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Project Title

Calpains 8 and 9 are Expressed in the Murine Intestines and Regulated by wnt3a in Colon Cancer Cells

Abstract

Objectives

Calpains are calcium dependent proteases that are reported to be implicated in processes such as signal transduction, cytoskeleton remodeling and apoptotic cell death. Upregulation of calpains have been seen in tumors and cancers including gastric cancer, breast, and ovarian cancer suggesting its activity in cancer development. However, their activity in colon cancer remain unknown. To investigate a potential role in colon cancer, we have stimulated human colon cancer cell line with WNT3A, Tumor Necrosis Factor alpha (TNF) and both for 24 hours. After WNT stimulation, the relative mRNA expression of calpain-8 and calpain-9 significantly increased, whereas TNF did not affect the expression of calpain-8 and 9. However, when stimulated with both; TNF has suppressed the ability of WNT to upregulate, suggesting an interaction between the two. We also found that calpain-9 to be highly expressed in murine colon through our immunohistochemical analysis. Taken together, our result suggest a possible role for calpains-8 and 9 that warrants further investigation.

Methods

Quantitative real-time PCR was used to analyze our stimulated RKO (colon cancer) cells. Slides of wildtype mouse colon tissue were treated with specific calpain antibodies to analyze calpain expression

Results

Wnt3a increased the expression of calpains 8 and 9 in RKO cells. The expression of calpains 8 and 9 in colon and stomach was made evident through our IHC results.

Conclusions

After wnt3a stimulation, the relative mRNA expression of calpains-8/9 increased by 18-fold. In contrast, stimulation with TNF did not affect calpain 8/9 expression in colon cancer cells. Interestingly, concurrent stimulation of TNF with wnt3a abrogated the ability of wnt3a to upregulate calpains 8/9, suggesting an interaction between the wnt3a and TNF signaling pathways. No significant change in calpains 1/2 were found with any stimulation, consistent with their known ubiquitous expression in most tissues. Furthermore, both calpains 8/9 were found to be expressed in the epithelium of murine colon as well as stomach. Previously reports describe an increase of calpain-2 activity in colorectal adenocarcinomas compared to normal mucosa While the expression of calpain-9 was reported to be decreased in gastric cancer, the expression in colorectal cancer is unknown. Further research is needed to determine the expression and consequence of calpain-8/9 in the normal colon and colon cancer.

Summary Statement

The roles of calpains in colon cancer is unknown, therefore we looked at their genetic expression in RKO cells and their localization in wildtype mouse colon.

Help Received

All the materials were provided by the research facility at University of Southern California. The project was designed by principle investigator, Dr.Shao. I performed the experiments under the supervision of mentor.