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CUTTING EDGE

Cutting Edge: Transgenic Expression of Human MUC1 in IL-10$^{-/-}$ Mice Accelerates Inflammatory Bowel Disease and Progression to Colon Cancer[1]

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***Epithelial cell MUC1 is aberrantly expressed on human epithelial adenocarcinomas where it functions as a regulator of immune responses and an oncogene. Normally expressed at low levels in healthy colonic epithelium, MUC1 was reported to be overexpressed in human inflammatory bowel disease (IBD) and thus may be expected to play an important role in regulating chronic inflammation and its progression to colitis-associated colon cancer. Studies in the immunobiology and pathology of IBD and colitis-associated colon cancer have been done in various mouse models but none could properly address the role of MUC1 due to low homology between the mouse and the human molecule. We report that IL-10$^{-/-}$ mice, a widely accepted mouse model of IBD, crossed to human MUC1-transgenic mice, develop MUC1^{+} IBD characterized by an earlier age of onset, higher inflammation scores, and a much higher incidence and number of colon cancers compared with IL-10$^{-/-}$ mice. The Journal of Immunology,* 2007, 179: 735–739.**

Human chronic inflammatory bowel diseases (IBD),[3] such as Crohn's disease and ulcerative colitis (UC), affect more than two million people worldwide. Although the etiologies of these disorders remain unknown, a prevailing hypothesis is that in genetically predisposed individuals, persistent intestinal inflammation results from enhanced or aberrant immunologic responsiveness to the normal microbial constituents of the gut lumen. IBD patients are at increased risk for developing colorectal cancer (1, 2). The risk of colitis-associated colorectal cancer (CACC) among these patients differs according to the extent of colitis and it further increases with the duration of the disease. The pathogenesis of CACC, although unknown, is thought to be related to an increased rate of epithelial proliferation associated with the repetitive cycles of inflammation, damage, and regeneration. These destructive cycles can lead to protein and DNA alterations resulting in increased risk of cancer development (3). No effective preventive or therapeutic modalities are currently available for either IBD or CAAC.

MUC1 is an epithelial cell glycoprotein that is overexpressed and profoundly hypoglycosylated on the majority of human adenocarcinomas and their precursor lesions (4). The peptide backbone of MUC1 is dominated by a variable number of tandem repeat (VNTR) regions composed on an average of 80–200 repeats that are 20 aa long. In healthy colon, MUC1 is expressed at low levels and its VNTR region is heavily glycosylated with long, branched *O*-linked carbohydrates. On colonic adenomatous dysplasia and colorectal adenocarcinomas, however, MUC1 is overexpressed and hypoglycosylated (5, 6). MUC1 overexpression and hypoglycosylation have also been reported in human IBD (7, 8), and autoantibodies specific for MUC1 have been eluted from inflamed colonocytes (9).

The IL-10 knockout (IL-10$^{-/-}$) mouse is a well-established model of IBD where the development of chronic intestinal inflammation is considered to be the result of an exaggerated immune response to intestinal microflora due to the complete lack of IL-10 that prevents immunoregulation. IL-10$^{+/-}$ mice are resistant to IBD because they can still produce adequate amounts of IL-10. At 3–6 mo of age, the majority of IL-10$^{-/-}$ mice on the C57BL/6 background develop spontaneous colitis characterized by inflammatory cell infiltration, goblet cell depletion, crypt abscess formation, and epithelial hyperplasia (10, 11). A small proportion of these mice develop adenocarcinomas in the colon. All of these characteristics are similar to human IBD except that this mouse model lacks MUC1 expression. The mouse homolog (designated Muc-1 to distinguish it from human MUC1) shares 87% homology in the short cytoplasmic domain with human MUC1. However, the extracellular VNTR region that differs between normal and diseased tissues and is involved in cell-cell and receptor-ligand interactions and immunoregulation has only 34% homology with human MUC1, and thus is not expected to play the same role in IBD as the human molecule. To explore the postulated importance of MUC1 in IBD, we introduced the human molecule into the

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[3] Abbreviations used in this paper: IBD, inflammatory bowel disease; UC, ulcerative colitis; CACC, colitis-associated colon cancer; VNTR, variable number of tandem repeats; Tg, transgenic.

IL-10$^{-/-}$ mouse model. We show here that the presence of MUC1 has a profound effect on the time of IBD occurrence, degree of inflammation, and progression to colon cancer. This new mouse model closer resembles human IBD and in addition to the differences in IBD pathology that we report here, it is likely to yield other important MUC1-related disease mechanisms and therapeutic targets.

Materials and Methods

Mice

IL-10$^{-/-}$ mice on a C57BL/6 background were purchased from The Jackson Laboratory and MUC1-trangenic (Tg) mice on a C57BL/6 background were purchased from Dr. S. J. Gendler (The Mayo Clinic, Scottsdale, AZ). All experiments were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh (Pittsburgh, PA).

PCR genotyping

PCR was used to identify the *MUC1* transgene as well as the presence or absence of the *IL-10* gene. The primer pairs for MUC1 Tg were 5′-CTTG CCAGCCATAGCACCAAG-3′ and 5′-CTCCACGTCGTGGACATTGA TG-3′. The IL-10 primers were 5′-GTGGGTGCAGTTATTGTCTTCCCG and 5′-GCCTTCAGTATAAAAGGGGGACC and 5′-CCTGCGTGCAA TCCATCTTG-3′. The PCR product of each reaction was analyzed on a 1% agarose gel. MUC1 amplification resulted in a 500-bp fragment and IL-10 amplification resulted in a 200-bp fragment if IL-10$^{+/+}$, 200 and 450 bp if IL-10$^{+/-}$, and 450 bp if IL-10$^{-/-}$.

Histology and colitis scores

The colon was divided into the ascending colon, descending colon, and cecum and fixed in 10% buffered formalin, and embedded in paraffin. Five-μm-thick sections were stained with H&E. Colitis scores (grades 0–4) were determined by a gastrointestinal pathologist who was blinded to the experimental protocol, using the criteria reported by Hegazi et al. (10). Briefly, 20–40 separate microscopic fields were evaluated for each mouse and graded 0–4. The total inflammation score for each sample was determined by taking the sum of the fields divided by the number of fields.

Immunohistochemistry

Five-μm-thick tissue paraffin sections were deparaffinized by baking overnight at 59°C. Endogenous peroxidase activity was eliminated by treatment with 30% H_2O_2 for 15 min at room temperature. Ag retrieval was performed by microwave heating in 0.1% citrate buffer. Nonspecific binding sites were blocked with Protein Blocking Agent (Thermo-Shandon). The anti-MUC1 Ab HMPV, which recognizes all forms of MUC1 by binding the epitope APDTR in the VNTR region in a glycosylation-independent manner, was purchased from BD Pharmingen. The anti-MUC1 Ab VU-4H5, which recognizes the epitope APDTRPAP in the VNTR region of hypoglycosylated MUC1, was purchased from Santa Cruz Biotechnology. The anti-β-catenin Ab H-102 was purchased from Santa Cruz Biotechnology. Staining was performed by the avidin-biotin-peroxidase complex method with a commercial kit (Vectastain ABC kit; Vector Laboratories). Color development was performed using a 3,3′-diaminobenzidine kit (BD Pharmingen) or a 3-amino-9-ethylcarbazole kit (Vector Laboratories).

Results and Discussion

To introduce the human MUC1 into the IL-10$^{-/-}$ mouse model of IBD, we took advantage of the existence of mice transgenic for human MUC1 (MUC1-Tg). These mice develop normally and express MUC1 under its endogenous promoter and thus in the same spatial and tissue distribution seen in humans (12). This includes low expression on healthy epithelia and overexpression of the hypoglycosylated form on epithelial tumor cells (13, 14). Importantly, these mice do not develop spontaneous IBD. We bred MUC1-Tg mice with IL-10$^{-/-}$ mice and used PCR to identify animals that carried the *MUC1* transgene and lacked *IL-10* genes.

We analyzed first the MUC1^{+}IL-10$^{+/-}$ mice that are not expected to develop IBD because they have one normal copy of the IL-10 gene, to make sure that the addition of MUC1 did not change their resistance to IBD. Mice were sacrificed at various time points and colonic tissue was stained with H&E (Fig. 1, *A–D*) to assess the presence or absence of inflammation. Colonic tissue was also stained with Ab HMPV to detect MUC1 expression (Fig. 1, *E–J*). HMPV is specific for a peptide epitope in the VNTR region that is independent of MUC1 glycosylation and thus can detect all forms of MUC1 (15). MUC1^{+}IL-10$^{+/-}$ mice did not exhibit colonic inflammation, as determined by H&E (Fig. 1*A*), and, as expected, we saw only very low, normal levels of MUC1 expression on the apical surface of the colonocytes (Fig. 1*E*).

Both MUC1^{+}IL-10$^{-/-}$ and IL-10$^{-/-}$ mice that are expected to develop IBD were monitored and sacrificed at the onset of diarrhea and rectal prolapse, the first external signs of disease. They developed the typical segmental, patchy inflammation, with areas of healthy-appearing colon (Fig. 1*B*) adjacent to areas of severe inflammation (Fig. 1*C*). We found that in areas with no inflammation, there was little MUC1 expression (Fig. 1*F*), similar to levels observed in MUC1^{+}IL-10$^{+/-}$ mice (Fig. 1*E*). MUC1 expression was very high, however, at sites of severe inflammation (Fig. 1*G*). IL-10$^{-/-}$ mice developed severe inflammation (Fig. 1*D*) but there was no MUC1 staining (Fig. 1*H*), consistent with the absence of the human *MUC1* gene in these animals. Expression of MUC1 in MUC1^{+}IL-10$^{-/-}$ mice is consistent with what we see in tissue sections from human IBD. In uninflamed human colonic mucosa, low MUC1 expression is detected in the colonic crypts within mature goblet cells toward the mucosal surface (Fig. 1*I*). In inflamed sections corresponding to UC, MUC1 is overexpressed on many cells, including the crypt epithelial cells of the expanded regenerative zones, in addition to mature goblet cells (Fig. 1*J*). Our histology results are consistent with the recent publication by Longman et al. (16) reporting increased MUC1 mRNA expression in crypts and goblet cells in colon samples from a large number of patients with severe UC.

Given only limited information about the role of MUC1 in inflammatory diseases (17, 18) and given the reported importance of MUC1 in cancer initiation and progression (4, 19),

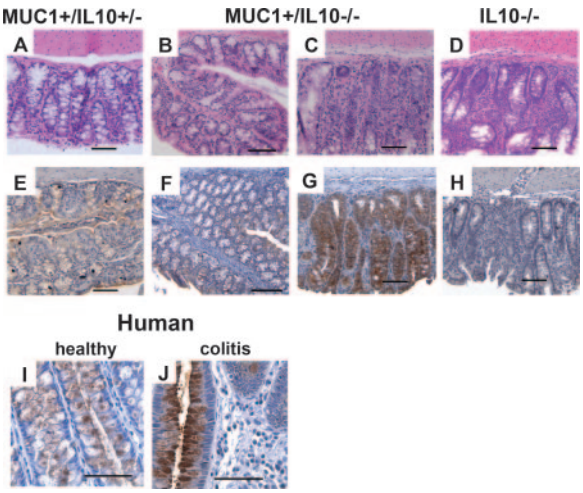

FIGURE 1. IBD in MUC1^{+}IL-10$^{-/-}$ mice and humans is characterized by a high level of MUC1 expression at sites of severe inflammation. H&E-stained mouse colon sections (*A-D*); immunostaining (hematoxylin counterstained) of mouse colon sections (*E–H*) and human colon sections (*I* and *J*) with anti-MUC1 Ab HMPV. Bar, 200 μm.

overexpression of MUC1 in human IBD begged the question of whether MUC1 was contributing to the initiation and the intensity of colonic inflammation, progression to cancer, or both. We thus compared the course of IBD in MUC1^{+}IL-10$^{-/-}$ mice vs IL-10$^{-/-}$ mice. MUC1^{+}IL-10$^{-/-}$ mice developed progressive disease much earlier than IL-10$^{-/-}$ mice (Fig. 2*A*). Median time to rectal prolapse was 11 wk for MUC1^{+}IL-10$^{-/-}$ mice compared to 16 wk for IL-10$^{-/-}$ mice. This difference was highly significant ($p = 0.0006$) and reproducibly observed. Furthermore, when we compared early inflammatory changes in the colon in MUC1^{+}IL-10$^{-/-}$ and IL-10$^{-/-}$ mice by sacrificing them at 5–6 wk of age before external signs of disease, we found that MUC1^{+}IL-10$^{-/-}$ mice had a higher total colonic inflammation score compared to IL-10$^{-/-}$ mice (Fig. 2*B*). They also had fewer fields of no inflammation (colitis score 0) and significantly more fields with moderate inflammation (colitis score 1–2; Fig. 2*C*). The underlying mechanism driving earlier disease and more severe inflammation appears to be the drastic difference in the intensity of the cellular infiltrate between MUC1^{+}IL-10$^{-/-}$ mice and IL-10$^{-/-}$ mice (Fig. 2*D*). Most significantly, in the presence of MUC1, neutrophils predominate in the infiltrate, while they are virtually absent in the IL-10$^{-/-}$ mouse

We were also interested to know what form of MUC1, normal or hypoglycosylated, was expressed early in disease. Thus, we analyzed colon sections from MUC1^{+}IL-10$^{-/-}$ mice at 5 and 8 wk of age using Abs that can distinguish between normal and hypoglycosylated forms of MUC1. Representative sections are shown in Fig. 2*E*. At 5 wk, we see little inflammation and low levels of primarily normal MUC1 detected by the Ab HMPV that sees all MUC1 forms. The lack of staining with the VU-4H5 Ab confirmed that expression of the hypoglycosylated MUC1 was absent at 5 wk of age. In contrast, at 8 wk, the colon is more inflamed, shows the presence of cellular infiltrate, and displays increased levels of MUC1 expression, including the hypoglycosylated (tumor) form.

Considering that the presence of MUC1 in this new mouse model of IBD was associated with earlier onset of inflammation, we hypothesized that this might also have an effect on the progression from IBD to colon cancer. MUC1^{+}IL-10$^{-/-}$ and IL-10$^{-/-}$ mice were sacrificed at various times after the first signs of rectal prolapse, and colon sections were examined under a dissecting microscope for the presence of tumors. Fig. 3, *A* and *B,* shows the gross morphology of tumors that developed in MUC1^{+}IL-10$^{-/-}$. We found that 88% of MUC1^{+}IL-10$^{-/-}$ mice with colonic inflammation had tumors compared with only 20% of IL-10$^{-/-}$ mice (Fig. 3*C*). In addition, MUC1^{+}IL-10$^{-/-}$ mice had a significantly higher tumor burden (three to four tumors per colon on average) compared with IL-10$^{-/-}$ mice (one tumor per colon) and accelerated tumor development. Tumors in MUC1^{+}IL-10$^{-/-}$ mice developed as early as 8–12 wk of age while most IL-10$^{-/-}$ mice developed tumors between 25 and 35 wk of age. Histologic assessment revealed that the majority of tumors were invasive adenocarcinomas (Fig. 4, *A–C*). The tumors from MUC1^{+}IL-10$^{-/-}$ mice stained strongly for MUC1 expression with both anti-MUC1 Abs HMPV (Fig. 4, *D* and *E*) and VU-4H5 (Fig. 4, *G* and *H*). Adenocarcinomas from IL-10$^{-/-}$ mice served as negative controls for MUC1-specific staining (Fig. 4, *F* and *I*). Both, MUC1^{+}IL-10$^{-/-}$ mice as well as the IL-10$^{-/-}$ mice develop many lesions that can be characterized as mild, moderate, or severe dysplasias, according to published histologic criteria (20) Unlike the fully developed tumors, these lesions have been very difficult to diagnose and enumerate precisely in human IBD or in animal models. MUC1^{+}IL-10$^{-/-}$ mice will be a good model for potentially validating aberrantly expressed MUC1 as a marker of such lesions. MUC1 plays a role in tumorigenesis through alterations in intracellular signaling, adhesion and migration properties of tumor cells, and increasing resistance to

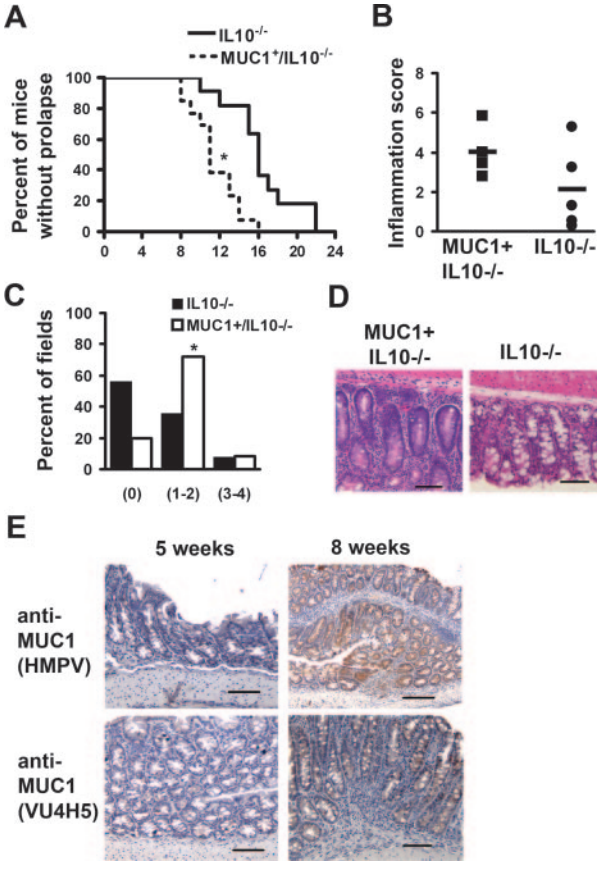

FIGURE 2. Development of IBD and progression to colon cancer differ between MUC1^{+}IL-10$^{-/-}$ mice and IL-10$^{-/-}$ mice. *A,* Kaplan-Meier survival curve. Dashed line, MUC1^{+}IL-10$^{-/-}$ mice ($n = 13$); solid line, IL-10$^{-/-}$ mice ($n = 11$); *, $p = 0.0006$. *B,* Total colonic inflammation scores: MUC1^{+}IL-10$^{-/-}$ mice ($n = 4$), IL-10$^{-/-}$ mice ($n = 5$). *C,* Colitis score: MUC1^{+}IL-10$^{-/-}$ mice ($n = 4$); IL-10$^{-/-}$ mice ($n = 5$); *, $p = 0.032$, two-tailed unpaired *t* test. *D,* H&E-stained sections showing the difference in the intensity of the cellular infiltrate into inflamed sites at 8 wk of age. *E,* Immunostaining (hematoxylin counterstained) of 5- and 8-wk-old mouse colon sections with anti-MUC1 Abs HMPV and VU-4H5. Bar, 200 μm.

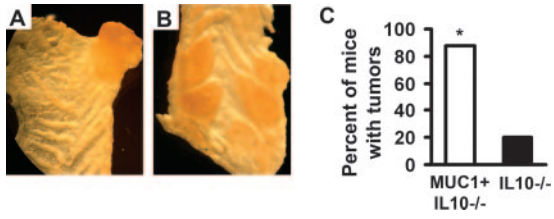

FIGURE 3. MUC1^{+}IL-10$^{-/-}$ mice have a higher incidence of colon tumors and a greater tumor burden compared with IL-10$^{-/-}$ mice. *A* and *B,* Whole mount of colons with tumors from two MUC1^{+}IL-10$^{-/-}$ mice. *C,* MUC1^{+}IL-10$^{-/-}$ mice ($n = 8$), IL-10$^{-/-}$ mice ($n = 5$); *, $p = 0.0319$, two-tailed Fisher's exact test.

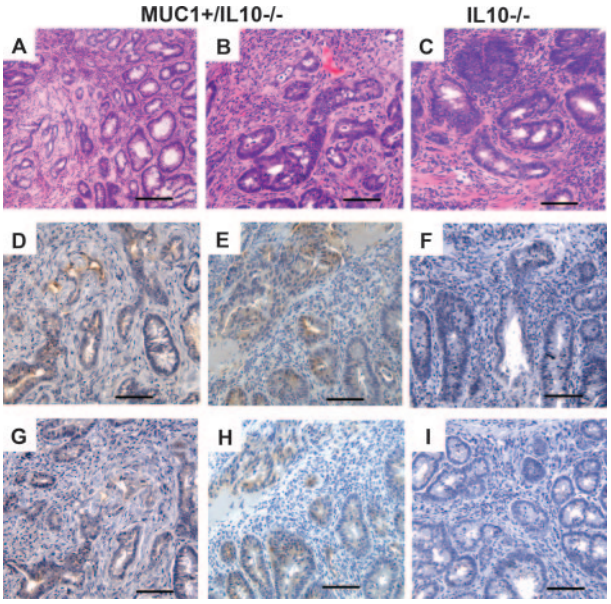

FIGURE 4. Colon tumors that develop in MUC1$^+$IL-10$^{-/-}$mice are MUC1$^+$ invasive adenocarcinomas. *A* and *B*, Colon tumors from two MUC1$^+$IL-10$^{-/-}$ mice; *C*, an IL-10$^{-/-}$ mouse; *D–F*, immunostaining (hematoxylin counterstained) with anti-MUC1 Ab HMPV. MUC1 expression (dark brown) is seen in the tumors of MUC1$^+$IL-10$^{-/-}$ mice (*D* and *E*) and absent in IL-10$^{-/-}$ mice (*F*). *G–I*, Immunostaining (hematoxylin counterstained) of the same tumors with anti-MUC1 Ab VU-4H5 specific for the hypoglycosylated (tumor) form of MUC1. Hypoglycosylated MUC1 expression (dark brown) is seen in the tumors of MUC1$^+$IL-10$^{-/-}$ mice (*G* and *H*) and absent in IL-10$^{-/-}$ mice (*I*). Bar, 100 μm.

apoptosis (21–23). Specifically, MUC1 has been shown to promote epithelial cell transformation through its ability to bind to and block the degradation of β-catenin (23). Accumulation of cytoplasmic β-catenin and subsequent translocation to the nucleus leads to multiple target gene activation, which has been linked to the majority of colon cancers. In Fig. 5, we see that MUC1$^+$IL-10$^{+/-}$ mice, which do not develop IBD, show low levels of β-catenin in their colon. Similarly, we see low levels of β-catenin in the colon section from the IL-10$^{-/-}$ mouse that includes a small tumor. In contrast, we see strong staining of β-catenin in the colon section with a tumor from a MUC1$^+$IL-10$^{-/-}$ mouse.

These studies reveal that MUC1 expression influences IBD pathogenesis and progression to CACC. We have previously published that hypoglycosylated forms of MUC1 are chemotactic to immature dendritic cells and capable of inducing their aberrant maturation and their cytokine profile (24). Sialic acid residues on the prematurely terminated carbohydrate chains on

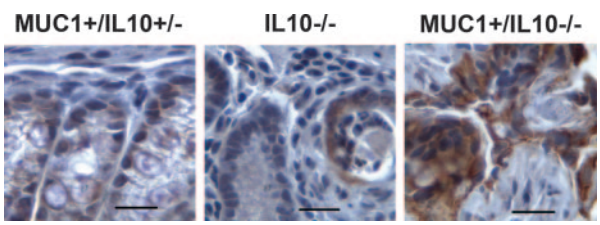

FIGURE 5. Colon tumors that develop in MUC1$^+$IL-10$^{-/-}$ mice have increased levels of cytoplasmic and nuclear β-catenin. Immunostaining (hematoxylin counterstained) of colonic tissue sections with anti-β-catenin Ab. Isotype control is negative (data not shown). Bar, 50 μm.

MUC1 are important in these interactions, implicating receptors such as Sig-lecs on dendritic cells and macrophages. Other receptors on these cells have also been shown to bind MUC1, including the mannose receptor (25, 26) and the C-type lectin receptor macrophage galactose-type lectin (27). Many of these receptors are conserved between mice and humans and thus the human MUC1 can exert many of its function in mice, as we see in the IBD model. Human MUC1 has previously been shown to have immunostimulatory activity on mouse innate and adaptive immune cells (28). Regardless of the etiology of IBD, the increased expression of the hypoglycosylated MUC1 that we show, even in the early stages of IBD, could cause continued accumulation and activation of various cells of the innate immune system, thus driving chronic inflammation and profoundly changing its outcome (3). An increase in MUC1 expression is most likely mediated by inflammatory cytokines produced during initial inflammation, such as IL-6, TNF-α, and IFN-γ that have been shown to induce MUC1 expression in epithelial cells (29, 30).

Changes in activities of various glycosyltransferases that result in the expression of hypoglycosylated MUC1 have been described in many malignancies, including colorectal cancer (31). We show here that hypoglycosylated MUC1 is expressed in IBD as well, indicating that these changes are not limited to fully transformed cancer cells. Hypoglycosylated MUC1 presents new epitopes to B cells and is more efficiently processed and cross-presented to T cells, which may bring cells of the adaptive immunity as well to the site of inflammation. This may lead to specific destruction of the colonocytes. Increased MUC1 expression in damaged colonic epithelial cells can in turn promote tumorigenesis through a number of well-documented signaling pathways (21–23). One known mechanism is through its interaction with β-catenin (22, 23), which we show is greatly increased in tumors from MUC1$^+$IL-10$^{-/-}$ mice.

Our results validate MUC1$^+$IL-10$^{-/-}$ mice as a model relevant to human IBD and CACC. By showing the importance of MUC1 in this disease, the model may encourage and facilitate studies on the identification of new therapeutic approaches that target MUC1 or MUC1-related molecules and pathways.

Disclosures

The authors have no financial conflict of interest.

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