be reported. We present a lifelong iron deficiency microcytic anemia associated with symptoms suggestive of neurotransmitter dysregulation and perceptual size distortion in a 54-year-old white female. She had a total absence of sweating, severe constipation, and intolerance to heat and cold and episodes of odourometer bias with size underestimation lasting 15 to 20 minutes. The anemia and symptoms resolved with daily administration of 10 mg of transferrin receptor (manganese). Premanifest levels for haptoglobin, direct bilirubin, and immunoglobulin were within the normal range. Erythrocyte sedimentation rate and platelet and white cell counts were within the normal range. Erythropoiesis levels were 11 ml/m^3 (ref. 4–21 ml/m^3). Blood transfusions but not oral iron improved her symptoms but not the red cell indices and a consistently low reticulocyte count (0.4%). She had been off oral iron for a few months prior to her visit. Manganese was started in May 2005. In December 2005 (and to the present) she has not experienced the typical manganese-induced changes in her hematologic profile and iron status from April 2005 to November 2006 are presented in Table 1. Studies performed in March and April in 2005 show iron studies consistent with appropriate iron absorption from the gut and inefficient incorporation of iron in the erythron, possibly from decreased TIR expression. Her tissue iron stores measured by ferritin levels were significantly increased beyond normal levels. The 5-allele homozygous genotype at 794 STR was associated with an OR = 11.9 (95% CI 1.4–98.3; p = 0.01) after inclusion of covariates in the multiple logistic regression model. Sequencing revealed a total of 45 polymorphic loci (13 novel) that were also analyzed for association. None of these were significantly associated with ALI, either individually or in haplotypes. This result is congruent with the fact that neither SNPs nor haplotypes are in strong LD with the STR. Conclusion: Our data suggest that the MIF gene is associated with susceptibility to ALI and support the causality of the STR locus. Genotyping of this STR is required for future association studies as the MIF SNPs examined were not in strong LD with the STR variants.

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### Table 1: Manganese-Induced Changes in Hematologic Profile and Iron Status

| Month | Haemoglobin (g/dL) | MCV (fL) | TIBC (µg/dL) | TIR (µg/dL) | Ferritin (ng/mL) | Manganese Receptor (µg/mg Hgb) |
|-------|-------------------|---------|--------------|-------------|-----------------|-------------------------------|
| Mar '05 | 7.4                | 68       | 26           | 3.77         | 0.6             |                                |
| Apr '05 | 9.7                | 72       | 32           | 4.53         | 0.6             | 268                           |
| May '05 | 11.0               | 84       | 37           | 4.15         | 0.6             | 248                           |
| Aug '05 | 13.6               | 87       | 39           | 4.46         | 0.6             | 46                           |
| Sep '05 | 14.4               | 94       | 42           | 4.45         | 0.6             | 81                           |
| Feb '06 | 12.7               | 88       | 40           | 5.09         | 0.6             | 388                           |
| Mar '06 | 12.7               | 85       | 39           | 5.09         | 0.6             | 395                           |
| Oct '06 | 14.7               | 89       | 43           | 4.73         | 0.6             | 21                           |
| Nov '06 | 13                 | 85       | 40           | 4.73         | 0.6             | 10                           |

**54 CRKL-LIKE FACTOR 4 REGULATES ENDOTHELIAL INFAMMATORY INFLAMMATION**

A. Hamak, Z. Lin, A. Kumar, M. Balzelli, S. Sinha, K. J. Gersten, R. E. Edelman, M. K. Jain. Case Western Reserve University, Cleveland, OH; Beijing Hospital, China. Background: The vascular endothelium plays a critical role in vascular homeostasis. A limited profibrotic, proinflammatory state is appropriate in the context of infection or wound healing, but sustained proinflammatory activity and endotoxin-induced conditions such as atherosclerosis and post-traumatic thrombosis. Inflammatory cytokines and nonlaminar blood flow induce endothelial dysfunction and confer a proadhesive and prothrombotic phenotype. Therefore, identification of factors that mediate the effects of these stimuli on endothelial function is of considerable interest. Kruppel-like factors (KLFs) are a subclass of the zinc-finger family of transcription factors. Previous studies demonstrate that KLF proteins typically regulate critical aspects of cellular differentiation and tissue development. Studies from our laboratory and others demonstrate an emerging role for this family of transcriptional regulators in vascular biology. KLF4/CRKL (gut-enriched Kruppel-like factor) was first identified as being highly expressed in epithelial cells, and subsequent work has verified a critical role in skin and intestinal development. KLF4 has also been identified in endothelial cells, yet its function in vessel biology has yet to be elucidated. Methods and Results: Immunohistochemical analysis of mouse and human vascular tissues demonstrated the expression, in vivo, of KLF4 in the murine and human cerebral blood vessels. Northern blot analysis of total mRNA harvested from primary endothelial cell lines derived from various human arterial vascular beds (sorta, pulmonary artery, and umbilical artery) shows expression of endothelial KLF4 in a variety of arterial and venous cell lines. Furthermore, we demonstrate that endothelial KLF4 is induced by proinflammatory stimuli and shear stress. Overexpression of KLF4 induces expression of multiple anti-inflammatory and anti-thrombotic factors, including eNOS and thrombomodulin, and inhibits basic fibroblast growth factor expression. A decrease in the number of proinflammatory factors, including MCP-1, RANTES, CRP, PAI-1, IL-6, and IL-8. The significance of this expression of KLF4 on target genes was assessed in experiments using siRNA-mediated knockdown of KLF4. These experiments demonstrate that KLF4 depletion leads to enhancement of TNF-α-induced VCAM-1 and tissue factor expression. In addition to the determination of target genes, we have verified the functional importance of KLF4 by demonstrating that KLF4 expression markedly decreases inflammatory cell adhesion to the endothelial surface and prolongs clotting times under inflammatory states. As a first step toward understanding the molecular basis of KLF4-mediated regulation of endothelial target genes, we assessed the effect of KLF4 on target gene promoters. KLF4 differentially regulates the promoter activity of pro- and anti-inflammatory genes in a manner consistent with its anti-inflammatory function. Conclusion: Inflammatory cytokines and the biomechanical effects of laminar shear stress are the most potent effectors of endothelial homeostasis identified to date. Perturbation of endothelial function by proinflammatory cytokines or nonlaminar...
interaction affects cellular transformation and cancer-affecting processes. Our preliminary data suggest that there is a strong interaction between Myc and MBP1. We have characterized antibodies and siRNA sequences that can be effectively used to detect and knock down MBP1. Using these tools and others, we will confirm the Myc-MBP1 interaction using coimmunoprecipitation (coIP) experiments. We will also use coIP to identify mutants of each protein that cannot bind the other. We will use quantitative PCR and chemostat IP to establish if MBP1 affects Myc gene regulation, particularly gene repression. We will use the TUNEL assay to determine if Myc and MBP1 affect each other's apoptosis-inducing properties. Finally, we will assay the effect that the Myc-MBP1 interaction has on cellular transformation, cell-growth-arresting anchorage-independent growth, and tumorigenicity and metastatic ability to produce a tumor in nude mice. These experiments will be complemented with parallel studies in nonprostate and noncancer cells to determine if the interaction, or its properties, are prostate or more importantly cancer specific. These studies should provide new avenues to detect and treat prostate cancer. They can also expose an Achilles' heel of prostate cancer against which drugs can be targeted.

49 EFFECT OF PKC STIMULATION ON SODIUM CHLORIDE COTRANSPORTER FUNCTION AND SURFACE EXPRESSION IN MAMMALIAN CELLS: S. Ko, I. Joshi, L. Cooke, M. Mutsch, R.S. Hoover, University of Chicago, Chicago, IL.

The sodium chloride cotransporter (NCC) belongs to the family of electroneutral cation-chloride transporters (SLC12). It is the site of action of one of the most effective classes of antihypertensive medications, thiazide diuretics. NCC is the principal salt-absorptive pathway in the mammalian distal convoluted tubule, and its regulatory mechanisms have been principally studied in the Xenopus oocyte expression system and have not been well defined in mammalian models. Therefore, we have developed a cell model system to assess NCC function in a mammalian cell line that natively expresses NCC, the mouse distal convoluted (mDCT) cell line. We now use this system to test the effects of activations of PKA ( forskolin, PFA), (TPA, or are (44-PDD, a non-PKC-stimulating phorbol ester) on function and surface expression of NCC. This is the first reported correlation of NCC function (22naTime) with surface expression in mammalian cells. This cell line contains 90% confluence in 12 well plates and was then placed in -fre media for 30 minutes. The cells were then incubated in medium containing vehicle (DMSO), forskolin, or 44-PDD. For functional assessment, cells were then incubated in 24-well plates and then washed with PBS and incubated with NHS-SS-biotin at 40°C for 30 minutes. The cells were washed, incubated in NHS-SS-biotin at 40°C for 30 minutes, incubated with PBS, and placed in -free media for 30 minutes. The cells were then incubated in medium containing vehicle (DMSO), forskolin, or 44-PDD. For functional assessment, cells were then incubated in 24-well plates and then washed with ice-cold wash buffer and lysed. Radioactivity was counted, protein concentrations of the lysates were determined, and uptake were normalized to mRNA. Thiazide-sensitive uptake was given by the difference between in the presence and absence of thiazide. We have used the TUNEL assay to detect apoptosis in these systems and have used the biotinylated protein was labeled with protein. The biotinylated protein was eluted in SDS sample buffer with TDT. Protein concentration was determined using the lysate from each group. Biorelated surface protein and the corresponding amount of total NCC in the cell lysates were analyzed by Western blotting and were quantified by densitometric analysis. TPA completely suppressed NCC function, essentially eliminating thiazide-sensitive uptake. In addition, uptake was normalized to mRNA in these systems.

50 A NOVEL REGION OF THE MYLIC LIGHT CHAIN KINASE IS ASSOCIATED WITH SUSCEPTIBILITY TO ACUTE LUNG INJURY IN A SPANISH POPULATION: S. Ma, C. Flores, K. Matsuoka, M. Benitez, R. Mardones, R. Bieber, V. Galghet, J. Villanueva, J. Ciarlet, J. Bosch, J. Paricio, D. Bermejo, E. Balcells, J. de la Torre, M. Rosales, J. Muñoz, S. Colome, K. Wernick, S. Toubi, J. Valls, L. Díaz, A. Balcells, S. Tanaka, V. Natarajan, University of Chicago, Chicago, IL.

Rationale: Acute lung injury (ALI) is a life-threatening syndrome with both susceptibility and outcome to be influenced by genetic factors. Nonmuscle myosin light chain kinase (NMMLK) encoded by MYLCK is known to be a cytoskeletal protein that is involved in multiple cell functions. We hypothesized that SNPs in MYLCK could influence the susceptibility to ALI.

Methods: We performed a genome-wide association study in a Spanish population (n=96 controls and 80 cases) to test this hypothesis. SNPs were selected using the high-throughput approach in the breast cancer cell line MCF-7 and its drug-resistant variant MCF7/Adr and could be validated by a different method. The approach may greatly facilitate the analysis of combinatorial expression of known genes in many important applications with a limited amount of RNA, such as molecular diagnosis of cancer patient with samples of fine-needle aspiration prior to surgery.

51 CLC-3 IS REQUIRED FOR NADPH OXIDASE-DEPENDENT NUCLEAR FACTOR KB ACTIVATION BY SIGNALING ENDOSOMES: F. Miler, M. Filali, G. Huis, B. Stanic, J. Matsuda, T. Barna, F.S. Lamb, University of Iowa, Iowa City, IA.

Recent studies suggest the existence of a c-Myc expression is important in maintaining and preventing the progression of prostate cancer. Separate studies have shown that MBP1 may be a specific target of c-Myc. The work of others led us to hypothesize that MBP1 and Myc are interacting in prostate cancer cells to affect tumor progression. You've identified two regions of the genome that interact with Myc and yield several independent isolates of MBP1. Interestingly, Myc and MBP1 are already sharing morphological features with regard to apoptosis and gene repression, processes that have direct implications for cancer progression. Considering the role of MBP1 in the early development of prostate cancer cells, we hypothesized that the interaction between MBP1 and Myc is a key determinant of malignancy in prostate cancer. We aim (1) to confirm the interaction between Myc and MBP1 and determine if this interaction is prostate cancer specific; (2) to determine if MBP1 affects Myc's regulation of target genes; and (3) to determine if the MBP1-Myc interaction affects cellular transformation and cancer-affecting processes. Our preliminary data suggest that there is a strong interaction between Myc and MBP1. We have characterized antibodies and siRNA sequences that can be effectively used to detect and knock down MBP1. Using these tools and others, we will confirm the Myc-MBP1 interaction using coimmunoprecipitation (coIP) experiments. We will also use coIP to identify mutants of each protein that cannot bind the other. We will use quantitative PCR and chemostat IP to establish if MBP1 affects Myc gene regulation, particularly gene repression. We will use the TUNEL assay to determine if Myc and MBP1 affect each other's apoptosis-inducing properties. Finally, we will assess the effect that the Myc-MBP1 interaction has on cellular transformation, cell-growth-arresting anchorage-independent growth, and tumorigenicity and metastatic ability to produce a tumor in nude mice. These experiments will be complemented with parallel studies in nonprostate and noncancer cells to determine if the interaction, or its properties, are prostate or more importantly cancer specific. These studies should provide new avenues to detect and treat prostate cancer. They can also expose an Achilles' heel of prostate cancer against which drugs can be targeted.

52 A NOVEL ROLE FOR MYC IN PROSTATE CANCER. A NOVEL ROLE FOR MYC IN PROSTATE CANCER. A NOVEL ROLE FOR MYC IN PROSTATE CANCER.

In this study, we examined the potential role of Myc in prostate cancer development.

Methods: We have identified two regions of the genome that interact with Myc in prostate cancer cells to affect tumor progression. Considering the role of Myc in the early development of prostate cancer cells, we hypothesized that the interaction between Myc and MBP1 is a key determinant of malignancy in prostate cancer. We aim (1) to confirm the interaction between Myc and MBP1 and determine if this interaction is prostate cancer specific; (2) to determine if MBP1 affects Myc's regulation of target genes; and (3) to determine if the MBP1-Myc interaction affects cellular transformation and cancer-affecting processes. Our preliminary data suggest that there is strong interaction between Myc and MBP1. We have characterized antibodies and siRNA sequences that can be effectively used to detect and knock down MBP1. Using these tools and others, we will confirm the Myc-MBP1 interaction using coimmunoprecipitation (coIP) experiments. We will also use coIP to identify mutants of each protein that cannot bind the other. We will use quantitative PCR and chemostat IP to establish if MBP1 affects Myc gene regulation, particularly gene repression. We will use the TUNEL assay to determine if Myc and MBP1 affect each other's apoptosis-inducing properties. Finally, we will assess the effect that the Myc-MBP1 interaction has on cellular transformation, cell-growth-arresting anchorage-independent growth, and tumorigenicity and metastatic ability to produce a tumor in nude mice. These experiments will be complemented with parallel studies in nonprostate and noncancer cells to determine if the interaction, or its properties, are prostate or more importantly cancer specific. These studies should provide new avenues to detect and treat prostate cancer. They can also expose an Achilles' heel of prostate cancer against which drugs can be targeted.