expressions. Studies showed that AI letrozole resistant breast cancer LTLT cells contain lower INrf2 and higher Nrf2 levels, as compared to drug sensitive MCF-7Ca cells. The removal of letrozole from LTLT cells led to increase in INrf2, decrease in Nrf2 and increased sensitivity to letrozole-induced death. Higher levels of Nrf2 were also observed in anestrozole resistant breast cancer AC1AnaR cells, as compared to sensitive AC1 cells. Further studies revealed that higher Nrf2-mediated activation of biotransformation enzymes, drug-transporters and anti-apoptotic proteins contributed to reduced efficacy of drugs and prevention of apoptosis that led to drug resistance. Current studies are investigating the mechanism of AI-mediated decrease in INrf2 gene expression. These together suggest that breast cancer cells during persistent treatment with AI drugs generate ROS and electrophiles that signal INrf2 down regulation and Nrf2 activation leading to reduced cell death and increased cell survival/drug resistance.

PS 2143 INHIBITION OF PARAQUAT-INDUCED OXIDATIVE STRESS, PROINFLAMMATORY CYTOKINE EXPRESSION, AND FIBROBLAST-TO-MYOFIBROBLAST TRANSFORMATION BY RESVERATROL VIA THE NRF2 PATHWAY.

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Paraquat (PQ) is a most widely used herbicide in the world. PQ selectively accumulates in the lungs and induces lung injury and fibrosis in humans. Redox cycling has been linked with PQ pulmonary toxicity but no effective antidote is available. Resveratrol (Res) is a natural phytoalexin with multiple functions including antioxidant, anti-inflammatory in animals and humans. In this study, we found that Res at pharmacological doses effectively attenuated PQ-induced cell toxicity and fibrogenic response in human lung cells. PQ dose-dependently caused toxicity in normal human bronchial epithelium BEAS-2B cells including increased cell death, oxidative stress, and loss of mitochondrial inner membrane potential. Res at 10 to 20 uM markedly inhibited PQ toxicity. PQ at 10 uM induced transformation of normal human lung fibroblast WI-38 cells into myofibroblasts, as shown by the de nova synthesis of a-smooth muscle actin, and heightened production of inflammatory cytokines TNFa and IL-6 and growth factor TGFb1. On the other hand, pre- or co-treatment with Res blocked the fibrogenic reactions to PQ. Mechanistic analyses revealed that Res activated the oxidant/antioxidant-activated receptor Nrf2 to induce cytoprotective genes. Nrf2 was required for normal defense against PQ toxicity and fibrogenic reactions as loss of Nrf2 significantly increased PQ toxicity, myofibroblast transformation, and cytokine expression. Finally, Nrf2 mediated the protective response to PQ by Res because protection was lost in Nrf2-deficient cells. The study demonstrated that Res prevents PQ-induced ROS production, inflammation, and fibrogenic reactions in cultured cells by activating Nrf2 signaling. The findings provide new insights into the understanding and chemoprotection of PQ lung toxicity and potential intervention through Nrf2-based mechanisms.

PS 2144 INRF2 (KEAP1) AND NRF2 BOTH CONTROL BCL-2 AND CELLULAR APOPTOSIS.

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Nrf2:INrf2 complex serves as a sensor of chemical and radiation-induced oxidative stress. Under normal conditions, INrf2/Cul3-Rbx1 ubiquitin ligase complex constantly ubiquitinate and degrade Nrf2. When cells encounter stressor, Nrf2 is dissociated from INrf2/Cul3-Rbx1 complex, translocates in the nucleus and coordinately activate a battery of cytoprotective proteins that provide the critical protection against oxidative stress, cellular transformation and neoplasia. However, persistent exposure to stressors or mutational inactivation of INrf2/Cul3-Rbx1 complex cause nuclear accumulation of Nrf2 that leads to enhanced cell survival and drug resistance. In this study, we demonstrate that antioxidant control of both INrf2 and Nrf2 leads to increase in anti-apoptotic protein Bcl-2 that prevents apoptosis and promotes cell survival and drug resistance. INrf2/Cul3/Rbx1 complex ubiquitinate and degrade Bcl-2 protein as observed with Nrf2. The DGR domain of INrf2 interacts with the BH2 domain of Bcl-2 and facilitates INrf2/Cul3-Rbx1-mediated ubiquitination of Bcl-2 by the conjugation of ubiquitin molecules to lysine17 of Bcl-2. Antioxidant t-BHQ antagonized INrf2:Bcl-2 interaction, led to the release and stabilization of Bcl-2, increased Bcl-2:Bax heterodimers and reduced apoptosis. In addition, antioxidant-induced Nrf2 binds with an ARE located between nucleotides -3148 to -3140 on the reverse strand of Bcl-2 gene promoter and increase Bcl-2 gene transcription leading to elevated levels of Bcl-2. The antioxidant control of INrf2 and Nrf2 led to increased Bcl-2 that decreased etoposide-mediated accumulation of Bax, release of cytochrome c from mitochondria and activated caspase-3/7. These alterations led to significantly reduced DNA fragmentation and apoptosis. Together, these results provide the first evidence of INrf2 and Nrf2 control of anti-apoptotic protein Bcl-2 and apoptosis.

PS 2145 NRF2 PROTECTS HUMAN AND MOUSE ALVEOLAR EPITHELIAL CELLS AGAINST INJURY BY CIGARETTE SMOKE.

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Oxidative stress caused by cigarette smoke (CS) directly causes lung injury and cell death. The epithelium is the barrier between inhaled air, which contains the toxic compounds in CS and the underlying tissue. Nuclear factor erythroid 2-related factor 2 (Nrf2) is the principle transcription factor that regulates expression of phase II detoxifying antioxidant enzymes. We studied injury by cigarette smoke extract (CSE) of human primary alveolar cells isolated from lung donors in vitro and Nrf2-/- and wild-type C57Bl/6 mouse alveolar cells and lung tissue in vivo. We found that CSE induces Nrf2 translocation to the nucleus in human primary alveolar type I-like (ATI-like) cells. Moreover, Nrf2 overexpresion protected these cells against injury by CSE and Nrf2 knockdown sensitizes these cells to CSE as detected by propidium iodide and Hoechst 33342 double staining. We also found that necrosis of ATI-like cells induced by CSE was prevented by the antioxidant compounds NAC and trolox. Furthermore, we also studied lung injury by CS in Nrf2-/- mice and wild-type C57Bl/6 mice in vivo. We found Nrf2 activation and induction of Nrf2-dependent genes HO-1 and NQO1 by western blotting and real time-PCR in alveolar type II cells isolated from wild-type mice but not from Nrf2-/- mice. This suggests involvement of the Nrf2 pathway in protection against lung injury by CS. Moreover, oral administration of NAC or trolox decreased expression of Nrf2 and Nrf2-dependent genes in the lung tissue of wild-type mice but not in Nrf2-/- mice exposed to CS as detected by real time-PCR. Our results suggest Nrf2 protects against CS-induced injury in human alveolar cells in vitro and in mice in vivo.
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PS 2146 NRF2 DEFICIENCY ATTENUATES FAT ACCUMULATION IN WHITE ADIPOSE TISSUE BY INHIBITING SREBP1C TRANSCRIPTION IN LEPOB/OB MICE.

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It is well described Nuclear factor erythoid 2-related factor 2 (Nrf2) regulates cellular electrophilic and oxidative stress in liver and kidney. While our recent findings along with others indicate Nrf2 pathway is inducible in adipose tissue. Nrf2 signaling is enhanced in obese and diabetic animal models, and the transcriptional levels of Nrf2, Nqo1 and Gclc was induced by fating, suggesting Nrf2 signaling plays key roles in regulating glucose and lipid metabolism. However the exact function of Nrf2 on lipid metabolism is still ambiguous. CDDO-Imidazolide induced Nrf2 activation prevented high fat diet (HFD)-induced obesity, decreased hepatic fat accumulation and lipogenic gene expression (Zhang. YK, 2010). In contrast, recent work from Pi et. al illustrated Nrf2-null mice displayed less fat mass and smaller adipocytes formation, protection against weight gain and obesity (Pi. et. al., 2010). In the current study, we generated the double knockout mice of Nrf2 deficiency in leptin mutant mice (Lepob/ob). Deficiency of Nrf2 attenuated fat accumulation to white adipose tissue, described by less fat mass and decreased rate of fat gain in Lepob/ob mice. The decreased fat accumulation may be explained by suppression of lipogenic gene expression, PPARγ, C/ebpα, and HSL in white adipose tissue. Deficiency of Nrf2 attenuated the induction of Srebp1c by obesity in white adipose tissue, and subsequently blocked the induction of FAS, ACC-1, and SCD-1. Double knockout mice deposited less fat (triglyceride) in livers, suggesting Nrf2 deficiency prevented hepatic steatosis and fatty liver process in Lepob/ob mice. However Nrf2 deficiency impaired glucose tolerance and more severe diabetic status, which displayed higher hyperinsulinmia and hypertriglyceridemia in Lepob/ob mice. Overall, Nrf2 deficiency attenuated fat accumulation to adipose tissue; this attenuation may mediate by decreased Srebp1c transcriptional levels and the decreased lipid synthesis.

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