1479 Comparative Study of Microcystin-LR on PP2A in Various Tumor Cell Lines

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Protein phosphatase 2A (PP2A) is distinctive in its key role in cell and has been recognized as a tumor suppressor protein. Microcystin-LR (MCLR) produced by blue-green algae is a potent tumor promoter and the major mechanism has been known for its inhibition on PP2A by covalent binding to S269 of the PP2A/C as some cancer therapeutic medicine does for anti-cancer. Our interest is how MCLR affects PP2A and what role PP2A plays in MCLR treated cancer cell lines. Human hepatoma SMMC7721,laryngeal epithelial cells Hep2 and lung adenocarcinoma cell line A549 were treated with 0.1~10 μmol/L MCLR for 0~48h. The results show that MCLR entered into the cells and conjugated with PP2A/C. PP2A activities were all inhibited and A549 was the most sensitive cell line. The phosphorylation of PP2A/C at Y307, which is negatively related with PP2A activities, was increased in three cell lines but methylation of PP2A/C at L309 also responsible for PP2A activity was only changed in Hep2. Interaction between PP2A/C and PP2A regulator α4, which indicates a compensate mechanism for PP2A activity, was decreased in Hep2 and A549 but increased in SMMC7721. The disorganization of cytoskeleton can be found in three cell lines but the involvement of cytoskeleton-associated proteins dephosphorylated by PP2A were different, both p-VASP and p-Hsp 27 were increased in SMMC7721 and A549 but only p-VASP in Hep2. The change of cell proliferation by MCLR for more than 24 h was only observed in A549 but not in the others. In summary, our study suggests that MCLR can bind with PP2A/C and inhibit its activity in three cell lines but the underling mechanisms and subsequent affected PP2A substrates and cellular outcome were different. The study will be meaningful in further elucidating the mechanisms of MCLR affecting PP2A as well as the regulatory role of PP2A in tumor cell. Meanwhile, the information may be helpful in understanding the mechanisms of cancer drugs that act on PP2A.

1480 Exposure of the Human HepaRG Liver Cell Line to Pyrrolizidine Alkaloids Results in a Gene Expression Profile Characteristic for Genotoxic Carcinogens

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Pyrrolizidine alkaloids (PAs) are secondary metabolites found in many plant species and can be present as contaminants in food, herbal teas, plant food supplements, and animal feed. A large number of PAs are toxic not only to livestock and wildlife but also to humans. Hepatotoxicity, genotoxicity, and carcinogenicity are the main toxicities observed in experimental animals treated with PAs. However, there is little direct evidence for mutagenicity and carcinogenicity of PAs in humans and epidemiological data are lacking. The present study aimed to contribute to a better evaluation of the genotoxic and carcinogenic risks of PAs for humans. To that end, the human hepatoma cell line HepaRG was exposed *in vitro* for 72 hours to six PAs (riddelliine, retrorsine, echimidine, monocrotaline, lasiocarpine, senkirkine), and the genotoxic carcinogen benzo[a]pyrene, the non-genotoxic carcinogen bis(2-ethylhexyl) phthalate, and the non-carcinogen mannitol as controls. Upon exposure, RNA was isolated and effects on whole genome mRNA expression were analysed using DNA microarrays. Pathway analysis showed that several processes involved in genotoxicity, including DNA damage response, p53 pathway and cell cycle checkpoints, were modulated by the PAs. Comparison of the array data with transcriptome data obtained by others upon exposure of HepaRG to various model hepatocarcinogens showed that each of the PAs used in the present study generated a gene expression profile that is characteristic for genotoxic carcinogens.

1481 Punicic, Jacaric, and Lithocholic Acid Induce ER Stress, Mitochondrial Dysfunction, and Autophagy in Human Prostate Cancer Cells

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Punicic (PA) and jacaric (JA) acid are naturally-occurring fatty acids found in pomegranate and Jacaranda seed; lithocholic acid (LCA) is a secondary bile acid that undergoes enterohepatic circulation. Each of these compounds is selectively toxic to various human cancer cells and we have previously shown these compounds to induce apoptotic and necrotic death of prostate cancer cells, but not of normal prostate epithelial cells. Our objectives were to determine the involvement of ER stress, mitochondrial dysfunction and autophagy in the death triggered by these compounds in prostate cancer cells with various tumorigenic characteristics. PA reduced androgen-dependent LNCaP and -independent PC-3 cell viability with similar potency (IC50 18-27 μM), whereas JA was more toxic to these cells (IC50 8-10 μM). LCA killed PC-3 and DU-145 cells with IC50 values of 30-32 μM. All three compounds induced ER stress markers CHOP and phosphorylated eIF2α at relatively toxic concentrations (30-50 μM) after 24 h. The ER stress inhibitor salubrinal slightly reduced the cytotoxicity of PA, but exacerbated that of JA in the 1-10 μM range in LNCaP and PC-3 cells, with no effect at more toxic fatty acid concentrations. The toxicity of LCA was slightly enhanced by salubrinal in PC-3 and DU-145 cells. Inhibition of CHOP by siRNA had no effect on the toxicity of any of the compounds, regardless of cell type. PA and JA increased levels of reactive oxygen species in LNCaP and PC-3 cells within 1 h, as well as loss of mitochondrial outer-membrane potential (> 30 μM) and pretreatment of cells with the antioxidant tocotrienol protected against fatty acid-mediated cytotoxicity. We suggest that ER stress is a response to, but not essential for PA- and JA-mediated cell death, which appears to be triggered by ROS-induced mitochondrial dysfunction. LCA increased autophagy markers (GFP-LC3B punctuation and conversion of LC3BI to LC3BII) in PC-3 cells (but not in autophagy-deficient DU-145 cells). The autophagy inhibitor bafilomycin A1 increased the toxicity of subtoxic concentrations of LCA (3-10 μM), suggesting the autophagic response of PC-3 cells to LCA is protective in nature.

1482 Strategies to Protect Against Triptolide Based on Its Toxic Mechanism

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Triptolide is a major active ingredient of the Chinese herb *Tripterygium wilfordii* Hook f. (TWHF) and has risen to wide acceptance on its remarkable performance in recent scientific investigations, showing multiple biological activities, such as anti-inflammatory, anti-fertility, antineoplastic and immunosuppressive activities. However, the clinical applications of triptolide are limited by its narrow therapeutic window and severe toxicity, particularly nephrotoxicity, hepatotoxicity and cardiotoxicity. In previous study, we reported that apoptosis induced by triptolide was mediated by oxidative stress along with the damage of NF-E2-related factor 2 (Nrf2) system, which is the main antioxidant defense system. Meanwhile, autophagy, known as a cellular self-digestion process, is also involved in the effect of triptolide. Interestingly, Both Nrf2 system and autophagy are activated in a short time, while suppressed in a longer period of time. As basic defense mechanism, Nrf2 pathway and autophagy are adaptively activated in response to oxidative stress in a short time, while are overwhelmed by increasing stress that trigger the toxicity subsequently. Our lab focus on the role of Nrf2 and autophagy in triptolide-induced toxicity. Fortunately, activation of Nrf2 plays a protective role against triptolide-induced cytotoxicity in BALB/C mice, NRK-52E cells, HepG2 cells and H9c2 cells. Additionally, our recent study suggests that autophagy can selectively eliminate abnormal mitochondria damaged by reactive oxygen species (ROS). The induction of autophagy by rapamycin could ameliorate the detrimental effects induced by triptolide in BALB/C mice. Collectively, Nrf2 and autophagy may be the potential target for modulating toxicity induced by triptolide. More specific and low toxic agonists targeting Nrf2 and autophagy need to be further studied before the clinical application against triptolide-induced toxicity.