Title
Mutations in mitochondrial DNA are common and contribute to the altered redox state of human melanoma: A target for chemotherapeutic enhancement

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intrinsic apoptotic pathway, upstream of cytochrome *c* release from the mitochondria. the present study demonstrates that mitochondria isolated from the glucocorticoid-resistant variants are also resistant to cytochrome *c* release induced in *vitro by* addition of recombinant pro-apoptotic tBid protein. of interest, the amount of cytochrome *c* expressed in mitochondria varies, with the resistant CAT2 and CAT38 expressing more cyt *c* than sensitive WEHI7.2 cells. the interplay of the various pro- and anti-apoptotic Bcl-2 family members can control mitochondrial integrity and cytochrome *c* release; however, Western blots for Bcl-2 family protein expression levels do not explain the difference in apoptosis sensitivity in our model system. Differences in Uncoupler Protein 2 (UCP-2) expression between sensitive and resistant variants could provide a mechanism that links ROS signaling and apoptosis, and western blots for UCP-2 demonstrate a reduced expression of protein in the resistant 200R cells, as compared to sensitive WEHI7.2. 2-D gel analyses of mitochondrial proteins have shown differences between sensitive and resistant cells, and have afforded additional proteins of interest for further study. We expect that results from these experiments will suggest targets for modulation of the apoptotic response in lymphoma therapy.

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Targeting the Redox Achilles Heel of Malignancy: NQO1-Activated Phenothiazinium Redox Cyclers for the Bioreductive Induction of Cancer Cell Apoptosis

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Altered redox signaling and regulation in cancer cells represent a chemical vulnerability that can be targeted by selective chemotherapeutic intervention. Here, we demonstrate that phenothiazinium-based redoxcyclers (PRC) induce selective cancer cell apoptosis by NAD(P)H:quinone oxidoreductase (NQO1)-dependent bioreductive generation of cellular oxidative stress. Using methylene blue and more advanced PRC lead compounds against human metastatic G361 melanoma cells in the low micromolar range, apoptosis occurred with phosphatidylserine-externalization, loss of mitochondrial transmembrane potential, and cytochrome C release. PRC-induced apoptosis was accompanied by massive ROS production as assessed by 2',7'-dichlorodihydrofluorescein diacetate staining. Consistent with reductive activation and subsequent redoxcycling as the mechanism of PRC cytotoxicity, partial protection was achieved by co-incubation with catalase, whereas reductive antioxidants such as NAC enhanced PRC-cytotoxicity. Unexpectedly, human A375 melanoma cells were highly resistant to PRC-induced apoptosis, and PRC-sensitive G361 cells were protected by preincubation with the NQO1-inhibitor dicoumarol. Indeed, NQO1 specific enzymatic activity was at least 15 fold higher in G361 than in A375 cells. the critical role of NQO1 in PRC-bioactivation and apoptogenicity was confirmed, when NQO1-transfected human breast cancer cells (MCF7-DT15) stably overexpressing active NQO1 displayed strongly enhanced PRC-sensitivity as compared to vector-control transfected (MCF7-neo) cells with base line NQO1 activity. Based on the known overexpression of NQO1 in various tumors (e.g. breast/colon/pancreas carcinoma, and melanoma) these findings suggest the feasibility of developing PRC lead compounds into tumor-selective bioreductive chemotherapeutics. Supported in part by grants from NIH (SPORE in GI Cancer CA95060; ES06694) and ABRC (0721).

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N-Acetylcysteine Inhibits the Tumor Suppressive Effect of MnSOD and GPx1 on MDA-MB231 Cell Line

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Manganese-containing superoxide dismutase (MnSOD) has been shown to inhibit MDA-MB231 (MB231) breast cancer growth but mechanism for this is still unclear. Our lab is interested in whether the H_2O_2 is responsible for the MnSOD induced cell growth inhibition. This study had demonstrated that BSO, an inhibitor of GSH synthesis, reduced GSH levels dramatically but growth inhibition was not increased significantly when combined with MnSOD. in contrast, overexpression of GPx-1 by adenovirus infection inhibited cell growth when combined with or without MnSOD overexpression. GSSG concentration was increased in both treatments. Catalase overexpression in MB231 did not reverse MnSOD-induced cell growth inhibition and by it self did not affect cell growth. in contrast, N-acetylcysteine (NAC) markedly reversed growth inhibition caused by the GPx1 and/or MnSOD overexpression. These results suggested that MnSOD and GPx1 overexpression cause growth inhibition by a thiol-sensitive pathway that is inhibited by N-acetylcysteine.

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Mutations in Mitochondrial DNA are Common and Contribute to the Altered Redox State of Human Melanoma: A Target for Chemotherapeutic Enhancement

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Studies have shown that mutations of mitochondrial DNA (mtDNA), which occur earlier and more extensively than nuclear lesions, subsequently initiate a cascade of events leading to increased ROS production. We found that 3 of 4 tested human melanoma cell lines exhibited specific mtDNA mutations (in cytochrome b, ND1 and 12S rRNA respectively), but none of them were typical UV signature mutation. Measurements of ROS production by dihydrorhodamine123 probing have revealed significant increases of ROS level associated with mtDNA mutation in these melanoma cells. Relative to normal melanocytes, melanoma cells exhibited higher sensitivity to antimycin, a specific complex III inhibitor; mitochondrial transmembrane potential (MMP) were reduced significantly. Our data also showed that intracellular ROS levels were further dramatically elevated with mitochondrial dysfunction by mtDNA depletion, and cells undergo apoptosis after 2-4 passages (varies from cell lines).

In melanoma, the metal chelators, O-phenanthroline (OP) and deferoxamine (DEF), produced significant decreases in MMP as well as a reduction in cell viability, while normal melanocytes were more resistant to OP- and DEF-induced MMP changes. More detailed studies demonstrated that the toxic effects of chelators were preceded by an increase in cellular Bax/Bcl-2 ratio. Notably, the decrease in MMP was rescued by the addition of equi-molar ferrous and ferric ions to cells exposed respectively to OP and DEF.

In toto, our data suggested that melanoma cells possess an alternative survival mechanism at the mitochondrial level in the face of uncoupling of oxidative phosphorylation, and implicate the mitochondria as a potential target for therapeutic intervention in human melanoma. We further hypothesized that treatment with an anti-mitochondrial or mitochondrial-interfering agent, to chemo-sensitize the cells, followed by chemo-therapeutic agents, should

enhance cytotoxic potential. This novel approach may help to overcome the resistance of melanoma to current therapeutic regimens.