Functional Consequences of CAPN14 Mediated Proteolysis of Catenins in the Esophageal Epithelium in Eosinophilic Esophagitis

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Introduction: Eosinophilic esophagitis (EoE) is a food-related, chronic, allergic disorder that affects 1 in 2000 individuals and costs the United States about 1 billion dollars annually to treat and manage. EoE is characterized by chronic esophageal inflammation that can make it difficult and painful to swallow. In children, this inflammatory process leads to decreased appetite, resulting in inadequate nutrition and ultimately failure to thrive. Patients must be placed on a restricted diet and undergo numerous endoscopies throughout their lifetime, resulting in a low quality of life. At a cellular and molecular level, esophageal tissue exhibits epithelial barrier dysfunction, increased epithelial cell proliferation, and a block in epithelial differentiation. Through genome-wide association studies, the calpain 14 (*CAPN14*) locus was identified to be associated with EoE risk. Additionally, CAPN14 is upregulated in esophageal epithelial cells in response to IL-13 stimulation. However, the substrate of CAPN14 and its effects on epithelial barrier dysfunction remain unknown. In this study, we aimed to test the hypothesis that catenin proteolysis is mediated by CAPN14 in esophageal epithelial cells in EoE, resulting in impaired barrier function, increased proliferation, and altered differentiation in the esophageal epithelium.

Methods: First, EPC2 cells were cultured with and without IL-13, a cytokine shown to upregulate expression of CAPN14. We generated protein lysates from these cells and conducted SDS-PAGE followed by western blot analysis to identify any change in catenin levels or size in the presence of CAPN14. Next, we co-expressed CAPN14 and alpha catenin in HEK293T cells by transfecting them with mammalian expression constructs. We then generated protein lysates and conducted SDS-PAGE and western blot analysis to determine if cells co-expressing CAPN14 and alpha catenin had lower levels of alpha catenin compared to controls. Lastly, we obtained esophageal biopsies from patients with and without active EoE, from which we generated lysates and conducted SDS-PAGE and western blot analysis. Additionally, we used immunofluorescence staining to observe the pattern of alpha catenin in patients with active EoE.

Results: In EPC2 cells expressing CAPN14, we observe no change in alpha catenin levels or size compared to controls. In HEK293T cells, we observed no change in alpha catenin in cells co-transfected with CAPN14 and alpha catenin compared to controls. In human biopsy lysates, we observed no change in alpha catenin levels or size in patients with active EoE compared to controls. Immunofluorescence staining showed no observable difference in alpha catenin localization pattern or quantity in patients with active EoE compared to controls.

Conclusion: In this study, we were unable to determine whether CAPN14 has an effect on alpha catenin quantity or expression patterns. Further work is required to determine the substrate(s) of CAPN14 and its implications in the changes observed in the esophageal epithelium in EoE.

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