velop hypertrophy, fibroblasts contribute to fibrosis during heart injury. We tested the response of these two cell types to oxidants by analyzing their gene expression profiles and identifying the transcription factors responsible for the changes in gene expression. Microarray analyses using Affymetrix chips containing 20K genes found that a sublethal dose of H_2O_2 (100 μM) caused about 1% of genes to increase expression to 2-fold or higher in cardiomyocytes and cardiac fibroblasts. Among those induced are antioxidant and detoxification genes containing the antioxidant response element (ARE) in their promoters, such as NAD(P)H:quinone oxidoreductase-1 (NQO1) and heme oxygenase-1 in cardiomyocytes and NQO1 and glutathione *S*-transferase in fibroblasts. ARE promoter-luciferase reporter assays demonstrated that the ARE is activated in an H_2O_2 dose-dependant manner in cardiomyocytes. The peak activation of ARE, an average of about 5 fold, occurred at 4 hrs post 10-min treatment with 100 μM H_2O_2. This level of activation was comparable to the positive control *tert*-butylhydroquinone. No induction was detected with a luciferase reporter plasmid containing an empty or a mutant ARE promoter after H_2O_2 treatment. NF-E2-related factor 2 (Nrf2) is a basic leucine zipper transcription factor that can bind to the ARE. Expression of dominant-negative Nrf2 abolished ARE activation by H_2O_2. A dominant negative form of c-Jun, which is thought be a dimerization partner of Nrf2 in certain experimental systems, did not affect ARE activation by H_2O_2. These data suggest that oxidants can induce survival or adaptive responses by activating the expression of antioxidant and detoxification genes in cardiomyocytes and cardiac fibroblasts.

1180 DIFFERENTIAL REGULATION OF THE NRF2- AND AP-1-RESPONSE ELEMENTS BY 4-HYDROXY-2-NONENAL, t-BUTYLHYDROQUINONE AND PHORBOL 12-MYRISTATE 13-ACETATE IN CULTURED HEPATIC STELLATE CELLS.

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Stellate cell activation is a central event in liver fibrosis characterized by increased extracellular matrix production and cell proliferation. Oxidative stress has been implicated as an activator of the bZIP transcription factors Nrf2 and AP-1 through shared kinase signaling pathways that purportedly mediate stellate cell activation. The present study examined Nrf2- and AP-1-mediated gene expression following oxidative stress in cultured hepatic stellate cells. Reporter constructs generated by mutation of the AP-1- and Nrf2-binding elements of the human nqo1 promoter demonstrated that the Nrf2 element was fully responsible for mediating inducible gene expression in response to the prooxidants 4-hydroxy-2-nonenal and tert-butylhydroquinone. Contrastingly, the AP-1 element was incapable of mediating inducible expression in response to either oxidative stress or PMA, despite the presence of Fos- and Jun-related nuclear proteins. When present together, the Nrf2 and AP-1 elements acted synergistically to mediate high-level basal promoter activity. Identification of AP-1 family proteins revealed heterodimeric binding complexes consisting predominantly of Fra-1, Fra-2, JunB and JunD proteins, with little c-Fos, FosB or c-Jun. With exception of a 2-fold increase in nuclear c-Fos following PMA treatment, neither electrophiles nor PMA treatment appreciably altered the presence of AP-1 proteins. Taken together, these data demonstrate that, although overlapping kinase-signaling pathways activates AP-1 and Nrf2 transcription factors, oxidative stress discretely activated Nrf2, while AP-1 was insufficient to convey independent reporter induction. This observation is significant in context of the physiologic importance attributed to the AP-1 in mediating essential profibrogenic gene expression during liver fibrogenesis.

1181 INDUCTION OF CYCLOOXYGENASE-2 BY H_2O_2 AND CORTICOSTERONE IN CARDIOMYOCYTES.

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Heart disease is the number one cause of natural death worldwide. A large body of evidence has indicated a role of oxidants in myocardial infarction and other types of heart disease. Although cyclooxygenase-2 (COX-2) can contribute to an inflammatory response, induction of COX-2 has been shown to be cytoprotective in cardiomyocytes *in vitro* and *in vivo*. Oxidants and the stress hormone corticosterone are thought to be detrimental to the heart. However, our gene array work has found COX-2 and a number of cytoprotective genes induced by these two stimuli in cardiomyocytes. An increase of COX-2 mRNA and protein was detected with either oxidants or corticosterone treatment by RT-PCR and Western blot. The optimal dose for COX-2 induction was 50-100 μM for H_2O_2 and 0.25-1 μM for corticosterone. The peak increase of COX-2 protein occurred at 2-4 hour after 1 hour treatment with 100 μM H_2O_2 or at 32 hour in cells treated with 1 μM corticosterone. While the induction of COX-2 is transient and returned to basal level within 10 hours for H_2O_2, elevation of COX-2 is sustained for 3 days with corticosterone treatment. The p38 MAPK inhibitor SB202190 but not inhibitors of JNKs, ERKs or PI3K prevented H_2O_2 from inducing COX-2 elevation. In contrast, the glucocorticoid receptor antagonist mifepristone inhibited COX-2 induction by corticosterone. Using the TranSignal array technique to screen for activated transcription factors, we found that oxidants and corticosterone activated several transcription factors in common. The roles of these transcription factors in elevation of COX-2 expression are under investigation.

1182 INDUCTION OF METALLOTHIONEIN I BY PHENOLIC ANTIOXIDANTS REQUIRES METAL-ACTIVATED TRANSCRIPTION FACTOR 1 (MTF-1) AND ZINC.

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Phenolic antioxidants, such as tert-butyl-hydroquinone (tBHQ), induce metallothionein (Mt1) gene expression. Induction of Mt1 mRNA correlates with oxidation-reduction functions of the antioxidants. Here we analyzed the biochemical pathway of the induction. Induction depends upon the presence of MTF-1, a transcription factor that is required for metal-induced transcription of Mt1, but does not require Nrf2, a tBHQ-activated CNC bZip protein that is responsible for regulating genes encoding phase II drug-metabolizing enzymes. Moreover, tBHQ induces the expression of MRE-bGeo, a reporter gene driven by five metal regulator elements (MREs) that constitute an optimal MTF-1 binding site. Reconstitution of Mtf1-null cells with MTF-1 restores induction by both zinc and tBHQ; however, reconstitution with only the DNA-binding domain of MTF-1 fused to VP16 activation domain allows in induction by zinc but not by tBHQ. Unlike activation of phase II genes by tBHQ, induction of Mt1 expression does not occur in the presence of EDTA, when cells are cultured in zinc-depleted medium, or in cells with reduced intracellular 'free' zinc due to overexpression of ZnT1, a zinc-efflux transporter, indicating that induction requires zinc. These findings establish that phenolic antioxidants activate Mt1 transcription by a zinc-dependent mechanism that involves MTF-1 binding to MREs.

1183 PI3-KINASE REGULATES CA^{2+}-DEPENDENT NRF2 TRANSLOCATION FROM THE CYTOPLASM TO THE PLASMA MEMBRANE PRIOR TO ITS NUCLEAR TRANSLOCATION.

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Expression of phase II detoxifying genes is regulated by NF-E2-related factor 2 (Nrf2)-mediated antioxidant response element (ARE) activation. Phosphatidylinositol 3-kinase (PI3-kinase) regulates nuclear translocation of Nrf2 through the rearrangement of actin microfilaments in response to prooxidants. We further studied the role of PI3-kinase signaling pathway in the rise of cellular Ca^{2+} and the translocation of Nrf2 to the plasma membrane. Immunocytochemistry and subcellular fractionation analyses revealed that pretreatment of cells with tert-butylhydroquinone (t-BHQ) inhibited the translocation of Nrf2 to the plasma membrane, whereas treatment of cells immediately after addition of PI3-kinase inhibitors (control experiment) caused Nrf2 to migrate to the plasma membrane before nuclear translocation. t-BHQ treatment stimulated a rise in $[Ca^{2+}]i$, which was prevented by PI3-kinase inhibition. Chelation of cellular calcium suppressed Nrf2 migration to the plasma membrane. Conversely, A23187 a calcium ionophore, allowed cytoplasmic Nrf2 to move to the plasma membrane in the cells exposed to both t-BHQ and PI3-kinase inhibitor. Immunoblot analysis confirmed that either PI3-kinase inhibition or Ca^{2+} chelation prevented GSTA2 induction by t-BHQ. These results demonstrated that the PI3-kinase pathway regulates Ca^{2+}-dependent translocation of Nrf2 from the cytoplasm to the plasma membrane prior to its nuclear translocation.

1184 NICOTINE USES T CELL ANTIGEN RECEPTOR-INDEPENDENT AND -DEPENDENT SIGNALING PATHWAYS TO AFFECT INFLAMMATORY AND ADAPTIVE IMMUNE RESPONSES.

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Development of adaptive T cell immunity requires activation of the T cell antigen receptor (TCR), leading to increased intracellular Ca2+ levels ([Ca2+]i), T cell proliferation, and differentiation, while activation (nuclear translocation) of the transcription factor NF-κB is the hallmark of an inflammatory response. Activation of TCR through ligation by anti-TCR antibodies, increases Ca2+ and nuclear localization of NF-κB. Chronic exposure to cigarette smoke/nicotine suppress both

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Preface

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An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 443.

The document also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 473.

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