Drug Mediated Oxidative or Nitrosative Stress Induces the Unfolded Protein Response

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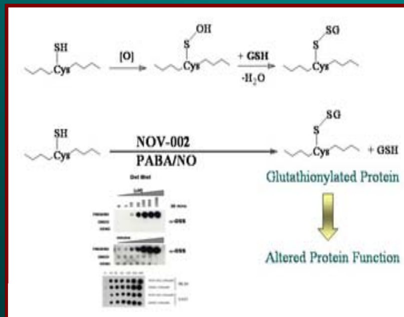
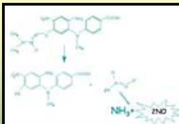
Abstract

The rapid proliferation of cancer cells mandates a high protein turnover. The endoplasmic reticulum (ER) is an organelle that is intimately involved in protein processing. An accumulation of unfolded or misfolded proteins in the ER leads to a cascade of transcriptional and translational events collectively referred to as the unfolded protein response (UPR). Protein disulfide isomerase (PDI) is one of the most abundant ER proteins and maintains a sentinel function in organizing accurate protein folding. Two novel drugs, a platinum-stabilized form of oxidized glutathione (NOV-002) and a novel nitric oxide releasing glutathione S-transferase activated pro-drug, PABA/NO (*O*2-[2,4-dinitro-5-(*p*-methylaminobenzoato)] 1-(*N*, *N*-dimethylamino)diazen-1-ium-1,2-diolate) lead to S-glutathionylation at low pKa cysteine residues of a select group of target proteins in tumor cells. This post-translational modification causes significant changes in protein structure, cellular location and function. Immunoprecipitation of PDI following PABA/NO treatment in human ovarian cancer cells (SKOV3) showed that it is S-glutathionylated. Mass spectroscopy analysis confirmed that a single cysteine residue [+305.3 Da] within each of the catalytic sites of PDI is modified. Our hypothesis is that S-glutathionylation of PDI is an upstream signaling event in the UPR. Activation of the UPR in SKOV3 cells was assessed by immunoblot and real-time PCR following either 300 µM NOV-002 or 20 µM PABA/NO. Within 4h, each drug treatment led to translational attenuation as measured by the phosphorylation and activation of the ER transmembrane kinase, PERK, and its downstream effector eIF2. Cleavage of the transcription factor, XBP-1 was concurrent with transcriptional activation of the ER resident proteins, BiP, PDI, GRP94 and ERO1 (5-10 fold induction). For NOV-002, because it is not a cytotoxic agent, the induction of the UPR is facilitated independently of apoptosis; for PABA/NO, the induced nitrosative stress causes the UPR as a prelude to cell death. As such, transcriptional activation of CHOP (a pro-apoptotic cytosolic protein) was elevated 8-fold in PABA/NO treated cells but not by NOV-002. The common pathways leading to UPR involve alterations in redox balance, either through altered GSSG:GSH ratios (NOV-002) or through thiol changes brought about by nitrosative stress induced by nitric oxide (PABA/NO).

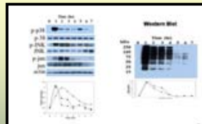
ROS/RNS induced S-glutathionylation



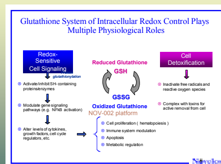
PABA/No - a GST pi Activated Prodrug



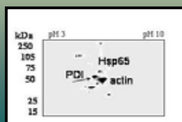
JNK Activation Concurrent With S-Glutathionylation



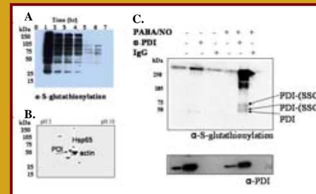
NOV-002 and intracellular redox



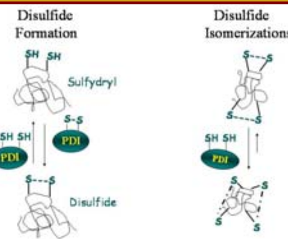
S-Glutathionylation of PDI

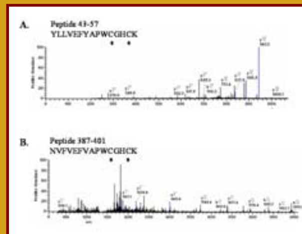


PABA/NO Induces S-Glutathionylation Of Protein Disulfide Isomerase



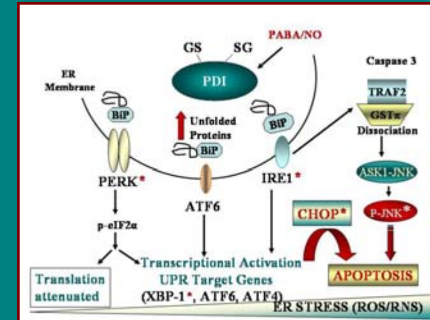
PABA/NO induces S-glutathionylation of PDI in HL60 and SKOV3 cells: (A) HL60 Cells were treated with 30µM PABA/NO. The lysates were separated under non-reducing conditions and immunoblotted with the Anti-glutathionylation antibody; (B) HL60 cells were treated with 30µM PABA/NO for 1h and separated by two-dimension SDS-PAGE.. The S-glutathionylated proteins were identified by mass spectrometry. (C) SKOV3 cells were treated with 20µM PABA/NO for 1h. PDI was immunoprecipitated and separated by SDS-PAGE. The precipitate was immunoblotted with the anti-glutathionylation antibody. The blot was stripped and probed for PDI.

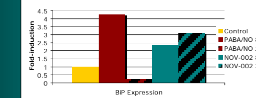


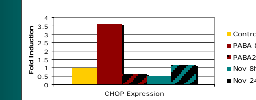


The active site cysteines in hPDI are targets for S-glutathionylation. hPDI was treated with increasing concentrations of PABA/NO for 1h. S-glutathionylation was detected by immunoblot (top). Control and PABA/NO-treated hPDI were digested with LysC under non-reducing condition and analyzed by tandem MS. Two fragments (43-57 and 387-401) were detected in the treated sample that was consistent with a single S-glutathionylated modification [+305.6] with a probability of 8.7 e-002 and 7.4 e-002, respectively

The Unfolded Protein Response

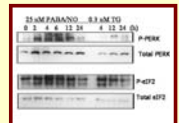


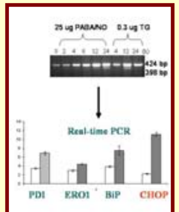




NOV-002 induces the UPR in the absence of cell death, as indicated by the lack of CHOP induction HL60 cells were treated with 30 µM PABA/NO or 300 µM NOV-002. Transcriptional activation of UPR responsive genes was assayed by Realtime PCR.

PABA/NO





SKOV3 cells were treated with 30µM PABA/NO for 1h. Translational attenuation was evaluated by phosphorylation of PERK and eIF2 (top) and transcriptional activation via real-time PCR (Bottom).

Conclusions:

- Both PABA/NO and NOV-002 cause S-glutathionylation of a variety of proteins
- S-glutathionylation of PDI is caused by both NOV-002 or PABA/NO and is upstream of the UPR
- Induction of the UPR by PABA/NO precedes cell death and activates CHOP
- Induction of the UPR by NOV-002 occurs through redox balance changes and is independent of cell death