XIAP MEDIATED DEGRADATION OF NOTCH PROTEIN AS A POTENTIAL MECHANISM IN PATHOGENESIS OF INFLAMMATORY BOWEL DISEASE

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Introduction

Inflammatory bowel disease (IBD) is characterized by chronic inflammation of the digestive tract and damage to the intestinal epithelium requiring constant regeneration and differentiation of the absorptive and secretory cells. The NOTCH pathway is known to regulate differentiation of intestinal epithelial cells and dysregulation of the NOTCH pathway has recently been implicated in IBD pathology. Genome-wide association studies have shown that a subset of patients with XIAP deficiency develop very early onset or adult inflammatory bowel disease (IBD). The transactivation domain of NOTCH has been reported to directly interact with the RING domain of XIAP to prevent ubiquitination of XIAP.

Hypothesis

We are challenging the outcome of the NICD and XIAP interaction. We have identified XIAP as a candidate molecular factor that targets NOTCH for proteasome degradation through its E3 ubiquitin-protein ligase domain. Our overall hypothesis is that patients with loss of function mutations in XIAP will show elevated levels of NOTCH activity, thereby perturbing cell fate decisions and increasing their risk of IBD and other clinical manifestations.

Methods

Co-immunoprecipitation and Western blot analysis was used to confirm the association between XIAP and NOTCH1 intracellular domain (NICD1) or SMAC. Steady state protein and RNA levels of Notch receptors and target genes in a parental HCT116 colorectal carcinoma and the genetically engineered HCT116 XIAP knockout cell line were determined using Western blot and quantitative real-time PCR analysis. We assessed true half-life levels of NOTCH1 in HCT116 and HCT116 XIAP knockout cell lines using cycloheximide to inhibit protein synthesis. The intestinal histopathology and Notch activity was investigated in mouse and human samples deficient for XIAP.

Results

The ring domain of XIAP is required for association with Smac. Although our studies are inconclusive for the association of XIAP and NICD1 at this time, Notch pathway activity is increased in the absence of XIAP.

Conclusions

Our data support the premise that in the absence of XIAP, NOTCH1 activity is increased.

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