Title

Pro-Oxidant Properties of Melanosomal Melanin from Melanoma Origin

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differentiate pathways which may play a role in MnSOD upregulation, we studied both the NFκB and RAR-α pathways. Using a reporter construct, driven by an intronic fragment known to contain the NFκB consensus sequence within the MnSOD gene, we show a contribution from the NFκB element. in addition, studies using dominant negative constructs to RAR-α suggest a decrease in ATRA-mediated increases in MnSOD protein levels. Upregulation of MnSOD is known to occur as a result of changes in cellular redox status. Our studies show a time dependent increase in protein carbonyl and 4-HNE adducted proteins with a decrease in GSH and copper-zinc, superoxide dismutase levels upon ATRA administration. Taken together these studies show alterations in cellular redox status as a result of ATRA administration followed by upregulation of manganese superoxide dismutase which may contribute to chemoresistance in neuroblastoma (Supported by 1P20RR020180).

285

Role of Arginine Metabolizing Enzymes and NADPH Oxidase in Statin-Induced Tumor Cell Cytotoxicity

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Statins (e.g., fluvastatin, simvastatin) inhibit the enzyme HMG-CoA reductase leading to decreased cholesterol synthesis. Statins also elicit vasoprotective, cardioprotective, and chemopreventive/chemotherapeutic effects that are independent of cholesterol-lowering effects (i.e., pleiotropic effects). This study is focused on understanding how statins exert cytotoxicity in breast adenocarcinoma cells (MCF-7 and MDA-MB-231). Both simvastatin and fluvastatin elevated nitric oxide (NO) in MCF-7 cells through stimulation of inducible nitric oxide synthase (iNOS). Statins along with sepiapterin (NOS co-factor) supplementation induced tumor cell death to a greater extent which was partially reversed by 1400W (specific inhibitor of iNOS), mevalonate (inhibitor of HMG-CoA reductase) and H89 (PKA inhibitor). These results suggest the involvement of protein isoprenylation in statin-induced cytotoxic effects in MCF-7 cells. However, statins (fluvastatin and simvastatin) downregulated iNOS and decreased NO production in MDA-MB-231, a metastatic breast cancer cell line. NO induces tumorigenesis in metastatic cancer cells. Both statins suppress the arginase activity in both cell types leading to decreased synthesis of polyamines and decreased cell proliferation. Statin-treatment inhibited superoxide formation via downregulation of the NADPH oxidase enzyme and decreased transferrin receptor expression in breast cancer cells. Isoprenylation inhibitors, especially the geranylgeranyltransferase inhibitor, mimicked statin-mediated effects. in conclusion, we report that statin's ability to regulate arginine metabolizing enzymes and NADPH oxidase is critical to elucidating its mechanism of cytotoxicity in breast cancer cells.

286

Curcumin Induced G2/M Arrest and Apoptosis by Enhancing Superoxide Generation and Inhibiting Akt Activity in Chemoresistant Human Ovarian Cancer Cells

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Curcumin, a major active component of turmeric, is well known to induce apoptosis in several cancer cells, but little is known about its activity in chemo-resistant cells such as cisplatin-resistant ovarian cancer cells. Hence the aim of the present study was to investigate the anticancer properties of curcumin in cisplatin-resistant human ovarian cancer cells in vitro. the results indicated that curcumin inhibited the proliferation of both cisplatin-resistant (CR) and sensitive (CS) human ovarian cancer cells with almost equal sensitivity. Enhanced superoxide generation was also observed in both CR and CS cells treated with curcumin but not in normal ovarian cells. Curcumin induced G2/M phase cell-cycle arrest in CR cells by enhancing the p53 phosphorylation. Curcumin induced apoptosis by the activation of caspase-3 followed by PARP degradation. the phosphorylation of Akt and ERK1/2 was inhibited while the phosphorylation of p38 MAPK and p53 was enhanced. Pretreatment with N-acetylcystiene attenuated the curcumin-induced inhibition of proliferation, caspase-3 activity and PARP degradation. in summary, our results showed that curcumin inhibits the proliferation of cisplatin resistant ovarian cancers through the induction of superoxide generation, G2/M arrest and apoptosis.

287

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Melanoma cells, in comparison to normal melanocytes, contain higher concentrations of redox active species and yet are inefficient in mediating oxidative stress. We attribute these features to changes of intracellular melanosomes and melanin reactivity during the pathogenic process. Eumelanins are redox active pigments containing hydroquinone, semiquinone, quinone and quinone-imine functionalities within their structure. We have previously shown that pro-oxidant properties of synthetic melanin are enhanced by stabilization of the oxidized form of melanin as a result of metal chelation. Chemical studies using DNA clipping activity of isolated melanosomes from different sources indicated enhanced pro-oxidant properties of melanosomes from melanoma origin compared to melanin from normal melanocytes and sepia origin. Electron paramagnetic resonance spin trapping studies confirm these results both in electrochemically polymerized dihydroquinones and in intact human melanoma cells and suggested generation of superoxide and hydroxyl radicals. Ultrastructural investigations of melanosomes by electron microscopy revealed abnormalities in melanin deposition and membrane of the melanoma melanosomes compared to melanocyte melanosomes, which may play a part in higher reactivity of melanoma melanosomes. in related experiments exposure of normal human melanocytes to ultraviolet light radiation –B plus copper and cadmium (but not zinc) salts led to cells that phenotypically resembled dysplastic nevus cells in culture.

288

The Regulatory Subunit Vb of Cytochrome C Oxidase is Required for Malignant Transformation

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Cytochrome c oxidase (COX) is an enzyme complex within the electron transport chain that is central to the regulation of aerobic metabolism. Recently, the expression of the nuclear encoded regulatory COX subunit Vb was found to be markedly increased in several tumor tissues as compared to matched normal tissues. the oncogene *ras* is activated in ~ 30% of all human neoplasms and is known to increase oxygen consumption in immortalized cells. We hypothesized that *ras* may increase oxygen consumption through upregulation of COX Vb expression. We found that ectopic expression of *ras* in immortalized human