potential candidates. A recent study provided evidence for a role of MRP1 in mediating both basal GSH release and the enhanced release of GSH that is observed in cells undergoing apoptosis (Hammond et al. J. Biol. Chem. 282:14337-14347, 2007). To test this hypothesis further, the present study characterized MRP1's role in basal and apoptotic GSH release using HEK293 cells stably transfected with human MRP1, or with MRP2, another member of the MRP family implicated in GSH export. The MRP1 overexpressing cells had significantly higher basal and apoptotic GSH release when induced with either Fas antibody or staurosporine. GSH synthesis rate in the HEK293-MRP1 cells was also markedly enhanced, and this higher GSH synthesis rate may have contributed to a lower level of apoptosis in these cells. Conversely, basal and apoptotic GSH release in the HEK293-MRP2 cells were similar to the vector-transfected cells, suggesting that MRP2 does not accept GSH on its own as a substrate. However, basal and apoptotic GSH release was enhanced with vincristine in the HEK293-MRP2 cells, indicating that MRP2 mediates GSH cotransport. Overall, these results suggest that MRP1 is a major contributor to both basal and apoptotic GSH release, whereas MRP2 is not involved in these processes. The enhanced GSH release and the concurrent decrease of intracellular GSH appear to be necessary for the progression of apoptosis. (Supported in part by NIH grants DK48823, ES01247, and ES07026).

352 P38 MAP KINASE MEDIATES APOPTOSIS THROUGH ACTIVATION OF FOXO3A AND INDUCTION OF BIM TRANSCRIPTION.

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Arsenicals are environmental toxicants. Sodium arsenite induces apoptosis in many different cell types and this apoptosis requires the stress-activated p38 MAP kinase and the pro-apoptotic BCL-2 family protein Bim. We demonstrated previously that p38 induces apoptosis by phosphorylating Bim at serine 65 and activating Bim's apoptotic activity. Here we report that sodium arsenite also stimulates both the transcription and protein expression of Bim. Ectopic activation of p38 is sufficient to stimulate Bim promoter, while blocking p38 signaling suppresses Bim induction. To identify mechanisms by which p38 regulates Bim expression, we examined if sodium arsenite activates FOXO3a, a member of the Fork head family of transcription factors, since Bim promoter contains two putative FOXO3a binding sites. Indeed, our data showed that FOXO3a translocates into the nucleus upon sodium arsenite treatment, indicative of its activation. Finally, RNAi knock down of FOXO3a inhibited Bim promoter activity, Bim protein expression, and apoptosis upon arsenite treatment. Our data reveal a novel apoptotic mechanism in which p38 induces apoptosis by activating FOXO3a which in turn induces the expression of Bim. Together with our previous study of post-translational regulation of Bim by p38, these results demonstrate that p38 induces apoptosis by regulating Bim two dimensionally at both the transcriptional and post-translational levels. Our data is also the first to report p38 as an upstream regulator of FOXO3a.

353 ARSENIC INDUCES DIFFERENT CELL SIGNALING PATHWAYS LEADING TO APOPTOSIS AND CELL CYCLE ARREST IN P53 +/+ AND P53-/- CELLS.

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The effects of arsenic on humans are well documented, but the mechanism of its perplexing paradoxical capability to act as both a carcinogen and a chemotherapeutic agent is unclear. The tumor suppressor gene p53 is crucial in maintaining genome integrity following exposure to environmental agents through the induction of cell cycle arrest, allowing for the DNA repair or apoptosis for cells with irreparable damage. In our previous study, we found As3+ uniquely caused toxicological changes in both genotypes, with more sensitivity in p53 knockout (-/-) cells illustrated by the dose-dependent increase in cytotoxicity and cell cycle alterations. Microarray-based gene expression analysis suggested that As3+ induced different pathways leading to cell cycle arrest and induction of apoptosis in the p53 wildtype (+/+) versus p53 -/- cells. In this study, we hypothesize that As3+-induced apoptosis in p53 deficient cells is through the activation of pro-apoptotic genes Noxa, and Puma. Cultures of p53 +/+ and p53 -/- mouse embryonic fibroblasts (MEFs) were treated with As3+, and cell viability and cytotoxicity were measured by Neutral red or LDH release assays. Total proteins were extracted and Western blot analyses of protein expression levels of Bcl-2 related proteins, including Puma and Noxa, were conducted. We observed differential dynamic changes of these proteins in p53 -/- as compared to p53 +/+ cells. This study suggests that cells with a functioning p53 protein utilize the pathway to mediate cellular defense and thereby mitigate damage. Alteration of p53-dependent cell cycle regulatory genes may play an important role in As3+ induced toxicity as well as carcinogenesis since most cells or tissues harbor the functional p53. Our current study also suggests that As3+ induces apoptotic process in p53 -/- MEF cells through the activation of p53-independent cell signaling pathway.

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354 TITANIUM DIOXIDE NANOPARTICLES INDUCE JB6 CELL APOPTOSIS THROUGH ACTIVATION OF THE CASPASE-8/BID PATHWAY.

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Titanium dioxide (TiO2) nanoparticles are manufactured worldwide in large quantities for use in a wide range of applications. Early studies have shown that the pathogenic effects of TiO2 depend on particle size. However, the molecular mechanisms involved in TiO2-induced cytotoxicity and carcinogenicity have not been clearly defined. The present study investigates TiO2 nanoparticle-induced cytotoxicity and the mechanism involved in this process in a mouse epidermal cell line, JB6 cells. For comparison, an equivalent concentration range of TiO2 fine particles was used in this study. We found that same mass of TiO2 nanoparticles exhibited stronger cytotoxity than that induced by TiO2 fine particles tested using the MTT assay. Moreover, a dual fluorescence dye assay indicated that TiO2 induced cell death through apoptosis in a time- and dose-dependent manner. Fluorescence staining using YO-PRO-1 or fluorescein isothiocyanate (FITC)–dextran dye demonstrated that TiO2 nanoparticles induced remarkable mitochondrial and lysosomal membrane injury. These results suggest the involvement of mitochondria and lysosomes in apoptosis induced by TiO2 nanoparticles. The signaling pathway involved in TiO2 particle- induced apoptosis was also investigated. Western-blot analysis showed an activation of caspase-8, caspase-3, Bid and Bax, and a decrease of Bcl-2, in JB6 cells treated with TiO2 particles. In conclusion, we have determined that caspase-8/Bid signaling may play a major role in TiO2–induced apoptosis and that mitochondrial and lysosomal injury may also be involved. Unraveling the complex mechanisms associated with these events may provide insights into TiO2-induced pathogenicity.

355 A COMPARATIVE EFFECT OF CADMIUM ON NON-TUMOR AND TUMOR DERIVED OSTEOBLASTIC CELLS.

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Cadmium is a known environmental and occupational toxin. There are multiple sources of cadmium, however, increasing discard of electronic products (e-waste) containing heavy metals makes cadmium exposure a growing public health concern. Humans are typically exposed to cadmium by consuming contaminated food or water, or smoking cigarettes. Exposure to cadmium is linked to bone diseases, including osteoporosis and osteomalacia. Previous studies in our lab have shown that cadmium induces apoptosis in the osteosarcoma cell line Saos-2 through a Caspase-3 dependent pathway. This study examined the effects of CdCl2 exposure on mouse non-tumor MC3T3-E1 osteoblastic cells compared to tumor derived Saos-2 cells. Our hypothesis is that non-tumor derived osteoblasts will react in a similar manner to tumor derived osteoblasts and undergo apoptosis when exposed to CdCl2. A cytotoxicity profile using the MTT assay was created for both cells lines exposed to 0-100 μM CdCl2 for 0-48 hours. An ApoPercentage kit was used to detect the translocation of phosphatidylserine to the outer leaflet of the cell membrane which is an indicator of apoptosis. The cytotoxicity profile indicated that MC3T3-E1 cells were more sensitive to CdCl2 exposure than Saos-2 cells, with EC50 values of 73 and 107 μM CdCl2 at 24 hours, respectively. Apoptotic induction was seen in both cell lines by 48 hours in response to 10 μM CdCl2 treatment. These results support our previous research demonstrating cadmium induced apoptosis in tumor derived osteoblasts and extend the work to also demonstrate cadmium induced apoptosis in non-tumor derived osteoblasts. Our long term goal is to develop an osteotoxicity model that will provide insight into how cadmium directly impacts bone and help in the creation of future treatments and prevention of bone diseases. Research funded by NIH-INBRE grant #P20RR016454.

356 LEAD INDUCES APOPTOSIS IN HUMAN LEUKEMIA (HL-60) CELLS *VIA* OXIDATIVE STRESS.

C. G. Yedjou and P. B. Tchounwou. *Biology, Jackson State University, Jackson, MS.*

Lead is a multi-targeted toxicant that affects many organ systems including; the gastrointestinal tract, hematopoietic system, cardiovascular system, central and peripheral nervous systems, immune system, and reproductive system. There are many published studies that have documented the adverse effects of lead in children and the adult population. Previous in vitro studies in our laboratory have shown that lead nitrate induces cytotoxicity to HepG2 cells in a dose-dependent manner.

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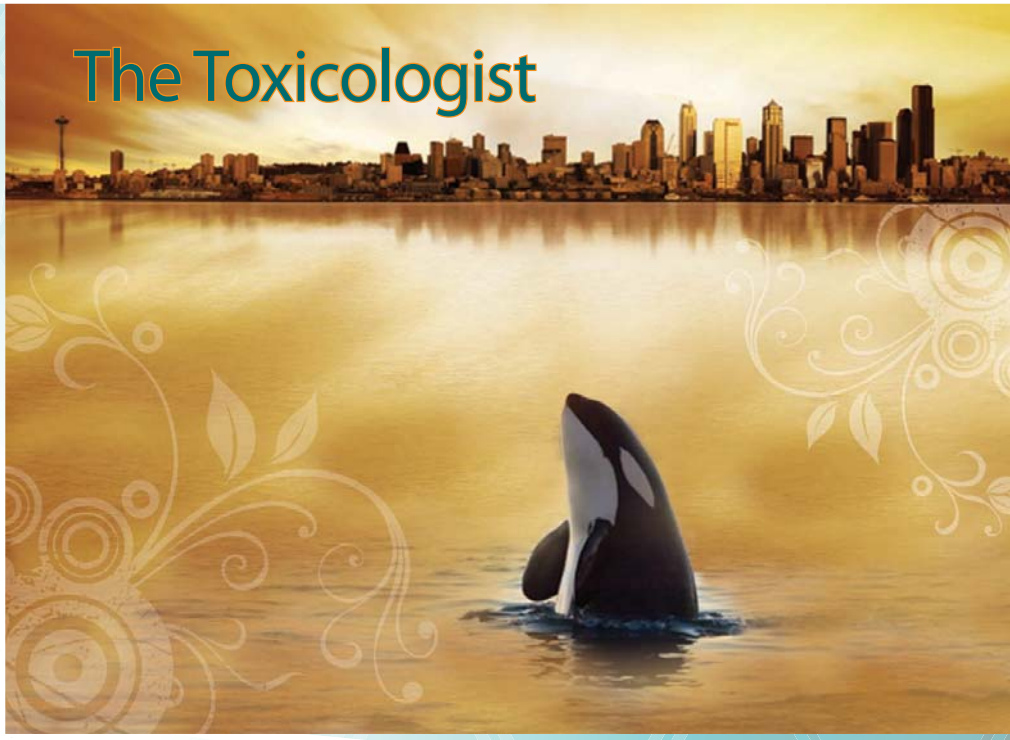

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