

Regulation of apoptosis, cell cycle progression and differentiation by SHetA2 in renal cancer cells is associated with alterations in Bcl-2, Cyclin D1 and E-Cadherin proteins.

Tongzu Liu, Patience Masamha, Shylet Chenedza, Stan Lightfoot, Doris M. Benbrook.
Oklahoma University Health Science Center, Oklahoma City, OK

Background and objective: The lead flexible heteroarotinoid (Flex-Het), SHetA2, is a promising compound for pharmaceutical development because it inhibited growth of each of the National Cancer Institute's 60 tumor cell lines, with the greatest activity against renal cancer cell lines. SHetA2 also induces apoptosis, differentiation and anti-angiogenesis. Apoptosis is induced to a much greater extent in cancer cells in comparison to normal cells. The objective of this study is to clarify the molecular mechanism of SHetA2 on renal cancer cell growth and differentiation.

Methods: The renal cancer (Caki-1) and normal human kidney proximal epithelial cell line (HK-2) cell lines, and primary cultured renal tubular epithelial cells (RTC) were treated with SHetA2 or solvent only. Flow cytometry was used to measure apoptosis in cells stained with Annexin-V-FITC and to measure cell cycle profile in cells stained with propidium iodide. The MTS assay was used to compare the cytotoxic effects between cancer and normal cells. E-cadherin mRNA levels were studied with real time rt-PCR. Western blotting was used to evaluate the expression of Bcl-2 family member proteins, caspase3, PARP-1 cleavage, Cyclin D1, E-cadherin and β -catenin. E-Cadherin/ β -catenin complex formation was studied with co-immunoprecipitation. *In vivo* evaluation of SHetA2 anti-tumor activity and induction of differentiation were studied in Caki-1 xenografts from nude mice with tumor measurements and H&E staining, respectively.

Results: Caki-1 cancer cells were much more sensitive to growth inhibition and apoptosis induced by SHetA2 in comparison to non-transformed HK-2 cells and RTC. Bcl-2 was down-regulated by SHetA2 in Caki-1 cells but not in HK-2 cells, while Bax expression was not changed in any of the cell types. Caspase3 activation and PARP-1 cleavage were significantly increased with the time of SHetA2 treatment. SHetA2 treatment induced G0-G1 arrest in Caki-1 and HK-2 cells in association with decreased cyclin D1 protein levels. *In vivo*, SHetA2 inhibited tumor growth and induced a more differentiated phenotype and tubule formation in two independent Caki-1 xenograft models. Expression of E-Cadherin mRNA and protein were significantly increased with SHetA2 treatment. SHetA2 treatment also increased the levels of β -catenin that co-immunoprecipitated with the anti-E-Cadherin antibody. Membrane protein isolation and western blotting also confirmed that both E-Cadherin and β -catenin proteins were increased in SHetA2 treated cell membranes.

Conclusions: The results of this study indicate that SHetA2 treatment can induce apoptosis, cell cycle arrest, and differentiation in renal cancer cells. The mechanism of apoptosis involves reduction of Bcl-2 protein levels, caspase3 activation and PARP-1 cleavage. Differential reduction of the Bcl-2 protein appears to be associated with the mechanism of the differential effects on cancer versus normal cells. Cell cycle arrest is associated with cyclin D1 down-regulation. Induction of differentiation was associated with up-regulation of E-Cadherin and E-Cadherin/ β -catenin complex formation.