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Cross-talk Between Notch and Akt/mTOR Signaling in Colon Cancer Cells

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Background: Colorectal cancer is the third most frequently diagnosed cancer and also the third leading cause of cancer-related deaths in men and women in the United States. The medicinal plant, *Withania somnifera*, is used extensively in Asian herbal medicines to treat a variety of ailments, including cancer. We determined that withaferin-A (WA), a major bioactive compound in *W somnifera*, exhibits potent anti-cancer effects on colon cancer cells, underscoring the need to study the molecular mode of action of WA to ascertain its potential clinical merit.

Methods: We studied the effect of WA on Notch signaling using three colon cancer cell lines (SW-620, SW-480 and HCT-116). Cell viability and apoptosis were determined using trypan blue exclusion assay and annexin V-FITC staining, respectively. Western blot analysis was performed to determine WA-mediated modulation in the expression of Notch signaling proteins. To determine whether WA transcriptionally regulates Notch and its downstream genes, we isolated total RNA and subjected it to reverse transcriptase polymerase chain reaction (RT-PCR) to determine WA-mediated modulation of mRNA expression of Notch and its downstream genes. We also studied the overexpression and knockdown of Notch in the colon cancer cells to delineate downstream effects.

Results: Our results suggest that WA inhibits cell proliferation and induces apoptosis in colon cancer cells (SW-480, SW-620 and HCT-116). While dissecting the mechanism of action of WA on colon cancer cells, we found that WA inhibited Notch-1 signaling, which resulted in the downregulation of pAkt, and Bcl-2 expression. In addition, inhibition of mTOR signaling by WA resulted in the downregulation of pS6K and p4E-BP1 expression in SW-480, SW-620, and HCT-116 colon cancer cells. We observed that WA activates caspase-3 and PARP cleavage, suggesting that it triggers the pro-apoptotic machinery in

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colon cancer cells. Interestingly, WA caused a strong mitotic catastrophe by arresting the colon cancer cells in the G2/M phase of cell cycle. Furthermore, inhibition of Notch-1 by siRNA resulted in the downregulation of Akt and mTOR signaling pathways in colon cancer cells.

Conclusions: These results suggest that WA inhibits cell viability and induces apoptosis in colon cancer cells. Our observations indicate that the biologic effects of WA are due to inhibition of Notch and its downstream signaling molecules. It is therefore reasonable to study WA for its potential as a targeted therapy for colon cancer.