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Expression of IRBIT Along the Rat Gastrointestinal Tract

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IRBIT EXPRESSION ALONG THE GASTROINTESTINAL TRACT

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Introduction

IRBIT (inositol-1,4,5-trisphosphate (IP3) receptors binding protein released with IP3) is a powerful regulator of fluid and electrolyte transport in the pancreatic duct and other epithelial cells (1). It functions by competing with IP_3 for binding on the IP_3 receptor, a calcium channel found in the endoplasmic reticulum of the cell (figure 1). Calcium is important for the regulation of fluid secretion in the pancreatic duct and epithelial cells. Most research focuses on what IRBIT regulates, but much less is known about what regulates IRBIT.

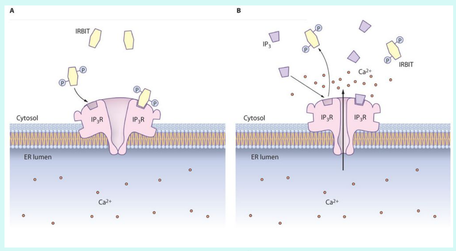


Figure 1. IRBIT competes with IP_3 to inhibit Ca^{2+} releasein the membrane of the endoplasmic reticulum. (Chi-un Choe and Barbara E Ehrlich, "The Inositol 1,4,5-Trisphosphate Receptor (IP3R) and Its Regulators: Sometimes Good and Sometimes Bad Teamwork" *Science's STKE* 28 (2006).

IRBIT is found in two isoforms, long and short. The two are 80% homogenous, and have nearly identical C terminuses, which is important for multimer formation. The N terminus is also similar, containing a serine rich region important for IP_3 receptor binding. However, the long form also contains a unique appendage of unknown function, which has actually been shown to prevent binding to the receptor. Investigation into the distributions of long and short IRBIT throughout the tissues may give some insight into the differences in function and regulation of the two isoforms.

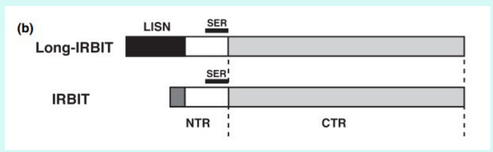
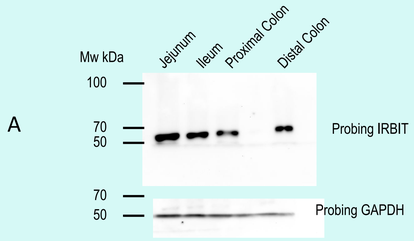


Figure 2. Comparison of the long and short versions of IRBIT. The LISN domain is long-IRBIT specific (Ando Hideaki, et al, "An IRBIT Homologue Lacks Biding Activity to INositol 1, 4, 5-triphosphate receptor due to the unique N-terrminal appendage", *Journal of Neurochemistry* 109 (2009).

This Study aims to investigate the differential expression of IRBIT along different segments of the GI tract, as well as to distinguish between the two isoforms in those tissues using PCR on cDNA library from the different parts of the GI tract.

Results

We have harvested stomach, duodenum, jejunum, ileum, proximal and distal colon from male rats. Using a phosphate buffered saline solution containing zero calcium, we have selectively isolated the epithelial cells from each of the aforementioned segments of the GI tract. A western blot was performed on each sample in order to determine IRBIT expression in each section of the GI tract.



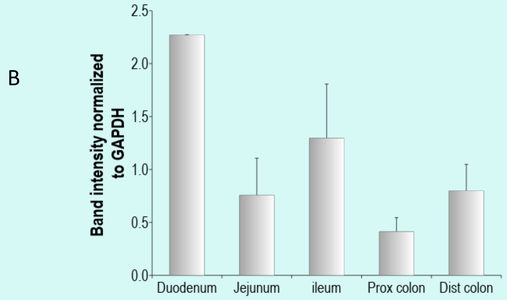


Figure 3. Intestinal epithelial cells from the duodenum, jejunum, ileum, proximal and distal colon were isolated and IRBIT expression was tested by immunoblot as shown in panel A. Panel B represents the result summary of three experiments similar to A except for duodenum where n = 2. Bars represent mean ± standard error of bands intensity.

Methods

Using Trizol-chloroform we have selectively isolated the total mRNA from the pancreas, liver, stomach, duodenum, jejunum, ileum, proximal and distal colon of male rats.

cDNA library Creation

1. Using total mRNA from tissues, a reverse transcriptase and random hexamers or oligodT, we will generate a complementary DNA library according to manufacturer recommendation.

Quantitative Real Time Polymerase Chain Reaction (Rt-PCR)

1. Use cDNA library and unique primers for the isoforms
2. Use Real time PCR we will quantify the expression of the two isoforms by comparing expression to an internal standard

Future Direction

In future experiments, we plan to create the above mentioned cDNA library from the mRNA samples that were extracted. We will then perform quantitative real time PCR in order to determine the distribution of short and long IRBIT throughout the tissues which may help determine the function and regulation of these two isoforms.

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- Choe, Chi-un and Barbara E. Ehrlich. "The inositol 1,4,5-trisphosphate receptor (IP3R) and its regulators: sometimes good and sometimes bad teamwork." *Science's STKE : signal transduction knowledge environment* 2006 363 (2006): re15 .
  Homologue Ando, Hideaki, et al. "An IRBIT Lacks Binding Activity to Inositol 1,4,5-Trisphosphate Receptor Due to the Unique N-Terminal Appendage." Journal of Neurochemistry, vol. 109, no. 2, 2009, pp. 539–550., doi:10.1111/j.1471-4159.2009.05979.x.