enzymes and xenobiotic transporters by binding to the cis-antioxidant response elements in the promoter region of these genes. Previous works by our lab and others demonstrated that Nrf2 is subject to poly-ubiquitin-mediated proteasomal degradation in a Keap1-dependent manner. Here we report that active form of XBP1, XBP1s suppresses Nrf2 and its downstream signal under ER stress condition, through activation transcription of synovial apoptosis inhibitor 1 (SYVN1). SYVN1, also known as Hrd1, is an ER-associated degradation (ERAD) ubiquitin ligase. We have determined that SYVN1 directly interacts with the Neh4 and Neh5 domain within Nrf2. Overexpression of SYVN1 attenuates Nrf2 signaling, whereas knockdown of SYVN1 enhances expression of Nrf2 and its downstream genes. Furthermore, SYVN1 accelerates the clearance of Nrf2 protein through promoting ubiquitination of Nrf2. These findings demonstrate that XBP1 and SYVN1 are involved in regulating the Nrf2 pathway in a new Keap1-independent mechanism. Moreover, our data revealed a possible crosstalk between ER stress pathway and oxidative responses via UPR and Nrf2 signaling pathway in order to protect cells against environmental stress.

PS 2012 Sulforaphane Stimulates Basal but Inhibits Glucose-Stimulated Insulin Secretion in Beta-Cells: Role of Reactive Oxygen Species and Induction of Endogenous Antioxidants.

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Excessive oxidative damage by reactive oxygen species (ROS) is a major contributor to pancreatic β-cells dysfunction. Interestingly, ROS are also involved as a second messenger in glucose-stimulated insulin secretion (GSIS) in β-cells. This paradox obscures the regulatory role of antioxidants in insulin secretion. In the present study, we used an integrated mathematical modeling and in vitro approach to understand the effects of antioxidant sulforaphane (SFN) on insulin secretion. Experiments with INS-1(832/13) cells and isolated mouse islets showed that 30-min SFN treatment stimulated basal insulin secretion in a concentration-dependent manner at low glucose conditions (3 mM). This acute stimulatory effect resulted from an initial SFN-elicited increase of ROS, and when suppressed with cell-permeable ROS scavenger N-acetylcysteine or glutathione ethyl ester, SFN-stimulated insulin secretion was diminished. Due to the negative feedback and incoherent feedforward loops comprising the redox homeostatic control circuit, cells can adapt to prolonged SFN treatment and settle to a steady state exhibiting strong induction of antioxidants but only marginally increased ROS levels. This adapted state slightly increased basal insulin secretion in INS-1(832/13) cells. More importantly, the model predicted that the elevated antioxidant capacity at the adapted state attenuates glucose-stimulated ROS signal and GSIS. This effect was validated in INS-1(832/13) cells exposed to low, non-cytotoxic concentrations of SFN. Despite suppressing GSIS, prolonged exposure to SFN protected INS-1(832/13) cells from cytotoxicity induced by exogenous H2O2. Taken together, our studies demonstrated that SFN has divergent effects on basal and glucose-stimulated insulin secretion in β-cells. Although the adaptive induction of endogenous antioxidants by SFN enhances β-cell survival, it suppresses GSIS.

PS 2013 Generation of Reactive Oxygen Species by Process Materials from Indium-Tin Oxide Production.

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The transition metal indium has been used for decades for various applications including electronics, fusible alloys, and solar cells. Indium compounds usage has increased dramatically based on the rise in demand of touch screens and flat panel displays (LCD's). With this growth of industry, there is potential for increase of indium lung disease among workers who produce, use, or reclaim indium-tin oxide (ITO). Inhalation exposure of indium samples can occur during various times of manufacturing. Materials from different process stages were collected from an ITO production facility. While the pathogenesis of indium lung disease is unknown, previous work has suggested a role for reactive oxygen species (ROS). Chemical characteristics of the process materials will aid in determination of reactivity differences between compounds. Electron spin resonance (ESR), a common tool used for measuring ROS, was used in both acellular and cellular exposures. Acellular samples were evalyated by combining 10 mg/mL process material, phosphate buffered saline (PBS), 10mM hydrogen peroxide (H_2O_2), and 100mM DMPO (spin trap). RAW 264.7 mouse monocyte macrophages, DMPO, and the same concentration of composite were used in the cellular samples conducted in ESR. Scavengers and chelators were used to define radical mechanisms. Results indicated that ventilation dust (VD), tin-oxide (SnOX), and unsintered ITO (UITO) cause a greater increase in ROS production than the other process material. H_2O_2 and O_2 consumption measurements were used to determine the source of the ROS. ESR studies combined with investigation of ROS production will help to determine the mechanisms behind indium lung disease. Data from this study will be used to determine possible hazards in occupational exposure of indium process material while increasing the understanding of indium lung disease.

PS 2014 Lower Expression of Nrf2 Promotes Proliferation, Migration and Invasion of Prostate Cancer Cells.

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The nuclear factor Nrf2 is known to play a critical role in cellular protection against oxidative stress and cellular transformation. However, unabated nuclear accumulation of Nrf2 is also known to reduce apoptosis, promote cancer cell survival and drug resistance. Mutations in INrf2 and Nrf2 leading to nuclear accumulation of Nrf2 in many cancers including prostate and breast cancer are known. These also raise interesting questions regarding the role of Nrf2 in cancer metastasis and/or metastasis progression that remains elusive. In this study we have investigated the hypothesis that loss of Nrf2 is associated with metastasis/metastasis progression. We used less metastatic LNCaP and highly metastatic LNCaP derived C4-B2 prostate cancer cell lines to test our hypothesis. The analysis revealed that highly metastatic C4-B2 cells expressed higher INrf2 and lower Nrf2 levels as compared to less metastatic LNCaP cells. We used control, INrf2 and Nrf2 shRNA to generate C4-B2 and LNCaP derived cells with altered levels of INrf2 and Nrf2 to determine the role of Nrf2 in metastasis and metastasis progression in Soft agar colony formation and X-CELLigence proliferation, migration and invasion assays. C4-B2-INrf2shRNA cells expressing inhibited levels of INrf2 and higher levels of Nrf2 showed fewer colonies in soft agar and proliferated faster but did not migrate as compared to C4-B2-Control shRNA cells. Similarly, LNCaP-Nrf2shRNA cells expressing inhibited levels of Nrf2 in less metastatic LNCaP cells demonstrated significantly higher number of colonies in soft agar, proliferated faster and showed greater migration and invasion as compared to LNCaP-Control shRNA cells. These results together suggest that lower Nrf2 levels are associated with higher proliferation, migration and invasion or metastatic progression. Currently, we are investigating the mechanism of the role of Nrf2 in metastasis progression and plan in vivo experiments to test our hypothesis of the association of lower Nrf2 with metastasis progression in mice.

PS 2015 Mechanisms of Oxidative Stress Promoted by 1, 4-Diamino-2-Butanone in Trypanosoma cruzi and Mammalian Cells.

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The putrescine analogue 1,4-diamino-2-butanone (DAB) is highly toxic to pathogenic microorganisms, including various fungi and Trypanosoma cruzi. Similar to other α-aminocarbonyl metabolites such as aminoacetone and 5-aminolevulinic acid, DAB exhibits pro-oxidant properties. DAB reportedly undergoes metal-catalyzed oxidation in aerobic medium yielding H2O2, NH4+ ion, and 4-amino-2-oxobutanal, a highly toxic α-oxoaldehyde. Administered to mammaliian cell cultures, DAB decreases the cell viability which was shown to be associated with changes in redox balance. Thus, treatment of RKO cells derived from human colon carcinoma or cultured LL-MK2 Rhesus epithelial cells with millimolar DAB caused significant decline in cell viability, which was inhibited by pre-addition of catalase, aminoguanidine (an α-oxoaldehyde trap), N-acetyl cysteine or reduced glutathione. Now we explore the mechanisms by which DAB exhibits pro-oxidant effects on trypomastigotes and on intracellular T. cruzi amastigotes. DAB (0.05-5.0 mM) exposure in trypomastigotes, the infective stage of T. cruzi, leads to a decline in parasite viability (IC50 c.a. 0.2 mM DAB; 4 h incubation), changes in morphology, thiol redox imbalance, and increased TcSOD activity. Medium supplementation with catalase (2.5 μM) protects trypomastigotes against DAB toxicity, while host cell invasion by trypomastigotes is hampered by DAB. Additionally, intracellular amastigotes are susceptible to DAB toxicity. Furthermore, pre-treatment with 100-500 μM buthionine sulfoximine (BSO) of LLC-MK2 potentiates DAB cytotoxicity, whereas 5 mM N-acetyl-cysteine (NAC) protects cells from oxidative stress. Together, these data support the hypothesis that redox imbalance, not only the long reported DAB-promoted inhibition of polyamine metabolism, contributes to its cytotoxicity in both T. cruzi and mammalian host cells.

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