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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Epithelial Smad4 Deletion Up-Regulates Inflammation and Promotes Inflammation-Associated Cancer

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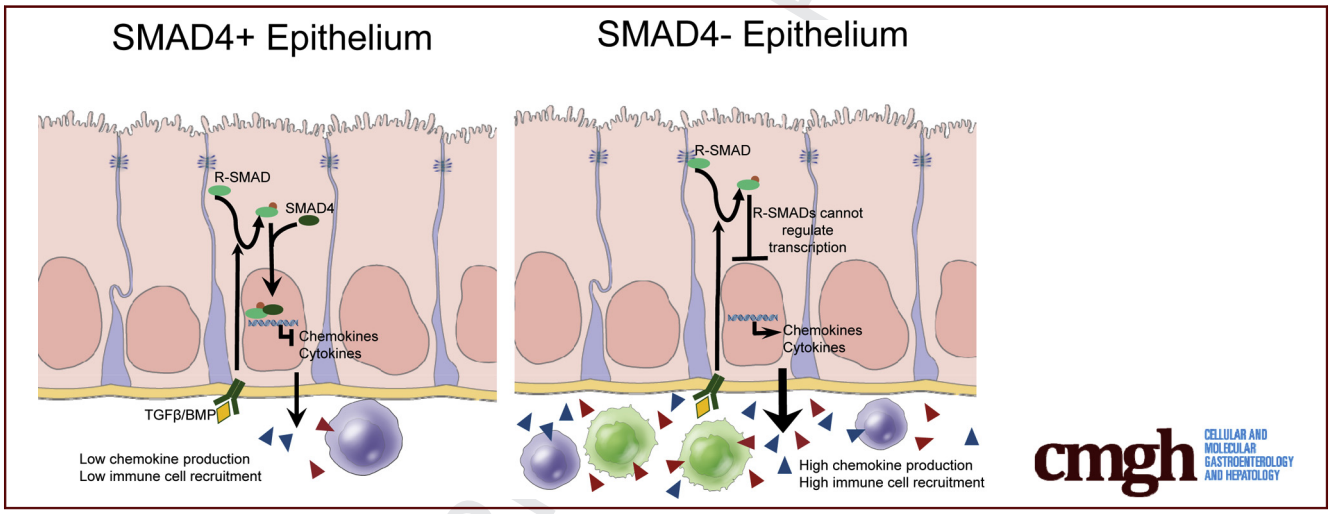

SUMMARY

This study reports the novel observation that canonical TGFβ family signaling through SMAD4 inhibits inflammatory signaling within colonic epithelium. Epithelial Smad4 deletion cells up-regulated proinflammatory gene expression, increased submucosal immune cell numbers, and promoted colitis-associated neoplasia. This correlated with SMAD4 loss in ulcerative colitis–associated colorectal cancers, relative to sporadic colorectal cancers, in patients.

BACKGROUND & AIMS: Chronic inflammation is a predisposing condition for colorectal cancer. Many studies to date have focused on proinflammatory signaling pathways in the colon. Understanding the mechanisms that suppress inflammation, particularly in epithelial cells, is critical for developing therapeutic interventions. Here, we explored the roles of transforming growth factor β (TGFβ) family signaling through SMAD4 in colonic epithelial cells.

METHODS: The *Smad4* gene was deleted specifically in adult murine intestinal epithelium. Colitis was induced by 3 rounds of dextran sodium sulfate in drinking water, after which mice were observed for up to 3 months. Nontransformed mouse colonocyte cell lines and colonoid cultures and human colorectal cancer cell lines were analyzed for responses to TGFβ1 and bone morphogenetic protein 2.

RESULTS: Dextran sodium sulfate treatment was sufficient to drive carcinogenesis in mice lacking colonic *Smad4* expression, with resulting tumors bearing striking resemblance to human colitis–associated carcinoma. Loss of SMAD4 protein was observed in 48% of human colitis–associated carcinoma samples as compared with 19% of sporadic colorectal carcinomas. Loss of *Smad4* increased the expression of inflammatory mediators within nontransformed mouse colon epithelial cells in vivo. In vitro analysis of mouse and human colonic epithelial cell lines and organoids indicated that much of this regulation was cell autonomous. Furthermore, TGFβ signaling inhibited the epithelial inflammatory response to proinflammatory cytokines.

CONCLUSIONS: TGFβ suppresses the expression of proinflammatory genes in the colon epithelium, and loss of its downstream mediator, SMAD4, is sufficient to initiate inflammation-driven colon cancer. Transcript profiling: GSE100082. *(Cell Mol Gastroenterol Hepatol 2018;■:■–■; https://doi.org/10.1016/j.jcmgh.2018.05.006)*

Keywords: TGFβ; Colitis-Associated Carcinoma; Tumor Necrosis Factor.

Chronic inflammation is a predisposing condition for many cancers.[1] Ulcerative colitis is an inflammatory condition of the colon predisposing patients to colitis-associated carcinoma (CAC).[2–4] CAC arises from a different sequence of mutation events than most sporadic colorectal cancers (CRCs). For example, sporadic CRC cases frequently have early mutation of adenomatous polyposis coli (APC) and late mutation of p53. However, in CAC, p53 is thought to be an early mutational event and loss of APC is found late or not at all.[5,6] Although CAC clearly arises in an inflammatory microenvironment, mouse models have shown that multiple etiologies of CRC are either promoted or repressed by specific inflammatory responses.[7–10] Furthermore, there is compelling evidence that CRC can be triggered by a combination of microbiota-dependent and host-dependent mechanisms.[7,11] Multiple levels of regulation have evolved to precisely coordinate the extent of an inflammatory response, allowing for necessary antimicrobial and reparative responses while suppressing inappropriate and rampant responses that lead to disease. Many prior studies have focused on factors influencing the initiation and maintenance of gut inflammation. Given that minor mucosal injuries occur with regularity, it is remarkable that these rapid inflammatory responses are usually transient and extinguished promptly after the inciting cause is resolved without causing overt systemic and organism-wide inflammation with its attendant damaging effects. A better understanding of how this homeostatic balance is maintained may lead to more precise therapeutic interventions.

Transforming growth factor β (TGFβ) pathway signaling has important roles in regulating immune cell responses through its direct regulation of lymphoid and myeloid cell proliferation, differentiation, and survival,[12] which in turn leads to suppression of inflammation. Homozygous germline loss of *Tgfb1*[13] resulted in a marked increase in inflammatory cell infiltration throughout alimentary tract mucosal tissues. In addition, Kim et al[14] found that conditional loss of *Smad4* in T cells with intact epithelial expression of Smad4 in mice caused increased T-cell expression of interleukin (IL)5, IL6, and IL13, phenocopied familial juvenile polyposis, and resulted in epithelial cancers throughout the gastrointestinal tract. In contrast, they did not observe spontaneous gastrointestinal tumors when epithelial *Smad4* was disrupted using epithelial-specific promoters to drive expression of Cre recombinase (*MMTV-Cre* or *Transthyretin-Cre*). In that study, loss of epithelial *Smad4* was not examined in the setting of chronic inflammation and the mice were not examined for gene expression changes in the colon epithelium.

TGFβ family members act via interaction with multimers of type I and type II receptors that then phosphorylate R-SMAD proteins in the cytoplasm.[15] TGFβ1, β2, and β3 bind TGFβ receptors that, in turn, phosphorylate R-SMADs SMAD2 and SMAD3 (SMAD2/3). Bone morphogenetic proteins (BMPs) are TGFβ family members that activate related receptors but lead to the phosphorylation of SMAD1/5/9. Once phosphorylated, R-SMADs bind SMAD4, translocate to the nucleus, and regulate transcription, acting as transcriptional activators of some genes and repressors of other genes. This canonical signaling activity downstream of all TGFβ family receptors is dependent on the common mediator SMAD4. These pathways have multiple levels of redundancy at the levels of ligands, receptors, and R-SMADs, but SMAD4 is uniquely required for transcriptional activity of this pathway. Thus, loss of SMAD4 abrogates all canonical signaling by TGFβ family members.

Previous studies have implicated TGFβ signaling to epithelial cells in inhibiting cell proliferation, modulating differentiation, and inducing epithelial-to-mesenchymal transition.[16,17] We previously found that tissue-specific inactivation of the *Smad4* gene in adult intestinal epithelium in the context of *Apc* mutation led to increased WNT signaling and increased size and numbers of small intestinal and colonic adenomas as compared with *Apc* mutation alone.[18] However, loss of *Smad4* without *Apc* mutation did not result in increased β-catenin protein, likely owing to degradation by the β-catenin destruction complex. We now report a novel homeostatic role for TGFβ signaling in suppressing colonic epithelial cell inflammatory responses. SMAD4-mediated signaling in both human and mouse colonic epithelial cells suppresses inflammation-associated gene expression, including chemokine production, and blocks specific epithelial responses to inflammatory signals. Epithelial-specific loss of *Smad4*, without the introduction of any other targeted mutation, initiates inflammation-driven carcinogenesis in the colon. Furthermore, we observed a significantly increased frequency of SMAD4 loss in ulcerative colitis–associated carcinomas compared with sporadic CRCs in human beings, linking this pathway to epithelial regulation of inflammatory responses.

Abbreviations used in this paper: **AOM, azoxymethane; APC, adenomatous polyposis coli; BMP, bone morphogenetic protein; CAC, colitis-associated carcinoma; CCL20, _____; CRC, colorectal cancer; CRISPR/Cas9, _____; DMEM, Dulbecco's modified Eagle medium; DSS, dextran sodium sulfate; FBS, fetal bovine serum; FDR, false discovery rate; GFP, _____; HBSS, Hank's balanced salt solution; IBD, inflammatory bowel disease; IL, interleukin; $IMC^{S4fl/fl}$, _____; IMC^{S4null}, _____; LPS, lipopolysaccharide; mRNA, messenger RNA; PBS, phosphate-buffered saline; PE, _____; R-SMAD, _____; SFG, _____; sgRNA, single-guide RNA; STAT3, signal transducer and activator of transcription 3; TGFβ, transforming growth factor β; TNF, tumor necrosis factor; UC, ulcerative colitis; WNT, _____; YAMC, _____.**

2352-345X
https://doi.org/10.1016/j.jcmgh.2018.05.006