1* and both fasting insulin levels and insulin secretion in the Mexican American Coronary Artery Disease study, and of these same SNPs with diabetes risk in the San Antonio Family Diabetes Study. Identification of *SorCS1* as the gene underlying the *t2dm2* QTL and its validation in humans provides a novel window into the genetic contribution to obesity-induced type 2 diabetes.

Intestinal-specific PPAR γ Deficiency Enhances Tumorigenesis in ApcMin/+ Mice

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Multiple investigations of the effects of peroxisome proliferator-activated receptor gamma (PPAR γ) ligands on colon cancer have produced contradictory results. While some studies demonstrated increased numbers of colonic polyps in ApcMin/+ mice treated with various thiazolidinedione (TZD) PPAR γ ligands, others reported amelioration of tumor multiplicity and progression in both ApcMin/+ mice and in mice with chemically-induced colon cancer. We addressed the role of PPAR γ in murine intestinal tumorigenesis using gene knockout methodology. It was found that germline haploinsufficiency for PPAR γ elevated the number of tumors in ApcMin/+ females compared to PPAR γ +/+ ones, but had no effect on the number of tumors in ApcMin/+ males. Next, we observed that either heterozygous or homozygous intestinal-specific PPAR γ deficiency enhanced the number of Min-induced tumors in both the small intestine and colon, especially in the colon, where PPAR γ deficiency also modulated tumor incidence. Gender affected tumor multiplicity independent of PPAR γ genotype. Female ApcMin/+ mice developed more tumors in the small intestine and more tumors overall, whereas male ApcMin/+ mice developed more tumors in the colon. Nevertheless, intestinal PPAR γ deficiency enhanced tumorigenesis irrespective of gender. Our results suggest that PPAR γ functions as a tumor resistance factor in the mouse intestine and warrant further investigation of the PPAR γ -dependent and independent actions of TZDs in cancer.

Methyl-Deficient Diet increases imprinted gene expression in the prostate

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Folate deficiency has been linked to aging and cancer susceptibility in a number of organs, including the prostate. IGF2 (Insulin-like Growth Factor 2) is a potent inducer of neoplasia in vitro and in vivo. IGF2 levels have been previously found to increase in the prostate with aging. The goal of this study was to determine whether the expression of IGF2 and other imprinted genes can be modulated by a methyl-deficient diet in the prostate.

Three month old B6 mice containing a polymorphism for IGF2/H19/p57 were placed on a choline and methionine deficient (CMD) diet, 10% caloric restricted (CR) or 33% caloric restricted (CR) control diet. Animals were then assessed at 0, 4 and 7 months for alterations in global methylation, gene expression and genomic imprinting changes in the prostate.

Seminal vesicle and prostate weights decreased in the CMD animals at 4 and 7 months compared to other groups. Androgen signaling was intact as demonstrated by no alteration in probasin expression in the prostate. Global methylation loss (30%) was demonstrated in the animals receiving the CMD versus CR diets at both timepoints. Expression analysis in the dorsolateral prostate demonstrates significant increases in expression at the 4 month (IGF2 - fold, H19 -fold) and 7 months (IGF2 -fold, H19 -fold) timepoints compared to CR animals. No expression changes were noted for p57. Increased gene expression was mediated, in part, by an increase in the IGF2 P1, P2 and P3 promoters. Analyses by fluorescent primer-extension demonstrate no significant changes in IGF2 imprinting in CMD animals compared to control.

This study for the first time demonstrates that methylation deficient diets modulate imprinted gene expression and significantly increase IGF2 and H19 expression synchronously. This synchronous increase suggests an alternate model not utilizing the H19 imprint control region (ICR). This data provide evidence for a mechanism linking age-related changes in prostate cancer with dietary modification of an epigenetic basis. Further analysis of this mechanism is ongoing.

Phage Expression Arrays to Detect Potential Prostate Cancer-Testis Antigens

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Cancer-testis antigens (CTA) represent a family of proteins that are normally expressed in the testis but which can be aberrantly expressed in malignant tissues. The use of antibody-based screening methodologies using sera from patients with cancer to probe expression libraries from malignant tissues or normal testis tissue has led to the identification of over 50 members of this family. The expression of CTA in multiple different tumor types has suggested that some CTA may represent “universal” tumor antigens that could be targeted by tumor vaccines for multiple tumor types. In the current study, we wished to identify whether patients with prostate cancer have IgG responses to known CTA. To test this, we cloned 29 known CTA into lambda phage expression vectors and confirmed transcription of the individual antigens. Phage were then used to transfect *E. coli* for individual CTA expression. Using a high-throughput immunoblotting procedure, phage expression arrays were then screened with sera from 98 patients with prostate cancer and 50 male control volunteer blood donors. Of the 29 CTA, the antigen most commonly recognized in patient sera was NY-ESO-1 (3/98 patients, 0/50 controls). These results support the findings of other investigators, and support the ongoing investigation of NY-ESO-1 as a potential tumor antigen in prostate cancer. In addition, the methodology we report could be applied to the identification of CTA using sera from patients with other tumor types.

A Comparative Analysis of the Mammalian Recombinational Landscape

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Linkage disequilibrium analyses and sperm-typing of targeted regions have unveiled a strikingly punctuated distribution of recombination rates across the human genome. This heterogeneity in rates is characterized by a prominent bimodal pattern, with the overwhelming majority of recombination events occurring in a small fraction of the genome. Comparisons of recombination rates in orthologous human-chimpanzee 1-2 kilobase windows reveal little selective constraint on the maintenance of these so-called “hotspots” of recombination. In contrast, we have previously identified a clear phylogenetic component across eutherian mammals in the total level of recombination per genome. The discrepancy between the net and fine-scale patterns of phylogenetic conservation in recombination rate suggests that this trait may be subject to distinct evolutionary pressures operating on different physical scales. Here, we adopt a comparative approach to characterize evolutionary patterns in recombination rates at an intermediate scale. We compare recombination activity at the megabase level across homologous genomic regions of human, mouse, rat, and cow, four mammals that feature dense genetic maps integrated with genome sequences. We use this approach to ask whether genomic regions inferred to harbor recombination hotspots in humans are characterized by elevated levels of recombination in other species. Although we focus on three orders of mammals, our sampling spans a broader portion of the mammalian phylogeny than has been considered by previous studies of localized variation in recombination. Our preliminary results indicate relatively low levels of rate conservation at the resolution of megabases. Together, our findings suggest that recombination rates may evolve rapidly within genome regions, even as selective pressures act to maintain a minimum total amount of recombination throughout the genome.

Brain-Computer Interfaces for Severe Motor Disabilities

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Brain-computer interface (BCI) technology has the potential to provide individuals with severe motor disabilities greater independence, functionality, and a higher-quality life. A BCI is a communication system that does not depend on the brain's normal input/output pathways of peripheral nerves, muscles, and sensory organs. Instead, a direct connection is made between a computer and signals recorded from the brain. Specifically, recorded neural signals are translated into real-time actions, which can give a person with a motor disability the ability to answer simple questions quickly, perform word processing, and adjust environmental variables (e.g. lights, temperature, television) by controlling the movement of a cursor on a computer screen. Other future BCI applications include using neural signals to operate a robot, wheelchair, or neuroprosthetic limb.

Electroencephalography (EEG) and electrocorticography (ECoG) are two methods of recording neural signals for use with a BCI. EEG electrodes are scalp-based, while ECoG electrodes are neurosurgically implanted on the surface of the cerebral cortex. This study investigates target acquisition performance and learning using BCIs controlled by EEG and ECoG signals. EEG subjects included able-bodied and severely motor disabled individuals, and ECoG subjects were neurosurgery patients with temporary intracranial electrode implants. First, screening data was collected while subjects imagined movements or sounds in response to a cue. This data was analyzed to find signal features that could be modulated by the subject, and 3-5 Hz frequency bands (chosen based on their correspondence to imagery) of individual electrodes were assigned a direction of cursor movement. Subjects then performed a closed-loop target acquisition task by using imagery to self-modulate their neural signals to move the cursor. Measures included human information processing rate in a Fitts' law paradigm and learning rate, which are used to make comparisons across EEG, ECoG, and manual cursor control modalities. The next phase of this study will use mental effort and perturbation response data to increase BCI usability. The results of this study will be used to better understand and improve current BCI technology so that it will be a practical and useful means of communication and independence for individuals with severe motor disabilities.

High Throughput Screening to Identify Small Molecule Modulators of Biological Processes

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The Keck-UWCCC Small Molecule Screening Facility is a full service assay development and small molecule screening facility at the University of Wisconsin's Comprehensive Cancer Center. Our small molecule screening service has been used to perform assays to identify small molecules that inhibit UDP-Galactopyranose Mutase, UGM being an important component for the onset of Tuberculosis. Using fluorescence polarization (FP) 16,000 molecules were screened and 3 UGM inhibitors were identified(1). Using our expertise in performing cell based assays we developed several services to analyze the effects of small molecules on a small panel of cancer cell lines representing the cell lines used by the National Cancer Institute for their 60 cell line screen (2). This service has been utilized to determine a cytotoxicity profile of 81 compounds that are derived from Digitoxin, a cardiac glycoside that exhibits anti-cancer activity. The cytotoxicity assay service helped determine that the chemical modifications enhanced the anti-cancer properties of digitoxin (3). We present here two examples of the utilization of high-throughput screening to identify not only new chemical modulators of biological systems, but also new uses for existing chemical entities.

(1)J. Am. Chem. Soc., 126 (34), 10532 -10533, 2004.

(2)Cancer Research 48: 589-601, 1988.

(3)Proc Natl Acad Sci U S A.2005 Aug 30;102 (35):12305-10

DLL3 as a Candidate Gene For Human Congenital Scoliosis and Vertebral Malformations

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Prior investigations have not identified a major locus for congenital vertebral malformations and congenital scoliosis. Because congenital vertebral malformations represent a sporadic occurrence, linkage approaches to identify genes associated with human vertebral development are not feasible. Based on observations in mice and humans, we hypothesized that mutations in *DLL3*, a member of the notch signaling pathway might account for a subset of human vertebral malformations. We therefore performed DNA sequence analysis of the *DLL3* gene in 50 patients with congenital vertebral malformations spanning the entire vertebral column. A Caucasian male patient with a T5-T6 block vertebrae, ventricular septal defect, left renal agenesis, hypospadias, bilateral clubfoot, left thumb hypoplasia and a syrinx was found to be heterozygous for a "G" to "A" missense mutation resulting in the substitution of glycine by arginine at amino acid codon 269 of exon 5. This residue is conserved in the chimpanzee, mouse, dog and rat, but not in chicken, fugu or zebrafish. The mother of the patient, who is clinically asymptomatic, was also heterozygous for the missense mutation. Additional testing on this patient included normal 46, XY male karyotype, negative breakage study for Fanconi Anemia and negative fluorescence in-situ hybridization for 22q deletion. The mutation was not found in a control population of 85 anonymized individuals (81 Caucasians, 2 Hispanics and 2 Asians). Since this mutation was not observed in a control population, and leads to a non-conservative amino acid change, we suggest that this mutation may be clinically significant. Several established mechanisms could explain the existence of the mutation in both the patient and his asymptomatic mother (decreased penetrance, somatic mosaicism, multigenic inheritance). Documenting the absence of the mutation in a larger control population or the presence of the mutation in additional affected patients, or documenting a functional difference in *DLL3* function would provide further evidence supporting its pathogenicity.

Molecular-Epidemiological Study of Estrogen-Metabolizing Enzymes in Women with Myoma and Endometrial Carcinoma

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At the present time the ability of estrogens to stimulate the growth of a number of endocrine cancers is well established. Excess exposure to endogenous and exogenous estrogens increases endometrial cell division and is suggested to be the mechanism through which established risk factors for myoma and endometrial cancer influence risk. Genetic polymorphisms in estrogen metabolizing enzymes contribute to susceptibility to disease. Identification of certain allelic variants of these enzymes as genetic susceptibility markers can provide an important tool in early diagnosis and prevention. We estimated a frequency of allelic variants of cytochrome P450: CYP1A1, CYP1A2, CYP19 (Aromatase) and II-phase enzyme SULT1A1 (Sulfotransferase) genes in the female Caucasian population of the Novosibirsk region of and their association with the elevated risk of endometrial cancer and myoma. In our study we had three experimental groups: the patients with endometrial cancer with different level of the tumor malignancy (n = 110), the patients with myoma (n = 172), and the control group consisted of healthy aged (not less then 55 years old) women who had an uncomplicated obstetric history (n = 172). No association of CYP19 and CYP1A1 polymorphisms with increased endometrial cancer and myoma risk was revealed. A positive correlation of C allele of CYP1A2*1F and G allele of SULT1A1*2 with studied hormone-dependent diseases was found. We carried out the epidemiological studies allowing to determine phenotypes of the hormonal status of patients, CYP1A2 enzyme activity and SULT, STS (steroid sulfatase) gene expression in tumor tissues. We have shown that among females with endometrial cancer and myoma those having an enhanced hormone level are much more frequent than in the control group (OR varied by a factor 4 - 5 for such parameters as late menarche, late menopause, amount of pregnancies, and number of abortions). The functional significance of polymorphism for CYP1A2 was confirmed by theophylline test: women with myoma had the increased ratio of poor metabolizers for this test drug. Gene expression of SULT1E1 was decreased in tumor endometrial and myoma tissues, whereas STS was increased compared to non-cancer tissues. Thus, CYP1A2, SULT, STS genes could be appropriate candidates for studying their contribution to endocrine disorder and environmentally determined diseases susceptibility.

Stochastic Kinetics of Virus Intracellular Growth and Production: Computer Simulations

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Viruses cause diseases such as influenza, AIDS and SARS and thereby create a major and ever expanding global threat to human health. The persistence, emergence and potential bio-terrorist use of viruses define urgent reasons to understand how viral growth and persistence are linked. Many viruses exist in nature as genetically heterogeneous populations, but it is not known to what extent genetic variation impacts their response to host or other environmental factors. Synchronous ‘one-step’ infections provide an average measure of virus growth across a large population of infected host cells, but they mask information about the distribution of growth behaviors. To better understand quantitatively how genetic and environmental factors contribute to distributions in virus production from individual cells we proposed to combine flow cytometric measurements of infected cells with computational models of virus intracellular development. As a model we study Vesicular Stomatitis Virus (VSV), a non-segmented negative-stranded RNA virus that encodes five genes: 3'-N-P-M-G-L-5'. Our past deterministic kinetic model of VSV growth accounts for the production, interactions and decay of essential VSV molecular species including VSV mRNA, genomic and anti-genomic full-length RNA, VSV proteins, several protein-RNA intermediates, and VSV progeny precursors; the model captures essential relationships among virus constituents. However, recent experiments have revealed broad distributions of virus-mediated gene expression, measured at the single-cell level by flow cytometry, as well as distributions of virus yields measured from single infected cells, features that deterministic models cannot explain. To better understand mechanistically how such distributions may come about, we have begun to develop a stochastic VSV infection model that allows for inherent fluctuations in the levels of viral constituents. Such effects will be most significant during the earliest stages of infection when levels of viral species are low; they may cause extinction of virus species from infected host cells or contribute to distributions in virus progeny production. Experimental data will be used to estimate parameters of the model, and a sensitivity analysis will be performed to gain insights into the processes that contribute most prominently to the observed distributions.

Discovering Structural Variants in Cancer Genomes via Single Molecule Analysis

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Cancer is a complex disease caused by an accumulation of somatic mutations, and tumors present a panoply of these mutations ranging from SNPs to cytogenetically visible rearrangements. The Human Cancer Genome Project will incorporate high-throughput methods for discovering somatic point mutations and copy-number polymorphisms via array-based and re-sequencing approaches. We present the optical mapping system as a platform for the discovery and characterization of acquired structural mutations in human cancer samples, ranging from small (5 kb) insertions and deletions to large-scale chromosomal rearrangements.

Optical mapping analysis of the breast cancer cell line MCF-7 has revealed hundreds of structural variants, the majority of which are undetected in other published datasets. The optical map data has also dissected complex breakpoints, at subgenic resolution, without any prior hypotheses or additional experiments. Candidate fusion genes have been identified, as well as large (50-100 kb) structural mutations in genes relevant to other cancer types. Chromosome arms 17q and 20q harbor copy-number increases in 20-40% of breast cancer cases including MCF-7; optical map data for MCF-7 shows that these two regions are involved in multiple complex translocations, resulting in several distinct structures within a single cytoband.

Successful therapeutics such as Gleevec, Herceptin, and Iressa are designed to target large-scale structural mutations. However, the prevalence and consequences of smaller structural variants in cancer samples is currently difficult to assess given the high costs associated with library construction and sequencing for genome-wide projects. As such, optical mapping complements existing technologies (SNP detection, CNP chips, FISH) in order to obtain a complete view of the mutational spectrum found in a cancer genome.

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Fetal Androgen Excess Accelerates Pulsatile Luteinizing Hormone (LH) Release in a Nonhuman Primate Model for Polycystic Ovary Syndrome (PCOS)

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Exposure of the developing female hypothalamus to androgens produces permanent alterations in the ability of gonadotropin-releasing hormone (GnRH) release, which can result in luteinizing (LH) hypersecretion. LH excess found in women with polycystic ovary syndrome (PCOS) may therefore be a consequence of programming of the developing fetal hypothalamus by androgens, leading to hypersecretion of GnRH in adulthood. To test this hypothesis, 7 adult, prenatally androgenized (PA) female rhesus monkeys that exhibit many of the pathophysiological features of PCOS, and 3 age- and weight-matched controls were studied. Four of the PA monkeys were exposed to androgen excess during early (E) gestation (25-35 days, starting on gestation days 40-43) and 3 were exposed during late (L) gestation (15-25 days, starting on gestation days 100-115). Plasma LH levels were determined from a series of blood samples obtained through an indwelling femoral catheter at 10-min intervals, followed by an additional GnRH challenge. LH levels in plasma were assessed by RIA and LH pulses were determined by PULSAR. Both EPA and LPA females did not differ from control females with regard to baseline and mean LH levels, LH pulse amplitude, and LH response to GnRH. However, the results suggested a ~2-fold increase in frequency of pulsatile LH release in EPA, but not LPA females. Early gestation exposure to androgen excess thus appears to increase the frequency of pulsatile LH release without apparent concomitant changes in pituitary gonadotrope responsiveness to GnRH. The acceleration of LH pulse frequency in EPA, but not LPA females, implies that early prenatal androgen exposure programs accelerated GnRH pulsatility in adult female monkeys. Our non-human primate study has direct implications for PCOS women, as these results suggest that not all women with PCOS will exhibit LH hypersecretion, a prediction that is borne out in the heterogenous presentation of elevated LH levels in women with PCOS.

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Use of N-15 Metabolic Labeling for Biomarker Discovery in the Apc^{min} Mouse Model for Colorectal Cancer

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Over the past several years a variety of isotope-assisted quantitative proteomics techniques have been developed, allowing use of tandem mass spectrometry for the simultaneous comparison of the identities and abundances of hundreds or thousands of proteins within pairs of biological samples. These approaches include in vitro labeling techniques such as ICAT and ¹⁸O labeling which introduce an isotopically-labeled tag onto each sample during sample preparation, as well as in vivo metabolic labeling techniques in which an isotopic label is ubiquitously incorporated into the organism from food during normal growth and development. For all of these approaches, after incorporation of either a light or a heavy isotopic tag, the control and experimental samples are combined and processed together. Because the tagged peptides are essentially chemically identical, they provide an excellent internal control for all subsequent steps in sample preparation and analysis. Yet using mass spectrometry we can differentiate among peptides from each sample due to the mass difference between the heavy and light isotopic tags. Because metabolic labeling allows combination of control and experimental samples through all steps of sample processing including tissue homogenization and protein extraction, it provides perhaps the ideal internal control.

While metabolic labeling provides an elegant control for all steps in sample preparation and is easily applied to yeast and prokaryotic model systems, technical challenges have limited its use in intact mammalian organisms. Having recently achieved efficient incorporation of ¹⁵N and developed the informatics tools required for automated analysis of data from this kind of experiment, we are using metabolic labeling with tandem mass spectrometry to characterize protein and peptide changes in plasma throughout early stages of tumorigenesis in the Apc^{Min} mouse model for colorectal cancer. While analysis is ongoing, we have achieved excellent incorporation (85-95%) of blood proteins and have identified and quantified hundreds of peptides at several timepoints during the course of tumorigenesis. Several peptides appear to show changes in abundance that are consistent across multiple timepoints and biological replicates. These results will not only enhance our understanding of tumorigenesis in the Min mouse, but demonstrate that we have established a system for using metabolic labeling to study other mouse models of human disease.

The Gamma Interferon Induced GTPase, Human Guanylate-binding Protein (hGBP-1), Plays a Role in Host Defense Against Chlamydia Trachomatis

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Chlamydia trachomatis(Ct) is an obligate intracellular parasite which exhibits a biphasic life cycle. A highly infectious extracellular elementary body (EB) infects cells, forms an inclusion membrane around itself, and converts to a noninfectious but metabolically active reticuloid body (RB) which multiplies within the inclusion. A major host response to Ct infection is the production of interferon gamma. IFN γ causes the induction of multiple genes including indoleamine 2,3-dioxygenase which depletes host tryptophan, and human guanylate-binding protein-1 (hGBP-1), a large GTPase. Human GBP-1 belongs to a family of hGBPs which are farnesylated proteins that localize to the Golgi upon activation. We found that hGBP-1 plays a role in inhibiting chlamydia trachomatis growth and development. We used siRNA technology to knock out hGBP-1 in HeLa cells after interferon gamma treatment and determined the effect on Chlamydia development. The cells were first treated with siRNAs, either mock or hGBP-1 specific. Subsequently, they were incubated in tryptophan depleted medium with 200units of IFN γ for 24 hours, infected with Ct, and maintained in the same medium. To assay for Ct infection, cells were stained with an anti-LPS antibody specific for Ct and flow cytometry analysis was performed. We found that Ct growth could not be detected unless the cells had been pretreated with the hGBP-1 siRNA. Both coding and noncoding siRNAs for hGBP-1 showed the same result. Constitutive expression of hGBP-1 in the HeLa cells by a retroviral vector with the hGBP-1 cDNA reversed the effect of the non-coding siRNA knock out and Ct was unable to grow in these cells again indicating specificity. However, while knockout of GBP-1 allowed inclusion formation and conversion to the RB form, these forms were aberrant and the recovered infectious forming units (EBs) was down by 100 fold. Thus, human hGBP-1 is one of the defense mechanisms that human cells use to inhibit the growth and development of Chlamydia trachomatis.

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Novel Modifiers of Apc^{Min} on Chromosomes 18 and 11

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The Apc^{Min} mouse model of intestinal cancer develops discrete polyps, affording a quantitative measurement of tumor multiplicity. This property has previously been used to uncover two polymorphic modifiers of the Apc^{Min} phenotype through quantitative trait locus analysis: *Mom1* and *Mom2*.

Here, we describe two new modifiers, *Mom3* and *Mom4*, localized to chromosomes 18 and 11, respectively. Three inbred Apc^{Min} strains have been used: C57BL/6J (~100 tumors), BTBR (~500 tumors), and AKR/J.*Mom1* (~15 tumors). Reciprocal congenic substitutions of *Mom3* among the B6, AKR, and BTBR backgrounds strikingly demonstrate that homozygosity or compound heterozygosity of the AKR and BTBR *Mom3* alleles enhances intestinal neoplasia >2.5-fold on the B6 genetic background. Conversely, one or two copies of the B6 allele of *Mom3* reduces tumor multiplicity >2.5-fold on the AKR and BTBR backgrounds.

Fine mapping has localized the *Mom3* locus to within 8Mb on proximal chromosome 18, and emerging recombinant lines may narrow it to within 6Mb. We hypothesize that *Mom3* may act by directly modulating the rate of loss of heterozygosity (LOH) of the distal *Apc* locus. The incomplete genome assembly of the pericentromeric region on chromosome 18 hinders identification and characterization of *Mom3*, requiring novel methods of sequence assembly. It is possible that the centromere itself is mitotically unstable, in that its repetitive nature is recombinogenic. It will be informative to determine whether tumor suppressor-linked modifiers are also found on metacentrics, as in the Apc^{Pirc} rat or in humans.

In contrast, homozygosity for the AKR allele of *Mom4* on chromosome 11 provides a 1.5-fold resistance to tumorigenesis over the B6 allele. This effect was highly significant ($p < 0.01$) in populations of 205 backcross and 92 intercross progeny. However, reciprocal congenic lines have not established a consistent modifier effect to date. It may be that complex allelic interactions at multiple loci are required; preliminary data from [B6 x AKR] F1 $Mom4^{AKR/AKR}$ mice suggest that a heterozygous background may uncover the modifier effect.

The hypothesis that *Mom3* may modulate the LOH of distal elements - or that *Mom4* modulates the LOH of unlinked loci - raises the possibility that the somatic LOH of canonical human tumor suppressors is similarly regulated.

Cell surface receptor characterization using whole cell-binding array

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The cell surface proteome plays an important role in fundamental biological processes such as cell signaling, cell-to-cell communication, and nutrient and metabolite transport. Specifically, cell surface receptors bind to extracellular ligands to initiate cellular responses. Because cells express different receptors at different times under various conditions on the cell surface, it is important to monitor and characterize these changes to better understand basic cellular functions. Our group has developed a system to investigate receptor expression patterns on cell surfaces without isolation or purification of the receptors. This system utilizes aldehyde-terminated self assembled monolayers (SAMs) on gold surfaces to react with amine-bearing biomolecules. Protein ligands can be attached to the SAMs through their lysine residues and then capture whole cells through a ligand-receptor interaction. The cell binding patterns can be monitored by phase contrast microscopy and the cell binding density can be determined by cell counting, which allows us to analyze the receptor expression. Currently, we are investigating cell surface receptors involved in stem cell self-renewal and angiogenesis of endothelial cells.

Indomethacin Reduces Skeletal Muscle Wasting in Mice Bearing the Lewis Lung Carcinoma But Not the B16 Melanoma

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Introduction: Cancer cachexia is associated with progressive weight loss and skeletal muscle wasting (SMW), is mediated in part by pro-inflammatory mediators such as tumor necrosis factor. Cyclooxygenase (COX) is an enzyme involved in synthesis of PGE₂. Our lab previously reported that treatment with 5 mg/kg/day indomethacin, a nonspecific inhibitor of COX1 and COX2 activity, preserved body weight and muscle mass in mice bearing the colon26 adenocarcinoma. Preservation of muscle mass was associated with reduced levels of TNF receptor type 1 (TNFr1) in muscle homogenates. The purpose of the present study was to determine if indomethacin has similar effects in other mouse models of tumor-induced SMW.

Methods: Female mice weighing 18-20 grams were inoculated subcutaneously with 5×10^6 B16 melanoma cells or Lewis Lung carcinoma cells (LLC). Tumor-bearing and control animals were given 5 mg/kg indomethacin or 7 mg/kg NS398, a COX2 specific inhibitor, in their food for 17 days. Mice were euthanized on day 17 of tumor growth, and the gastrocnemius muscles were removed and weighed. Muscle mass relative to body weight was analyzed using ANOVA.

Results: Growth of the B16 resulted in significant SMW that was not associated with an increase in muscle levels of TNFr1. Indomethacin had no effect on muscle mass of tumor-bearing or healthy control animals. Similarly, treatment with NS398 had no effect on muscle mass in mice bearing the B16. Growth of the LLC also resulted in significant wasting of the gastroc muscles and was associated with increased muscle levels of TNFr1. Treatment with either NS398 or indomethacin preserved muscle mass in the tumor-bearing mice, and had no effect on muscle mass of healthy control animals. Neither drug affected muscle levels of TNFr1 in mice bearing the LLC.

Conclusion: COX activity contributes to the catabolism of skeletal muscle in the LLC and colon26 models of tumor-induced SMW, but not in the B16. The beneficial effects of COX inhibitors in reducing SMW can occur independent of changes in muscle levels of TNFr1. Further work is needed to identify COX-independent mediators of tumor-induced SMW.

High Throughput Culture of Epithelial and Stromal Cell Lines in Microchambers

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The basic platform for performing cell-based experiments has changed little in almost a century. Cells in culture exist in a largely undefined milieu - both physically and biochemically. This is in contrast to microtechnology-based approaches, where surface patterning of molecules and cells, defined geometries, as well as spatial and temporal control of soluble factors is possible. In addition to improved control, microscale cell culture vessels are more efficient. Such vessels require fewer cells for each assay, and a smaller amount of reagents. Despite these advantages, microtechnology is still not widely used. Biologists do not have ready access to this technology because it generally requires specialized equipment and training. We have developed a microtechnology-based cell culture platform that can be interfaced with conventional hand-held pipettes as well as high performance automated liquid handling equipment. Using this platform we tested the response of NMuMG and 3T3-L1 cell lines to six different cell culture media. The cells were seeded in 48 chamber plates in regular culture medium in 3 microliter microchambers at a seeding density of 120 cells/mm. At 21 hours, the medium was replaced with one of six different recipes.

Phase contrast photomicrographs were captured at 14 and 48 hours after seeding and the cell number at each timepoint was used to assess proliferation. The cells were stained with live and dead cell probes at 48 hours. These images provided information about cell death. We found that the proliferation of NMuMG cells was significantly lower and cell death was increased in the absence of FBS or EGF when compared with the regular cell culture medium. Proliferation of 3T3-L1 cells was not significantly different between any of the media, but cell death was higher in the absence of FBS or EGF. Our future studies will include co-culture in separately addressable, but fluidically connected microchambers. We have demonstrated cell seeding in such chambers and the cell culture characterization discussed above will serve as a baseline for our co-culture work.

Human Loci Orthologous to the Rat Compound QTL Mcs5a Associate with Breast Cancer Risk in Women

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Previous attempts to locate human breast cancer susceptibility genes have found genes that greatly influence risk, but are rare in the population (i.e. BRCA1 and BRCA2). It has been hypothesized that there are many high frequency genes that only slightly increase a woman's risk of developing breast cancer. Instead of the commonly used candidate gene approach to look for breast cancer genes in humans we have chosen to use an animal model to locate areas of the human genome that may contain orthologous loci that are involved in breast cancer risk. In the rat it has been shown that some inbred strains are more resistant to the development of mammary tumors than others after exposure to carcinogens. When a comparison of strains with high and low susceptibility after carcinogen exposure was made, QTLs that conferred resistance were found. We have evaluated the human homologous region of a compound rat mammary cancer resistance locus Mcs5a. Two SNPs in the orthologous regions to rat Mcs5a1 and Mcs5a2 were found to associate significantly with breast cancer risk in a large case-control study (n~12,000 women).

In the MCS5A1 region there is a SNP with a minor allele that confers an increase in risk of ~19% to the 6% of women who are homozygous for this allele. Homozygote risk 1.19 (95% CI 1.03-1.38), p-value trend test (1 df) = 0.022. In the MCS5A2 region there is a SNP with a minor allele that confers a ~14% reduction in breast cancer risk to the 22% of women who carry at least one minor allele. Heterozygote risk 0.86 (95% CI 0.79-0.94), homozygote risk 0.77 (95% CI 0.57-1.04), p-value for the trend test (1 d.f.)= 0.0003. The identification of these two breast cancer modifier alleles by taking advantage of natural genetic variation in model organisms, reinforces the possibility that a sufficient number of modifier alleles can be identified using similar methods to impact breast cancer risk estimation in women.

A Novel Technique of Assisted In Vitro Fertilization with Cryopreserved Inbred Mouse Sperm

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The management of a research program using the genetics of an inbred mouse strain can be made markedly more efficient if sperm from a mutant derivative of the strain can be cryopreserved and the derivative line reconstituted by fertilization in vitro (IVF). IVF using cryopreserved sperm from certain inbred strains has previously been reported to yield inadequate fertilization rates. This study has expanded upon a novel technique that increases fertilization rates from cryopreserved inbred sperm frozen in raffinose and skim milk (Carlisle Landel, Jackson Laboratory, personal communication). Oocytes are pre-treated with an acid tyrode solution to thin the zona pellucida as a means of assisting in vitro fertilization (AIVF). Results show that treatment of C57BL/6J oocytes with acid tyrode increased two-cell embryo development post-IVF with frozen C57BL/6J sperm from 3.3% (203 two-cell embryos/6203 oocytes) to 29% (552 two-cell embryos/1896 oocytes). Similar two-cell embryo development was obtained using AIVF with cryopreserved 129/SvEvTac and AKR/J sperm, 23% and 24% respectively. Live offspring were produced from each strain in this study at mean rates for a strain ranging from 34% to 62% of implanted two-cell embryos. This strategy applies to genetics programs involving an inbred strain and its single-factor derivatives. By contrast, multi-factor derivatives such as recombinant-inbreds must be managed by more labor-intensive embryo cryopreservation.

Integrating Patterns of Polymorphism at Single Nucleotide Polymorphisms (SNPs) and Short Tandem Repeats (STRs)

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Single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs) differ in mutation rate and mechanism. These differences have led most researchers to analyze variation separately at these two types of loci. However, the simultaneous consideration of polymorphism at SNPs and STRs can provide important insights that are difficult to extract from analysis of either marker type in isolation. We use computer simulations to model the opposing effects of contrasting mutational dynamics and of shared genealogical history on the correlation between polymorphism at linked SNPs and STRs. Results show that polymorphism patterns are correlated only weakly despite the shared underlying genealogy, underscoring the importance of divergent mutational processes. We apply this new perspective to large-scale polymorphism datasets to interpret patterns of co-variation at SNPs and STRs throughout the human genome. Further examples illustrate how knowledge of this correlation can be helpful for several problems in human population genetics.

The Keck-UWCCC Small Molecule Screening Facility Provides Access to Chemical Genetics and Mechanism of Action Assays to UW Investigators

Noel Peters, Megan Fitzgerald, F. Michael Hoffmann

Keck-UWCCC Small Molecule Screening Facility

The mission of the Keck-UWCCC Small Molecule Screening Facility is to provide biologists in identifying new chemical modulators of biological processes and assisting chemists in screening new chemicals for biological activities. We have screened our compound libraries over 35 times and work with individual investigators to identify the strongest leads from our compound collections of over 43,000 unique small molecules. A major effort was made by facility staff in 2004-2005 to establish biological assays that can be used by campus chemists or other investigators to assess the biological activity of a compound. The first component of this service was to offer cytotoxicity assays in cancer cell line lines. The initial 9 cancer cell lines were provided by the National Cancer Institute and are representative cell lines from the NCI's 60-cell line screen. The service is available not only to users who would like to determine if hits from their screens are toxic to cancer cells, but also to chemists who would like to determine if molecules they have synthesized are toxic to cancer cells. In 2005, the service was re-optimized to have a multiplexed read-out. The assay now determines effects of compounds on cancer cells using 3 read-outs of cytotoxicity. We have also expanded the initial cell line panel to include 9 more cell lines bringing the total number of cell lines up to 18. We now offer apoptosis, chamber migration assays, wound healing assays, ELISAs and custom designed assays to meet the needs of investigators. All services performed at the screening facility are available to UW investigators.

For more information visit the facility website: www.hts.wisc.edu

Vascular Defects in Type 2 Diabetes: the Role of SorCS1

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Proper vascularization is required for growth, survival, and function of the pancreatic islet. This vascularization becomes especially important during insulin resistance-induced β -cell expansion. Vascular defects have been suggested to play a role in the development of Type 2 Diabetes. Our lab has recently identified SorCS1 as a modifier of Type 2 Diabetes susceptibility in obese mice. The 1339A strain of obese mice contains SorCS1 promoter polymorphisms, causing an 8 to 10-fold increase in islet-specific transcription of SorCS1. These mice have defective in vivo glucose-stimulated insulin secretion, leading to severe Type 2 Diabetes. The 1339A strain exhibits increased apoptosis in the islet that co-localizes with the microcapillary network. Morpholino-induced knock-down of SorCS1 in the zebrafish embryo disrupts head and intersegmental vessel development. Moreover, in vitro binding studies demonstrate that SorCS1 binds to platelet-derived growth factor (PDGF-BB), an angiogenic growth and survival factor that promotes pericyte and smooth muscle cell recruitment to the developing vessel. SorCS1 encodes a transmembrane receptor, the extracellular domain of which is proteolytically cleaved from the cell surface of murine Min6 β -cells in vitro. These data lead us to hypothesize that SorCS1 overexpression in β -cells causes excessive shedding of the SorCS1 exo-domain into the extracellular space. Shed SorCS1 may interfere with PDGF-BB signaling by acting as a soluble decoy receptor, thus contributing to vascular death and preventing proper islet vascularization. SorCS1 polymorphisms have also been detected in two diabetes-susceptible human populations, suggesting that this role of SorCS1 may be important for human health.

**S. Raines and O. Richards contributed equally to this work.

Proteomic comparison of mouse pancreatic islet cells using 2-D PAGE and MALDI TOF/TOF to identify proteins associated with type 2 diabetes

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Introduction Whereas >80% of people with type 2 diabetes mellitus (DM2) are obese, the majority of obese people never develop DM2. Understanding this dichotomy is a major objective of the entire diabetes research community and is the main focus of research in the Attie laboratory. We have modeled the obesity-diabetes dichotomy in mice and identified an insulin-resistant mouse strain, BTBR. In contrast to the C57BL/6 (B6) strain, when made obese with the leptin-*ob* mutation, the BTBR strain develops severe diabetes due to loss of b-cell mass.

The objective of this study is to compare the pancreatic islet proteomes from diabetes-resistant (B6) and diabetes-susceptible (BTBR) mice before and after the onset of diabetes.

Methods Intact islets are isolated from pancreata using collagenase digestion and hand-picked under a stereomicroscope. Approximately 1000 islets (pooled from 10-15 animals) are utilized to isolate the cytosolic protein fraction. Gels are run in sets to compare lean vs obese samples. A total of approximately 500ug total protein is loaded onto each 2-D gel and separated by size and isoelectric point. The gels are stained with sypro ruby and compared to determine unique features and differences in spot intensities. These spots are excised and undergo in-gel tryptic digestion. The digests are purified over a C18 spin column and spotted onto MALDI plates with alpha-cyano matrix, where they are analyzed using MALDI TOF/TOF. The MALDI data is searched against a mouse protein database to identify the proteins.

Identification and Characterization of Genomic Structural Mutations in the Solid Tumor Oligodendroglioma via Single Molecule Analysis

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Structural variation encompassing segments of DNA ranging from 1kb to several megabases in size are important mediators of both human disease and diversity. Cancer cells can acquire structural aberrations that include indels, inversions, and gross rearrangements. Each acquired aberration introduces an opportunity to affect gene structure and regulation, which can decide a specific course to malignancy or present novel targets for new therapies. Identification and characterization of the structural mutations found in cancerous tumors will contribute to the understanding of the mechanisms that promote tumorigenesis.

Loss of heterozygosity (LOH) of 1p and 19q is a hallmark of oligodendroglioma, a brain tumor for which the exact etiology is unknown. The frequency of LOH of 1p/19q found in these tumors indicates that these mutations are important in early tumorigenesis. Cytogenetic evidence has established that LOH of 1p/19q correlates with treatment responsiveness; however, molecular details are lacking despite studies with CGH microarrays, FISH, and Quantitative PCR of microsatellite repeats. To date, the genes or regulatory regions responsible for the clinical behavior of these tumors have not been identified. Difficulties in finding causative mutations might be due to the presence of additional genetic abnormalities that contribute to the malignant progression of these tumors. As a result a comprehensive analysis of all the structural alterations present in the solid tumors is required. Towards this goal, I plan to use the optical mapping system to discover and annotate structural alterations at sub-genic resolution using whole-genome scans.

Tumor samples taken from four individual patients displaying LOH of 1p/1q will be analyzed. DNA isolated from a 1-2 mm section of the first tumor was used to create high-resolution optical consensus maps covering approximately 90% of the available genome sequence. Comparison of each optical consensus maps to the reference sequence has identified multiple types of intermediate sized variants (~1kb-250kb) and two large deletion events (>3MB) on chromosomes 1p and 13. Future research will focus on molecular characterization of structural alterations in each of the four tumors.

Gene-driven Approaches In The Rat and Their Application To Study Mammary Cancer Susceptibility Candidate Genes

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Gene-driven or reverse genetic approaches are a powerful strategy to study gene function and have resulted in numerous comprehensive studies in various model organisms. In the laboratory rat however, such approaches are relatively uncommon. Since the rat is one of the most studied model organisms in a variety of aspects of human disease, we have recently developed tools to interfere with endogenous gene function.

Knockout technology based on homologous recombination in embryonic stem-cells, as it is done in the mouse, is not applicable to the rat. We have developed rat knockouts and mutants by ENU mutagenesis and subsequent high-throughput mutation discovery, a method also known as TILLING (Targeting Induced Local Lesions in Genomes). Induced mutations in genes of interest were traced by CEL I-mediated heteroduplex cleavage [Smits et al., (2004) *Genomics* 83(2): 332-334], and by high-throughput resequencing [Smits et al., (2006) *Pharmacogenet Genomics* 16(3): 159-169]. In this way, we have generated a total of 6 knockouts and 131 mutants, of which 68 harbor a missense mutation. An alternative, more rapid and versatile approach to interfere with gene-function relies on RNA-interference (RNAi). Double-stranded RNA with sequence complementarity to a gene of interest can trigger the RNAi-pathway to degrade the endogenous mRNA of that gene and thereby blocking the production of that specific protein product. Currently, we are setting up RNAi-based knockdown experiments to study the function of breast cancer susceptibility candidate genes resulting from QTL-mapping experiments. More specifically, ultra-fine mapping data suggests the possible involvement of two genes located in Mammary Carcinoma Susceptibility locus 5a (Mcs5a) [Samuelson et al., *Cancer Res* (2005) 65(21): 9637-9642]. The function of these two genes, *Fbxo10* and *Frmpd1*, is largely unknown. By stably knocking down these genes using lentiviral short-hairpin RNA constructs, initially in human and rat cell lines, and ultimately in rat mammary glands, we hope to gain insight in their function, and their potential role in breast cancer.

Population Dynamics of Defective Interfering Virus-like Particles

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Defective interfering (DI) particles are virus-like particles that are formed as a byproduct of natural and laboratory viral infections. The propensity to generate and propagate DI particles is a common feature of many viruses, including bacteriophage, influenza virus, poliovirus, and vesicular stomatitis virus (VSV). DI particles typically carry large deletions in essential functions, making their replication dependent on complementation by co-infecting infectious virus. Within the co-infected cell, faster replicating DI genomic templates compete with wild-type genomic templates for limited replication resources, interfere with wild-type replication, and reduce the overall productivity of wild-type progeny viruses. Over several generations such interactions can result in spontaneous curing of the infection.

It is well accepted that DI particles arise most readily under conditions of high multiplicity of infection (MOI) passage, where many virus particles infect a single cell. However, it is not known what MOIs are necessary or sufficient for DI formation and propagation. We have performed controlled MOI passaging of VSV on baby hamster kidney (BHK) cells at MOIs of 100 to 10^{-4} . We have found that the virus yield drops by two orders of magnitude (from $\sim 10^9$ to $\sim 10^7$ plaque forming units/ml) in four passages for a controlled MOI of 100 and in five passages for a controlled MOI of 10. For the lower MOIs, the yield has not been as significantly affected. Further, we have adapted a predator-prey model of Bangham and Kirkwood to aid in understanding the population dynamics of this system. Our model captures the observed yield reduction of the virus over multiple passages at various MOIs. These studies set a foundation for understanding the dynamics of natural virus infections, gaining insights into mechanisms of viral pathogenesis, and advancing the development of novel parasitic anti-viral therapies.

The Role of Interleukin 1 β in Prostate Development

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Interleukin 1-beta (IL-1 β) is a major inflammatory mediator in prostatic inflammation. While extensive research studies have focused on its role in prostate cancer progression, little is known about the role of inflammatory mediators in prostate development. Interleukin 1-beta increases cell proliferation in prostate cancer, suggesting that it may also regulate proliferation in prostate development. The developmental role of IL-1 β 's functional similar counterparts, such as IL-1 α and IL-6, implies that IL-1 β may also be involved in prostate development as a growth factor. Thus, it is of great interest to understand its role in prostate development as well as its influence on prostate cancer progression. We hypothesize that IL-1 β regulate cell proliferation and differentiation during prostate development. Epithelial cells were treated with IL-1 β and its cell proliferation rate is determined. Cancerous prostate cell line LNCaP and the BHP cell line taken from human prostate are treated with IL-1 β . We determined IL-1 β influence on the PI3K signaling pathway that involves in prostate cancer progression by western blotting. We detected increase in proliferation rate after IL-1 β treatment. We also detected decrease in expression of the tumor-suppressing protein PTEN in a dose dependent manner. Increased proliferation rate in epithelial cell line suggests that IL-1 β has an effect on cell proliferation. Decrease PTEN expression implies activation of PI3K and enhanced tumor growth. We suggest further study on the effect of IL-1 β in cell proliferation and the mechanism of PTEN suppression by IL-1 β .

The Human Zinc Transporter Ziip13

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Zinc is an essential metal cation needed for proper human nutrition. Proteins involved in most cellular pathways require zinc for function, whether zinc is utilized in a structural motif or as part of a catalytic enzyme core. In contrast to a normal functioning cell, zinc deficiency leads to cell death and is best characterized through the phenotype of patients with the genetic disease Acrodermatitis Enteropathica. This disease results in a systemic zinc deficiency leading to growth retardation, skin lesions and immune system dysfunction. To maintain the optimal cellular zinc status, human cells express zinc transporters called the Zrt-, Irt-like Protein family (ZIP). There are 14 predicted members of the ZIP family and here we present the initial characterization of the human zinc-specific transporter Zip13. Using a radioactive ⁶⁵Zn uptake assay we show that Zip13 transport is saturable, time- and concentration-dependent. The apparent $K_m = 2.5 \mu\text{M}$ and $V_{max} = 10 \text{ pmol/min/mg}$ were determined and these kinetic values are similar to the other characterized mammalian Zip transporters. Northern blot analysis of Zip13 mRNA showed expression in most human organs and ubiquitous expression in the intestine. The mRNA expression is increased in low zinc in HeLa cells but remains unchanged in Caco-2 cells or in the presence of a high zinc concentration. The mechanism regulating Zip13 expression levels in response to zinc is currently under investigation.

Photoreceptor Degeneration Affects Expression Pattern Of Melanopsin In The Rat Retina

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Participation of retinal ganglion produced melanopsin in circadian photoentrainment might be independent of classical rods and cones. However, no evidence shows that melanopsin-positive ganglion cells will always escape the influences of photoreceptors in other biological or pathological processes. In this study, we investigate the melanopsin production in retinal ganglion cells in a murine model of photoreceptor degeneration.

Methods: Photoreceptor degeneration in an animal model was induced by a single intraperitoneal injection of N-methyl-N-nitrosourea (MNU) in adult SD rats. 0.5, 1, 5, 7, 13 and 28 d after drug administration, expression of melanopsin was examined by fluorescent immunolabelling of fixed retina, and real-time quantitative RT-PCR for mRNA from the retina was performed.

Results: On d0.5, photoreceptors showed some signs of early apoptosis; on d7, the majority of photoreceptors had degenerated. Melanopsin mRNA decreased gradually associated with photoreceptor loss. At the same time, pituitary adenylate cyclase-activating polypeptide (PACAP), a protein colocalized with melanopsin in ganglion cells, did not exhibit much variability. During the process of photoreceptor degeneration, expression of melanopsin on ganglion dendrites faded away, while in the soma the expression persisted for a long time.

Conclusions: Our data suggest normal photoreceptors may be essential for the expression of melanopsin, at least in ganglion cell dendrites. This also means that melanopsin from different cellular locations may affect circadian systems in different ways. Because light stimuli also regulates production of melanopsin, photoreceptors might participate in circadian photoentrainment as well.

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Cell Behaviour in Convective free Microenvironments

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In vivo, the local microenvironment plays an active role in regulating cell behavior. A complex interplay of mechanisms ranging from direct cell-cell contact to autocrine/paracrine signaling via soluble factors determines cell function. Attempts to study these mechanisms in vitro begin a century ago when investigators first removed tissue from animals and begin to develop methods of in vitro tissue/cell culture. While most in vitro cell biology is still performed using similar monolayer cultures in well-shaped culture vessels, alternatives are emerging. However, the ability to study autocrine/paracrine soluble factor effects is still difficult. Insights into paracrine signaling are typically gained via in vivo experiments requiring extensive labor and time. Canonical in vitro culture vessels are not conducive to the retention and study of soluble factor effects at a local cellular scale due to convective flows arising from the media/air interface that rapidly mix local cellular secretions into the bulk media volume. Here we present the use of confined microchannel environments to establish convection free culture systems to reveal population dependent effects in primary mammary epithelial cell cultures. In this work, we explored the growth of primary mouse mammary gland epithelial cells (MECs) in a microchannel culture platform. Results show that cell proliferation is strongly influenced by the local microenvironment. For example, the frequency of media changes strongly influenced proliferation as did the culture vessel (microchannel vs. multi well plate). In addition, we have observed a biomodal growth pattern based on absolute cell number in the microchannel platform. Statistically, these observations are consistent with in vivo cleared fat pad transplant assays that suggest the presence of stem/progenitor cells in the mammary gland at a frequency of (1:1500). These observed growth responses suggest that the diffusion-dominated transport environment within microchannels provides a more sensitive system for elucidating soluble factor effects. The diffusion-mediated environment could help to identify specific soluble factors and related cell behaviors that are masked in canonical culture vessels due to larger media to cell volume ratios and convective mixing.

Stochastic Kinetics of Virus Intracellular Growth and Production: Experiments

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Many RNA viruses, such as HIV-1, influenza A virus, and Hepatitis C virus, exist as genetically diverse populations due to their error-prone replication and short generation times. Increasing experimental and clinical evidence indicates that the broad distribution of genotypes within natural virus populations enable them to develop resistance to drug therapies, evade vaccination strategies, and play a role in the emergence of new viral diseases. However, it is not known to what extent distributions in virus-host phenotype, such as virus fitness or viral pathogenicity, reflect viral genotypes, host-specific variations or other environmental factors. A major technical challenge is that viral phenotype distributions are labor intensive to measure. Here we begin to address this issue by measuring burst sizes (virus fitness or progeny production) from single infected cells. As a model system we study vesicular stomatitis virus (VSV), an RNA virus that has potential applications for the production of pseudotype vaccines and as an oncolytic agent for the treatment of tumors. VSV has also served as a model for studying the evolutionary dynamics of virus populations, and it is of economic importance because it causes foot-and-mouth disease like symptoms in livestock. For our studies we employ rVSV-GFP, a recombinant strain of VSV that expresses green fluorescent protein (GFP) during infection, and we use fluorescence-activated cell sorting (FACS) to isolate single infected baby hamster kidney (BHK-21) cells early in their infection cycle. Analysis by plaque assay of virus produced from single GFP-positive cells showed a distribution of burst sizes from 0 to more than 5000 active virus progeny per cell, with typically 20 percent of GFP-positive cells producing no detectable virus. For multiplicities of infection of 0.1, 1 and 5, average burst sizes were 1300, 1200, and 1500 respectively, though distributions of burst size were not significantly different based on a one-way analysis of variance ($p=0.1249$). Further passaging of virus progeny from high- and low-yield cells and measurement of their distributions should reveal to what extent high- and low-yield phenotypes are transferable. By such means, we are aiming to identify how genetic and environment factors can quantitatively affect distributions of virus fitness.

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NOTES

Directions to Best Western Inn Tower

- Take University Ave. west.
- Take the 1500 - 2600 University Ave. Exit.
(Do not take Campus Drive).
- Hotel is 8 blocks down on the right.



UNIVERSITY BUILDINGS & ACCOMODATIONS

Building	Location	Building	Location
1. Memorial Union	D1	A. Dahliman Campus Inn	E1
2. State Historical Society	D1	B. University Inn	E2
3. Overture Hall	F2	C. Best Western Inn Tower	See Map Inset

PARKING LOTS

- P1 Parking Lot 6
- P2 Parking Lot 46
- P3 Parking Lot 83



