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Regulation of Aop2: an antioxidant implicated in atherosclerosis-resistance in mice

Shelley A. Phelan
Fairfield University, sphelan@fairfield.edu

Mary Lou Lombardi

Ellen Neylon

constructs that consist of the 5' flanking region of either MUC5AC or MUC5B and luciferase, we have also observed an increase of mucin gene promoter activities in airway epithelial cells after a TS exposure. Co-expression of the dominant negative forms of ERK1, ERK2, JNK, and p38α are used to examine the signaling pathway that may be involved in TS-induced promoter activities. Expression of dn-ERK1 and dn-ERK2 strongly suppressed TS-stimulated promoter activity, while the constitutively active ERK1 augmented it. Moreover, ERK1 inhibitors UO126 and PD98095 suppress TS-induced mucin gene expression. However, results from using dn-c-Jun and dn-p38α are different between MUC5AC and MUC5B. Therefore, the transcriptional induction of mucin genes by TS exposure is not identical for MUC5AC and MUC5B although both are regulated through ERK1/ERK2 dependent signaling pathway.

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Genetic Heterogeneity of the Zonular Cataract with Sutural opacities

haesook Kim, Choun-Ki Joo, Lab. of ophthalmology & visual science, Catholic University of Korea, 505 banpo-dong, Seocho-ku, Seoul, 137-701 Korea

Congenital cataract is a frequent cause of hereditary visual impairment in infants. It is highly heterogeneous morphologically and genetically. And the mechanism of lens opacification in cataract remains unclear. Previously, autosomal dominant zonular cataracts with sutural opacities (CCZS) have been mapped to chromosome 17q11-q12 and the mutation in bA3A1-crystallin gene (CRYBA1) was shown to be responsible for CCZS. We investigated a three-generation family affected with autosomal dominant congenital cataract. The phenotype in this family showed a typical zonular cataract with sutural opacities (CCZS). In order to determine whether a mutation in CRYBA1 gene caused this phenotype, we performed PCR-SSCP and sequence analyses of this gene from the patients and control. We were not able to identify any mutation in CRYBA1 gene. This result demonstrated that a gene other than CRYBA1 gene must have caused the same phenotype, thus indicating heterogeneity of CCZS. Further linkage analysis is required to identify the cataract-causing gene in this family.

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Analysis of the Prostate Specific Membrane Antigen (PSMA) Promoter and Enhancer's Effect on Transcription in Prostate Cell Lines

Kenneth R. Noss[1], Steven A. Wolfe[2], Sidney R. Grimes[2], [1]Dept. Biochem. and Molecular Biology, LSU Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130, [2]Research Service (151), VA Medical Center

Prostate specific membrane antigen (PSMA) is a 100 kDA glycoprotein which is highly expressed in prostate epithelial tissue. PSMA expression is upregulated as prostate cancer progresses and upon androgen deprivation. We have analyzed the ability of 5.5 kb of PSMA promoter/leader region and a recently discovered PSMA enhancer to promote transcription. Fragments were subcloned into the promoterless luciferase expression vector pGL3-Basic for analysis. Prostate cell lines (LNCaP, PC3, DU145) and the nonprostate kidney (786-O) and cervical (HeLa) cell lines were transfected with these constructs, pGL3-Basic, and pGL3-Control, which contains the SV40 promoter and enhancer. The promoter/leader region sequence had little effect in transcription assays, while the enhancer activated transcription 41-fold in the LNCaP cell line. This enhancement of transcription was not found in nonprostate cell lines or prostate cell lines that do not express PSMA. The effect of androgens on transcription promoted by the PSMA promoter/leader region and PSMA enhancer was analyzed by transfecting LNCaP cells, an androgen-responsive prostate cell line, with the luciferase constructs, and growing the cells for two days in media containing charcoal-treated serum supplemented with dihydrotestosterone at 0, 1, 2, or 10 nM concentrations. No androgen response was detected in transcription assays using the PSMA promoter/leader region and a small downregulation of 42% was observed with the PSMA enhancer. This research was supported by the Department of Veterans Affairs and NIH grant HD29381.

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Mechanical Distention Increases IL-6, MIF and GRO mRNAs in Pulmonary Alveolar Epithelial Cells in Vitro

David J Williams[1], Deborah Donig[1], Jeff N Vanderbilt[2], Leland G Dobbs[3], Jorge A Gutierrez[1], [1]Pediatrics, UC San Francisco, 3333 California St. Suite 150, San Francisco, CA 94118, [2] Cardiovascular Research Institute, UC San Francisco, [3]Cardiovascular Research Institute, UC San Francisco

Recent studies demonstrating improved outcomes for acute lung injury patients who were ventilated with reduced tidal volumes has generated much interest in the role of mechanical distention of the lungs in ventilator-induced lung injury. Although the mechanisms that account for ventilator-induced lung injury are incompletely understood, proposed mechanisms include physical damage to alveolar epithelial cells as well as alveolar shear stress. Ventilator-induced lung injury has also been related to cytokine and chemokine production. Mechanical factors are potent regulators of gene expression. We have previously reported that mechanical distention of type II cells in culture altered the expression of surfactant protein mRNA (Am. J. Physiol. 274(18) L196-L202, 1998). The objective of the present study was to investigate the effects of mechanical distention on cytokine and chemokine mRNA expression in primary cultures of type II cells as well as in mouse and rat lung epithelial cell lines (MLE-15, X404). Primary cultures of rat type II cells and cultures of MLE-15 and X404 cells underwent tonic mechanical distention of 20% for 24 hours. RNA was isolated from the samples and analyzed by Northern blot analysis and RNAse Protection Assay (RPA) for mRNA content of interleukin-6 (IL-6), macrophage inhibitory factor (MIF) and the chemokine GRO. Mechanical distention resulted in a 42% increase in IL-6 mRNA content, a 63% increase in GRO mRNA content, and a 42% increase in MIF mRNA content in comparison to non-stretched controls. These data are consistent with the hypothesis that mechanical distention modulates expression of cytokines and chemokines in pulmonary alveolar epithelial cells. Mechanical distention may play an important role in the expression of these inflammatory mediators in ventilator-induced lung injury. Supported in part by NIH Grant HL-04372-01 and RWJ Grant 30805.

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Regulation of Aop2: an Antioxidant Implicated in Atherosicerosis-Resistance in Mice

Shelley A Phelan[1], Mary Lou Lombardi[2], Ellen Neylon[2], [1]Biology, Fairfield University, North Benson Road, Fairfield, CT 06430, [2]Fairfield University

AOP2 (Antioxidant Protein 2) is a thiol-specific antioxidant (TSA) capable of reducing hydroperoxides in the presence of a thiol-containing electron donor. We previously identified Aop2 as a candidate atherosclerosis-resistance gene in mice based on gene expression and protein sequence differences between resistant and susceptible strains. We thus sought to investigate the regulation of Aop2 in the liver, which plays a critical role in lipid metabolism. An analysis of the tissue distribution of the gene shows highest expression in the liver. We next examined the effect of oxidative stress on Aop2 mRNA and protein expression in the H2.35 mouse hepatocyte line. Aop2 levels were only slightly elevated by H202, while levels were significantly and transiently increased in response to glucose oxidase-induced H202 production. We also examined the effect of serum and KGF on Aop2 expression due to a previous report demonstrating a marked induction of Aop2 in serum-deprived keratinocytes. We found no induction of Aop2 with either treatment, however, we did observe significant induction of the highly related intronless gene Aop2-rs1 by both serum and KGF after only one hour of treatment. To understand the high basal and inducible expression of Aop2, 6.5 kilobases of the upstream regulatory sequence were determined and analyzed for putative transcription factor binding sites. Several sites were found, including multiple Sp1 consensus sites, and a potential NF-κB binding site, suggesting a possible mechanism for both types of regulation. Analysis of the proximal promoter in transient transfection assays showed this region is sufficient to trans-activate a downstream reporter gene. Together, our data support distinct roles for both Aop2 and the related Aop2-rs1 in the oxidative stress response, and possibly a coordinate function in atherosclerosis protection.

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Neuroendocrine Specific Protein A + B mRNAs are decreased in the brains of P20 Twitcher mice

Emma Kavanagh[1], Hilary Morrison[2], Robert M Gould[3], [1]Pharmacology, N. Y. S. Institute for Basic Research, 1050 Forrest Hill Rd, Staten Island, NY 10314, [2]Comparative Molecular Biology and Evolution, Marine Biological Laboratory, [3]Pharmacology, Institute for Basic Research

The twitcher mouse is an authentic animal model of globoid cell leukodystrophy (Krabbe disease) and has the same gene defect of a lack of functional ß-galactoceramidase. To identify the early molecular changes in Krabbe disease, we have used subtractive hybridization and differential screening, to look for messenger RNAs that are either enriched or depleted in twitcher versus normal mice. Poly A mRNA was isolated from total brain RNA from three normal and three twitcher 20d mice. Suppression Subtractive Hybridization was carried out using a kit from Clontech, and the subtracted products were ligated into pGEM TEasy vector (Promega) and transformed into competent cells JM1109 (Promega). Differential screening was carried out using a kit from Clontech. Clones that were possibly enriched were identified and isolated by miniprep, sequenced and Northerns carried out to confirm if they are enriched or depleted.

Using this approach we identified 56 clones that were possibly depleted in twitcher mice but only one that was depleted in wild type. Of the cDNAs that we have analyzed so far we have identified two as Neuroendocrine Specific Protein A and B, (NSP-A + NSP-B). The third smaller NSP mRNA, NSP-C, was also present but was unaffected. We have confirmed that these mRNAs are depleted in both 17d and 20d twitcher mice by Northern blot analysis. These changes occur in advance of marked changes in brain psychosine levels. This is an unexpected finding because NSP proteins are expressed in differentiated neurons and not in oligodendrocytes.

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KLF15 (Krüppel-like factor15) Represses Transactivation of *Rhodopsin* Promoter Constructs by Crx (cone rod homeobox) and Nrl (neural retina leucine zipper)

Deborah C Otteson, Donald J Zack, Guerrieri Center for Genetic Engineering and Molecular Ophthalmology at the Wilmer Institute, Johns Hopkins University School of Medicine, 600 N. Wolfe St, Baltimore, MD 21287

Transcriptional regulation of the photoreceptor-specific gene *rhodopsin* relies on combinatorial effects of transcription factors (e.g. Crx, Nrl) acting on specific