

## Activated Guanylate Cyclase C and its Effects on Intestinal Cancer Cell Proliferation

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**BACKGROUND:** There is an inverse relationship between the worldwide incidence of colon cancer and infection with enterotoxigenic *Escherichia coli* (ETEC). ETEC produce a heat stable enterotoxin agonist of the intestinal epithelial receptor guanylate cyclase C (GC-C). Although the GC-C signaling pathway mediates intestinal secretion, it also plays a role in intestinal epithelial cell (IEC) proliferation and perhaps cancer susceptibility. Based on studies that found a loss of endogenous GC-C ligands guanylin (Gn) and uroguanylin expression in adenomas as well as elevated IEC proliferation in Gn-null mice, it was hypothesized that activation of GC-C inhibits IEC proliferation. We investigated the effects of GC-C activation on proliferation in both a non-transformed cell line (IEC-18) and a colon cancer cell line CaCo-2 BBE (BBE) as well as involvement of cell cycle regulatory molecules ERK, PKC- $\alpha$  and Retinoblastoma (Rb). **METHODS:** IEC-18 cells lack endogenous GC-C, and thus were transduced with a retroviral construct expressing GC-C. Experimental groups of the transduced IEC-18 cells, BBE cells, or PKC- $\alpha$  inhibited BBE cells were stimulated with Gn and/or serum. Cell numbers and proliferation was measured via tetrazolium salt metabolism and ELISA-based BrdU incorporation assay. Western blotting was used to measure phosphorylated ERK and phosphorylated Rb. **RESULTS:** IEC-18 cells did not respond to GC-C activation with respect to cell cycle changes. BBE cells treated with Gn 1 hour prior to serum stimulation exhibited a statistically significant decrease in proliferation as compared to cells only stimulated with serum (P value = 0.0056). BBE cells stimulated with Gn prior to serum stimulation exhibited a marked decrease in pRb expression. While PKC- $\alpha$  inhibition did not affect proliferation with respect to Gn stimulation, we noted that pretreatment of BBE cells with Gn blocked serum-induced nuclear ERK activity and accumulation of nuclear cyclin D1. **CONCLUSIONS:** The ineffectiveness of IEC-18 as a model for this signaling pathway may indicate a role for the GC-C pathway only in times of overexpression, such as cancer. The Gn-stimulated CaCo-2 BBE decrease in proliferation supports this. While the role of PKC- $\alpha$  in the antiproliferative effects of this pathway are unlikely, the ability of GC-C signaling to block mitogen activated protein kinase activity and subsequent effects on cell cycle proteins provides an avenue for further study.