SHetA2 Effects on Thymidine Phosphorylase and NF-κB activity.

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INTRODUCTION: Current cancer chemotherapy strategies are toxic and demonstrate limited efficacy, especially in ovarian and renal cancers. Therefore, new molecular targets are needed for drug development. Thymidine phosphorylase (TP) is a potential target because it is overexpressed in ovarian cancer, induces angiogenesis, and suppresses apoptosis induced by hypoxia and chemotherapeutic drugs. TP catalyzes reversible phosphorolysis of thymidine, deoxyuridine and their analogues to their respective bases and 2-deoxyribose-1-phosphate. Microarray analysis indicated that TP mRNA expression is inhibited by the Flexible Heteroarotinoid (Flex-Het) drug, SHetA2, which targets the mitochondria leading to increased ROS production. NF-κB is another potential molecular target that is constitutively activated in many cancers, is regulated by the redox state of the cell and is implicated in regulation of TP expression.

HYPOTHESIS: It was hypothesized that SHetA2 effects on mitochondria alter the cellular redox state leading to inhibition of NF-κB activity, TP expression, and subsequent apoptosis.

METHODS: The effects of SHetA2 on TP expression and apoptosis in ovarian (name cell lines A2780, etc) and renal cancer (Caki) cell lines and normal endometrial cells were evaluated with Western blot, real time rt-PCR and flow cytometric analyses. SHetA2 effects of on NF-κB activity were measured in ovarian cancer cells using NF-κB specific reporter and gel retardation assays.

RESULTS: SHetA2 inhibited TP mRNA and protein expression in all cancer cell lines in dose and time-dependent manners. Inhibition was observed as early as 2 hours and was maximal by 24 hours. The lowest concentration that inhibited TP expression over 24 hours of treatment was 3 μM. Induction of apoptosis by SHetA2 could be observed within 2 hours. SHetA2 inhibited TP expression in the same dose and time frame as it induced apoptosis in both ovarian and renal cancer cell lines. Inhibition of both constitutive and TNF-induced NF-κB activity by SHetA2 was both time and dose dependent. SHetA2 affected NF-κB activity by altering the DNA binding activity of NF-κB transcription factors.

CONCLUSIONS: SHetA2 inhibits TP mRNA and protein expression in correlation with induction of apoptosis. Studies are planned to validate the role of TP in apoptosis by determining if inducible expression of exogenous TP cDNA can block SHetA2-induced apoptosis. SHetA2 also regulates NF-κB DNA-binding and transactivation activities. Current studies are underway to determine which of the five members of NF-κB family members are involved in SHetA2 action on ovarian cancer cells. Future studies are planned to evaluate SHetA2 effects on the TP gene promoter and to determine if NF-κB plays a role in the mechanism of TP inhibition.