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Induction of Apoptosis by Pectenotoxin-2 is Mediated with Induction of DR4/DR5, Egr-1 and NAG-1, Activation of Caspases and Modulation of Bcl-2 Family in p53-deficient Hep3B Hepatocarcinoma Cells

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Pectenotoxin-2 (PTX-2), isolated from marine sponges has been reported to display significant cytotoxicity to p53-deficient cancer cell lines. In this study, we compared the anti-cancer activity of PTX-2 in order to further test the status of p53 using two well-known hepatocarcinoma cell lines. MTT assay indicated that Hep3B cells were highly susceptible whereas HepG2 cells were more resistant to this compound, which was connected with the induction of apoptotic cell death in p53-deficient Hep3B cells but not in HepG2 cells. The apoptosis induced by PTX-2 in Hep3B cells was associated with the down-regulation of anti-apoptotic Bcl-2 members and IAP family proteins, up-regulation of pro-apoptotic Bax protein and DR4/DR5, and mitochondrial dysfunction. PTX-2 activated caspases and the blockade of the caspase-3 activity by caspase-3 inhibitor prevented the PTX-2-induced apoptosis in Hep3B cells. Additionally, the transcription factor Egr-1 gene was transcriptionally activated and the levels of NAG-1 protein were also elevated in PTX-2-treated Hep3B cells. This present study suggest that PTX-2 may be a good candidate for development as a potential anti-tumorigenic agent in p53-deficient tumors. [This research was supported by a grant (M2007-11) from Marine Bioprocess Research Center of the Marine Bio 21 Center funded by the Ministry of Maritime Affairs & Fisheries, Republic of Korea.]

Key words: Pectenotoxin-2, apoptosis, EGR-1, NAG-1, p-53, Hep3B, HepG2

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Induction of Apoptosis by Sanguinarine in C6 Rat Glioblastoma Cells is Associated with the Modulation of Bcl-2 Family and Activation of Caspases through Down-regulation of ERK and Akt

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Sanguinarine, a benzophenanthridine alkaloid derived from the root of *Sanguinaria canadensis* and other poppy fumaria species, is known to exhibit anti-microbial, anti-inflammatory and anti-oxidant properties. In the present study, it was investigated the possible mechanisms by which sanguinarine exerts its anti-proliferative action in cultured C6 rat glioblastoma cells. Exposure of C6 cells to sanguinarine resulted in growth inhibition and induction of apoptosis in a dose-dependent manner as measured by MTT assay, fluorescence microscopy, agarose gel electrophoresis and annexin-V-based assay. The increase in apoptosis by sanguinarine was associated with the up-regulation ofpro-apoptotic Bax expression as well as down-regulation of anti-apoptotic Bcl-2 and IAP family members such as XIAP and cIAP-1. Sanguinarine treatment induced the proteolytic activation of caspases and inhibitor of caspase-activated DNase/DNA fragmentation factor 45 (ICAD/ DFF45), which was associated with a concomitant degradation of PARP and PLC-γ1 protein and DNA fragmentation. z-DEVD-fmk, a caspase-3 specific inhibitor, blocked caspase-3 activation, PARP degradation, DNA fragmentation, and increased the survival rate of sanguinarine-treated C6 cells. Moreover, sanguinarine triggered the down-regulation of phosphorylation of extracellular regulated kinase (ERK) and Akt, and PD98059, a specific inhibitor of ERK, and LY294002, a specific inhibitor of Akt, but not SB203580 (an inhibitor of p38) and SP600125 (an inhibitor of JNK), sensitize sanguinarine-induced apoptosis. Taken together, it is suggested that sanguinarine can be a promising anti-cancer agent for glioblastoma cells and the modulations of Bcl-2 and IAP family, activation of caspase through down-regulation of the ERK and Akt signaling pathway may play critical roles in sanguinarine-induced apoptosis in C6 cells.

Key word: Apoptosis, sanguinarine, caspase, ERK, Akt