SHetA2 targets cyclin D1 for proteasomal degradation through a GSK3β-independent mechanism leading to G1 cell cycle arrest.

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Abstract

INTRODUCTION AND OBJECTIVE: Flexible Heteroarotinoids (Flex-Hets) are promising chemoprevention drugs that regulate growth, differentiation, apoptosis and angiogenesis. SHetA2 was chosen as the lead Flex-Het compound because it induced the highest level of growth inhibition against a broad spectrum of cancer cell lines with reduced effects against normal cells. Induction of apoptosis was shown to contribute to the SHetA2 mechanism of cancer cell growth inhibition.The aim of this project was to investigate whether cell cycle arrest also contributes to SHetA2 growth inhibition in ovarian cancer cell lines.

HYPOTHESIS: SHetA2 induces cell cycle arrest by altering specific cell cycle regulatory proteins.

METHODS: The proteasomal inhibitor MG132 and GSK3β inhibitors were used to treat ovarian cancer cell lines, A2780 and Skov-3 in the presence or absence of SHetA2. The cell cycle profile was determined using Flow cytometry with PI staining. Western blots analysis was used to investigate expression of cyclin D1, Cyclin E2, p21, GSK3β and the phosphorylation of cyclin D1 and GSK3β. Cyclin D1 mRNA expression was quantified by real time RT-PCR.

RESULTS: SHetA2 induced G0-G1 cell cycle arrest in a time-dependent and dose-responsive manner. This G0-G1 arrest was transient and fully reversible after how long? upon removal of the drug. Of the G0-G1 proteins examined, only cyclin D1 protein levels were significantly altered, with the protein disappearing from the nucleus earlier than in the cytoplasm. Cyclin D1 was also reduced at the mRNA level. MG132 blocked the G0-G1 cell cycle arrest and cyclin D1 degradation. Although SHetA2 increased cyclin D1 Thr286 phosphorylation in the ovarian cancer cell lines, GSK3β inhibitors did not abrogate the effects of SHetA2-induced cell cycle arrest and cyclin D1 degradation. Instead, SHetA2 inactivated GSK3β by phosphorylating the protein at Ser9.

CONCLUSION: SHetA2 induced G0-G1 cell cycle arrest is dependent on proteasomal degradation of cyclin D1 through classical cyclin D1 Thr286 phosphorylation, and proteasomal degradation. Ubiquitination is implicated in the mechanism. Our data agrees with current findings that question the role of GSK3β mediated cyclin D1 degradation. Ongoing studies are being conducted to investigate the mechanism of SHetA2 inhibition of Cyclin D1 mRNA expression and the kinase involved in cyclin D1 phosphorylation.