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 activatable pro-drug, PABA/NO (2-[5,6-dinitro-3-p-methylaminobenzoato]-5-p, 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 transcriptional activation as measured by the phosphorylation and activation of the ER transmembrane kinase, PERK, and its downstream effectors. Changes in the translation factor, eIF2α, was concurrent with transcriptional activation of the ER resident proteins, BiP, PDI, GRP78 and ERP5 (5-10 fold induction). For NOV-002, because it is not a cysteine agent, the induction of the UPR is independent of apoptosis. For PABA/NO, the induced nitrosative stress causes the UPR in a protracted cell death. As such, transcriptional activation of CHOP (a pro-apoptotic cytosolic protein) downstream of PERK was observed in NOV-002 but not for PABA/NO. The common pathway leading to UPR involves alterations in redox balance, either through altered GS/GSSG ratios (NOV-002) or through direct changes brought about by nitrosative stress induced by nitro-oxides (PABA/NO).

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