flow has been documented in most vascular disease states, including vasculitis, aneurysm formation, pathologic thrombosis, and atherosclerosis. The observations described above implicate KLF4 as a novel regulator of endothelial activation in response to proinflammatory stimuli. A greater understanding of KLF4 function is thus of considerable scientific and potentially therapeutic interest.

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A HIGHLY SENSITIVE AND SPECIFIC SYSTEM FOR LARGE-SCALE GENE EXPRESSION PROFILING. G. Hu, Q. Yang, H. Li, Princeton University, Princeton and Piscataway, NJ.

Gene expression profiling of very few or even single cells is of particular interest in many applications, including molecular diagnosis. However, detection of a large number of mRNA sequences from a small number of cells is limited by the sensitivity of available methods. High-throughput multiplex reverse transcription followed by PCR amplification (RT-PCR) has much to offer these studies owing to its inherent sensitivity, efficiency, and cost-effectiveness. A multiplex RT-PCR-based high-throughput gene profiling system is described in this communication. With this system, a breast cancer–specific microarray is developed. More than 1,000 different mRNA species can be amplified in a single tube to a detectable amount. By using specially designed PCR primers, the long-standing low-specificity problem associated with high-throughput gene expression profiling has been solved. Amplified sequences are then resolved by microarray with probes that only hybridize to sequences amplified from mRNA. The method is so sensitive that mRNA in single cells can be reliably detected. Differentially expressed genes identified with the high-throughput approach in the breast cancer cell line MCF-7 and its drug-resistant variant MCF-7/AdrR 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.

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ACUTE RESPIRATORY DISTRESS SYNDROME ALVEOLAR FLUID INDUCES MYOFIBROBLAST GENES BUT SUPPRESSES KERATINOCYTE GROWTH FACTOR IN NORMAL HUMAN LUNG FIBROBLASTS. M. Jain, L. Synenki, N.S. Chandel, G.R. Budinger, J. Eisenbart, H. Donnelly, Northwestern University, Chicago, IL.

Rationale: Alveolar repair is critical to ARDS outcome. Myofibroblast induction is integral to healing by facilitating wound apposition (α-smooth muscle actin (α-SMA), secreting matrix (collagens 1 and 3), cytokines (TGF-β1 and connective tissue growth factor [CTGF]), and epithelial mitogens (keratinocyte growth factor [KGF]). We hypothesized that bronchoalveolar lavage fluid (BALF) from ARDS patients would induce multiple features of myofibroblasts. **Methods:** ARDS BALF was collected within 48 hours of intubation. Patients with cardiogenic pulmonary edema were included as negative controls. ARDS and control BALF were incubated with normal human lung fibroblasts for 24 hours, and α-SMA, collagens 1 and 3, CTGF, TGF-β1, and KGF mRNA was measured by real-time RT-PCR. **Results:** Ten ARDS patients and five negative controls were enrolled. The average age for ARDS patients was 53 ± 16 years. The average PaO_2/ FiO_2 ratio, compliance, and APACHE 2 scores were 118 ± 70, 21 ± 10, and 28 ± 8, respectively. ARDS BALF induced 110% more α-SMA ($p < .05$) and 85% more CTGF ($p < .05$) than controls. In addition, ARDS BALF induced 43% more collagen 1 ($p = .11$) and 52% more collagen 3 ($p = .08$) than controls. In contrast, control BALF induced 70% more KGF ($p < .03$) than ARDS BALF. There was a significant inverse correlation between ARDS induction of α-SMA and KGF ($r = -.78$, $p < .01$). The TGF-β1 receptor inhibitor SB431542 significantly reduced ARDS BALF induction of α-SMA, CTGF, and collagens 1 and 3 ($p < .05$ for all) but had no impact on KGF expression ($p = .33$). **Conclusion:** Early ALI/ARDS BALF has predominant profibrotic characteristics and suppresses epithelial mitogen induction.

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ROLE OF ACYLGLYCEROL KINASE IN LYSOPHOSPHATIDIC ACID–INDUCED TRANSACTIVATION OF EPIDERMAL GROWTH FACTOR RECEPTOR AND EXPRESSION OF CYCLOOXYGENASE 2 AND INTERLEUKIN-8 IN PRIMARY HUMAN BRONCHIAL EPITHELIAL CELLS. S. Kalari, Y. Zhao, E. Berdyshev, P.V. Usatyuk, D. He, V. Natarajan, University of Chicago, Chicago, IL.

Rationale: Acylglycerol kinase (AGK) phosphorylates monoacylglycerol and diacylglycerol to form potent lipid second messengers, lysophosphatidic acid (LPA) and phosphatidic acid (PA), respectively. We have shown earlier that LPA induces transactivation of epidermal growth factor receptor (EGF-R) in human bronchial epithelial cells (HBEpCs). Here we provide evidence for the role of AGK in LPA-mediated expressions of COX-2 and IL-8 involving transactivation of EGF-R in HBEpCs. **Methods/ Results:** Expression of AGK in HBEpCs was detected by real-time PCR. The full-length cDNA of AGK was cloned from HBEpCs and inserted into lentiviral vector with V5-tag on the C-terminal. The overexpressed AGK was mainly localized in mitochondria as determined by immunofluorescence and confocal microscopy. Overexpression of lenti-AGK wild type increased intracellular LPA production (≈1.8-fold), enhanced LPA-mediated phosphorylation of EGF-R and ERK1/2, and stimulated expression of COX-2 and IL-8 secretion. Further, down-regulation of AGK expression by AGK siRNA decreased intracellular LPA levels by ≈2-fold and attenuated LPA-induced tyrosine phosphorylation of EGF-R and tyrosine/threonine phosphorylation of ERK1/2, COX-2 expression, and IL-8 secretion. **Conclusions:** These results suggest a role for AGK in LPA-mediated activation of COX-2 expression and IL-8 secretion via EGF-R transactivation and signal transduction in HBEpCs. Thus, AGK-mediated intracellular LPA production and signaling may play a potential role in innate immunity and airway remodeling during inflammation.

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A NOVEL ROLE FOR MYC IN PROSTATE CANCER. D.N. Khalil, W.P. Tansey, Cold Spring Harbor Labs, Cold Spring Harbor, NY.

Recent data suggest that excessive c-Myc expression is important in maintaining and preventing the regression of prostate cancer. Separate studies have shown that MBP1 can cause prostate cancer regression in mouse systems. A rapid convergence of evidence from our laboratory and others led us to hypothesize that MBP1 and Myc are interacting in prostate cancer cells to affect tumor progression. Yeast-two-hybrid screening for proteins that interact with Myc yielded several independent isolates of MBP1. Interestingly, Myc and MBP1 have striking functional similarities with regard to apoptosis and gene repression, processes that have direct implications for cancer progression. Considering the role of Myc and the specific role of MBP1 in prostate cancer, we therefore hypothesize 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 chromatin IP to establish if MBP1 affects Myc gene regulation, particularly gene repression. We will use the TUNEL assay to determine if MBP1 and Myc affect each other's apoptosis-inducing properties. Finally, we will assay the effect that the Myc-MBP1 interaction has on cellular transformation, examining anchorage-independent growth, loss of contact inhibition, and the 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, prostate cancer specific. These data can potentially yield a diagnostic assay for early detection of prostate cancer. They can also expose an Achilles' heel of prostate cancer against which drugs can be targeted.

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EFFECT OF PKC STIMULATION ON SODIUM CHLORIDE COTRANSPORTER FUNCTION AND SURFACE EXPRESSION IN MAMMALIAN CELLS. B. Ko, L. Joshi, L. Cooke, M. Musch, 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, but 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 assess the effects of activators of PKA (forskolin), PKC (TPA), or neither (4α-PDD, a non-PKC-stimulating phorbol ester) on function and surface expression of NCC. This is the first reported correlation of NCC function (22Na+ uptakes) with surface expression in mammalian cells. For this, mDCT cells were grown to 90% confluence in 12-well plates and then placed in Cl^--free media for 30 minutes. The cells were then incubated in medium-containing vehicle (DMSO), TPA, forskolin, or 4α-PDD. For functional assessment, cells were then incubated in a $22Na^+$-containing medium with or without thiazides and then washed with ice-cold wash buffer and lysed. Radioactivity was counted, protein concentrations of the lysates were determined, and uptakes were normalized to nmol/mg. Thiazide-sensitive uptake was given by the difference of the uptakes with and without thiazide. For assessment of surface expression, the cells were washed with ice-cold PBS and incubated with NHS-SS-biotin at 40°C for 30 minutes. The cells were lysed, and labeled protein was isolated. The biotinylated protein was eluted in SDS sample buffer with DTT. Protein concentration was measured using the lysate from each group. Biotinylated 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 (statistically no different from zero for TPA vs 43.4 ± 8.68 nmol/mg for vehicle, $p < .01$ by ANOVA). Stimulation of PKA did not significantly alter NCC function compared with vehicle or 4α-PDD. For the biotinylation study, the fraction of cell surface NCC over total NCC in the cell lysate was calculated for each group. Cell surface NCC protein represented 48.4 ± 8.9% and 45.6 ± 8.7% of total NCC protein in the DMSO and 4α-PDD groups, respectively. NCC surface expression in the TPA group was 16.9% (± 5.5) ($p < .05$ vs vehicle or 4α-PDD). This was a 65% reduction of surface expression in the TPA group compared with controls. The almost complete inhibition of function by TPA is mostly secondary to a reduction in cell surface expression. These findings suggest that PKC is a key determinant of NCC activity and surface expression in the DCT.

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A NOVEL REGION OF THE MYOSIN LIGHT CHAIN KINASE GENE IS ASSOCIATED WITH SUSCEPTIBILITY TO ACUTE LUNG INJURY IN A SPANISH POPULATION. S. Ma, C. Flores, K. Maresso, S. Devender, J. Villar, J.N. Garcia, University of Chicago, Chicago, IL; Canary Islands, Spain.

Rationale: Acute lung injury (ALI) is a life-threatening syndrome with both susceptibility and outcome known to be influenced by genetic factors. Nonmuscle myosin light chain kinase (nmMLCK) encoded by the MYLK gene is a cytoskeleton protein involved in the endothelial cell barrier regulation and in the inflammatory response. We have reported the association of MYLK variants with susceptibility to sepsis-induced ALI in African and European Americans (AJRCMB 2006;34:487). Here we tested the association of MYLK variants in ALI patients from Spain. **Methods:** DNA samples of 96 controls and 80 patients with severe septics were obtained from a Spanish ICU Network. Using TaqMan assays, we first genotyped 16 SNPs selected from our previous report and assessed individual SNPs and haplotype association by Armitage tests and a sliding-window approach, respectively. A new set of nine tagging SNPs (tSNPs) were further selected from the strongest associated region based on the HapMap and data were similarly analyzed. **Results:** Significant association was observed with an SNP ($p = .027$) previously associated with sepsis and ALI in European Americans with exploratory haplotype analysis of the first 16 SNPs identifying the strongest association ($p = .01$) in the specific region encoding the nmMLCK N-terminus. The additional nine tSNPs selected from this region and the 4 kb region upstream reinforced the association of the 5′ end of the gene (N-terminus) with susceptibility to ALI (permuted $p = .027$). **Conclusions:** Our haplotype analyses confirm that MYLK gene variants are associated with ALI in a well-phenotyped replicate population. A fine map genotyping with tSNPs has allowed us to identify a novel region of MYLK associated with ALI, which may be useful to delineate a smaller region for further functional studies of these susceptibility variants.

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

Reactive oxygen species (ROS) are mediators of intracellular signals for a myriad of normal and pathologic cellular events, including differentiation, hypertrophy, proliferation, and apoptosis. NADPH oxidases are important sources of ROS that are present in diverse tissues throughout the body and activate many redox-sensitive signal transduction and gene expression pathways. To avoid toxicity and provide specificity of signaling, ROS production and metabolism necessitate tight regulation that likely includes subcellular compartmentalization. However, the constituent elements of NADPH oxidase–dependent cell signaling are not known. Here we show that activation of NADPH oxidase by inflammatory cytokines generates ROS within early endosomes and requires ClC-3, a member of the chloride channel (ClC) family. Nox1, one of multiple membrane-bound catalytic subunits of NADPH oxidase, colocalizes with ClC-3 in early endosomes. Both Nox1 and ClC-3 are necessary for tumor