Smad7 Expression in T cells Prevents Colitis-Associated Cancer

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Abstract

Patients with inflammatory bowel disease (IBD) have an increased risk of developing colorectal cancer due to chronic inflammation. In IBD, chronic inflammation relies upon a TGFβ signaling blockade, but its precise mechanistic relationship to colitis-associated colorectal cancer (CAC) remains unclear. In this study, we investigated the role of the TGFβ signaling inhibitor Smad7 in CAC pathogenesis. In human colonic specimens, Smad7 was downregulated in CD4+ T cells located in the lamina propria of patients with complicated IBD compared with uncomplicated IBD. Therefore, we assessed CAC susceptibility in a transgenic mouse model where Smad7 was overexpressed specifically in T cells. In this model, Smad7 overexpression increased colitis severity, but the mice nevertheless developed fewer tumors than nontransgenic mice. Protection was associated with increased expression of IFNγ and increased accumulation of cytotoxic CD8+ and natural killer T cells in the tumors and peritumoral areas. Moreover, genetic deficiency in IFNγ abolished the Smad7-dependent protection against CAC. Taken together, our findings defined a novel and unexpected role for Smad7 in promoting a heightened inflammatory response that protects against CAC. Cancer Res; 71(24); 1–10. © 2011 AACR.

Introduction

Chronic inflammation is believed to be a leading cause of cancer in various organs (1). Indeed, chronic release of oxygen and nitrogen reactive species induces genomic damage thus contributing to tumor initiation and promotion, whereas proinflammatory cytokines stimulate dysplastic cell proliferation and neoangiogenesis thereby supporting tumor progression.

TGFβ1, hereafter indicated as TGFβ, is a crucial negative regulator of inflammation and the TGFβ knockout mice develop a fatal multiorgan inflammatory disease (2). The anti-inflammatory properties of TGFβ rely, at least in part, on its ability to suppress T-lymphocyte activities. TGFβ prevents the activation and differentiation of naive T cells in proinflammatory T-helper (Th) 1 and Th2 cells while inducing them to acquire a suppressive phenotype [i.e., regulatory T cells (Treg); 3, 4]. Moreover, TGFβ suppresses proliferation and effector/memory T-helper cell activity (5).

TGFβ signaling is tightly regulated in T cells. The binding of TGFβ with its specific receptor complex (i.e., TGFβ receptor type I and type II) causes the phosphorylation of the cytoplasmic molecules Smad2 and Smad3 which in turn migrate into the nucleus where they regulate the expression of several proinflammatory genes. TGFβ signal transduction is negatively regulated by Smad7 that prevents Smad2/3 phosphorylation by recruiting on the intracellular domain of the TGFβ receptor type I, the GADD34 complex, and the protein phosphatase 1 to dephosphorylate it (6). Moreover, Smad7 has been shown to promote the ubiquitination and proteasomal degradation of the TGFβ receptor complex (7–10).

Smad7-mediated suppression of the TGFβ signaling has been well documented in the inflamed gut of patients with Crohn disease and ulcerative colitis, the two major forms of inflammatory bowel diseases (IBD), which are associated with an increased risk to develop colitis-associated colorectal cancer (CAC). Downregulation of Smad7 with a specific antisense oligonucleotide restores TGFβ signaling thus suppressing proinflammatory cytokine synthesis in vitro (11). Moreover, Smad7 overexpression makes T cells resistant to the suppressive activity of Tregs, and Smad7 downregulation restores Treg-mediated suppression (12).

In vivo, the downregulation of Smad7 attenuates IBD-like experimental colitis in mice (13). Although the above findings suggest that high Smad7 can facilitate colorectal cancer progression by sustaining chronic inflammation, studies in mice with a nonphysiologic block of TGFβ activity in T cells operated by the dominant-negative

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TGFβ receptor have provided divergent results on the role of TGFβ in cancer cell growth and metastasis (14, 15). Therefore, this study investigated the role of Smad7-induced block of TGFβ signaling on the initiation and progression of CAC. Here, we show that the development of CAC in patients with IBD is associated with a significant downregulation of Smad7 in CD4+ T cells. Moreover, we have identified a novel and unexpected protective role of Smad7 against CAC development in a transgenic mouse model in which Smad7 is selectively overexpressed in T cells.

Materials and Methods

Cell lines

YAC-1 lymphoma cell cells were obtained from the American Type Culture Collection (ATCC-TIB-160). The abovementioned cell lines were procured more than 6 months ago and have not been tested recently for authentication in our laboratory.

Mice

C57BL/6 CD2-Smad7Tg mice were generated as previously published (12). C57BL/6 IFNy−/− mice were purchased from Jackson Laboratories, and IFNy−/− Smad7Tg were hosted in the SPF Animal Facility at the University of Rome Tor Vergata (Rome, Italy). See also Supplementary Materials and Methods.

Azoxy methane/dextran sodium sulphate protocol

All mice were male and 6 to 8 weeks old and experimental protocols were conducted according with the local institutional guidelines. To induce CAC, mice were injected intraperitoneally with a single dose (10 mg/kg) of the alkylating agent azoxymethane (AOM; Sigma-Aldrich) followed by 3 cycles of 2.5% dextran sodium sulphate (DSS; MW, 36,000–50,000 Da; MP Biomedicals) given in the drinking water for 3 days. Mice were kept on water for 2 weeks after each DSS cycle. In some experiments, mice were treated with DSS only to induce chronic colitis but not CAC.

Endoscopic procedures

Tumor development and colitis were screened by a miniaturized endoscope (Coloview System). Tumors were counted and scored as previously described (16). Colitis was graded according to the mouse endoscopic index of colitis severity (MEICS).

Histologic analysis and immunohistochemistry

Colon sections from mice were obtained for hematoxylin and eosin (H&E) staining and analyzed by light microscope (Olympus). Colitis was scored as previously published (17). In some cases, colonic sections were used for indirect immunofluorescence using rat anti-mouse CD8 or CD4 (BD Pharmingen) or CD68 (Acris) or Smad7 (Santa Cruz Biotechnology) or FOXP3 (eBioscience). Antigen detection was obtained with Tyramide [Cy3 and fluorescein isothiocyanate (FITC)] according to the manufacturer’s guidelines (PerkinElmer). Paraffin-embedded colonic sections from patients with active IBD, IBD-related CAC, and controls were obtained from the Institute of Pathology (Bayreuth, Germany) as previously reported (18). Patients’ characteristics are reported in Supplementary Table S1. Sections were used for H&E staining or stained with anti-human CD4 (BD Pharmingen) and anti-human Smad7–specific antibodies (R&D System) and analyzed by confocal microscope as previously published (18). The number of positive cells was quantified by ImageJ software (NIH, Bethesda, MD).

Isolation of lamina propria mononuclear cells and tumor-infiltrating lymphocytes

Lamina propria mononuclear cells (LPMC) and tumor-infiltrating lymphocytes (TIL) were isolated according to standard protocols. See Supplementary Material and Methods.

Flow cytometry

The following anti-mouse conjugated antibodies were used: CD4-FITC, CD8-phycoerythrin (PE), CD49b (DX5)-PE, CD3-FITC, IL-17A-PE, IFNγ-APC (BD Pharmingen), and Annexin V-PE (BD Pharmingen). Interleukin (IL)-17A- and IFNγ-expressing intracellular staining on LPMCs and TILs were analyzed after 5-hour stimulation with phorbol-12-myristate-13-acetate (PMA: 40 ng/mL) and ionomycin (1 μg/mL; Sigma-Aldrich), in the presence of monensin (2 μmol/L; eBioscience) according to standard protocols. Stained cells were analyzed by flow cytometry (FACScalibur; BD).

Cytotoxicity assay

Cytotoxic activity was measured by flow cytometry–based assay as previously described (19). See Supplementary Material and Methods for detailed description.

RNA extraction, cDNA preparation, and real-time PCR

Total RNA was isolated using TRIzol reagent (Invitrogen) and retrotranscribed with the M-MLV reverse transcriptase (Invitrogen). Real-time PCR was carried out using a SYBR Green–based PCR using iQ SYBR mix (Bio-Rad). β-Actin was used to normalize gene expression results. Specific primers were designed on the cDNA sequence provided by ENSEMBL database (Supplementary Table S2).

Statistical analysis

The Student t test and the nonparametric Mann–Whitney test were used to calculate statistical significance between groups. Spearman rank correlation test was used to calculate correlation between inflammation grade and tumor score.

Results

Smad7 is downregulated in inflamed colon of patients with IBD-related CAC

To assess Smad7 expression in patients with CAC, sections from patients with IBD with or without CAC and controls (Fig. 1A) were stained for Smad7. A marked accumulation of Smad7–expressing cells was seen in the lamina propria of patients with Crohn disease and ulcerative colitis without CAC in comparison to controls. In contrast, the number of
Smad7-positive cells did not differ between patients with IBD complicated by CAC and controls (Fig. 1B and C). As shown in Fig. 1D, the majority of the Smad7-expressing cells coexpressed the CD4. These data indicate that IBD not complicated by CAC is characterized by accumulation of Smad7⁺CD4⁺ T cells in the lamina propria and that the number of these cells is significantly reduced in patients with IBD with CAC.

**Smad7Tg mice develop severe DSS-mediated colitis**

To investigate the functional role of Smad7 in CAC development, we used a T-cell–specific Smad7 transgenic (Smad7Tg) mouse as previously described (12). We initially assessed whether, analogously to human IBD, high expression of Smad7 in T cells was associated with enhanced mucosal inflammation. To this end, 6- to 8-week-old wild-type and Smad7Tg mice received 3 cycles of 2.5% DSS to mimic human chronic-relapsing colitis (Fig. 2A). Endoscopy conducted at the end of the experimental protocol (day 75) showed colitis in both wild-type and Smad7Tg mice (Fig. 2B). However, Smad7Tg mice had a more severe colitis as shown by the endoscopic score of colitis severity (MEICS) applied to wild-type and Smad7Tg mice at day 75. Data were obtained from 2 independent experiments (Fig. 2C). H&E staining of representative colon sections collected from wild-type and Smad7Tg mice at the end of the experiment confirmed the presence of inflammation in both wild-type and Smad7Tg mice (Fig. 2D). However, Smad7Tg mice had a more severe colitis as shown by the endoscopic score of colitis (Fig. 2C). Histologic analysis of colonic specimens obtained at the end of the experiment confirmed the presence of inflammation in both wild-type and Smad7Tg mice (Fig. 2D), even though Smad7Tg mice had a significant higher histologic score (Fig. 2E). Both DSS-treated wild-type and Smad7Tg mice were characterized by high expression of the proinflammatory cytokines IFNγ, TNFα,
IL-6, and IL-17A, although these cytokines are more expressed in the Smad7Tg mice (Fig. 2F). Moreover, Smad7Tg mice were characterized by a higher number of CD4\(^{+}\) and CD8\(^{+}\) T cells accumulating in the lamina propria as compared with wild-type mice (Supplementary Fig. S1), with no differences in terms of CD68-expressing macrophages. Finally, as observed in human IBD, the stronger inflammation observed in the Smad7Tg was associated with the accumulation of a higher number of FOXP3\(^{+}\) regulatory T cells (20). These data indicate that, similar to humans, high Smad7 expression in T cells makes mice more susceptible to colitis.

**Smad7Tg mice are largely protected against CAC**

To investigate whether the more severe inflammation observed in Smad7Tg mice was associated with an increased susceptibility to CAC, mice underwent the AOM/DSS model of CAC (Fig. 3A) which is characterized by the induction of adenomas with histologic signs of high-grade dysplasia by 9 to 10 weeks (16, 21).

As expected, at the end of the experiment, wild-type mice developed multiple colonic tumors (Fig. 3B). As observed in humans, Smad7 was highly expressed by LPMCs of peritumoral areas. In contrast, Smad7 was mostly expressed by dysplastic epithelial cells, with significantly fewer positive cells infiltrating the tumor stroma than peritumoral LPMCs (Supplementary Fig. S2A). Surprisingly, Smad7Tg mice developed fewer tumors than wild-type (Fig. 3C). The tumor score, which takes into account not only the number of tumors but also their size, was significantly higher in wild-type than Smad7Tg mice (Fig. 3D). Histologic analysis of peritumoral areas showed higher inflammatory cell infiltrate in the mucosa and submucosa associated with modest mucosal hyperplasia, conserved crypt architecture, and minimal goblet cell depletion in wild-type mice [Fig. 3E (i)–(iii)]. In contrast, Smad7Tg mice were characterized by dense inflammatory cell infiltrate both in the mucosa and submucosa, mucosal hypertrophy, crypts distortion and rarefaction, and strong depletion of goblet cells [Fig. 3E (iv)–(vi)]. These differences were confirmed by the histologic score of sections of wild-type and Smad7Tg mice (Fig. 3F) and this inversely correlated with the tumor score of both groups of mice (Fig. 3G). Accordingly, CD4\(^{+}\) and CD8\(^{+}\) T cells seen in both tumor and peritumoral areas were more in the Smad7Tg than in the wild-type, whereas no differences were observed in CD68\(^{+}\) macrophages (Supplementary Fig. S2B).
To assess whether the higher inflammation and lower tumor incidence observed in the Smad7Tg mice were related to a defect in Treg accumulation, the expression of the Treg-specific transcription factor FOXP3 and TGFβ was evaluated in peritumoral LPMCs and TILs. Although TGFβ expression was higher in TILs than in LPMCs isolated from both the groups, there was no significant difference between wild-type and Smad7Tg mice (Fig. 4A, right). The data indicate that the higher inflammation seen in the Smad7Tg mice might interfere with CAC, despite the accumulation of FOXP3-expressing cells in the peritumoral areas.

**Smad7Tg mice exhibit a marked intratumoral Th1-mediated immune response**

Cytokine expression analysis in the LPMCs and TILs of wild-type and Smad7Tg mice showed IFNγ, TNFα, IL-6, and IL-17A to be the most expressed cytokines in both wild-type and Smad7Tg mice (Fig. 4A and B, left). In keeping with the more severe inflammation observed in the Smad7Tg, IFNγ, IL-6, and IL-17A mRNA levels were nearly 8-fold higher in the Smad7Tg LPMCs than in wild-type mice (Fig. 4A, right). In contrast, in the tumors of Smad7Tg, there is a prevalent IFNγ-associated immune response. Consistently, both Tbet and RORγt, the Th1- and Th17-specific transcription factors, were overexpressed in Smad7Tg LPMCs (Fig. 4C), whereas in the Smad7Tg TILs, there was a higher Tbet and lower RORγt expression than wild-type (Fig. 4D). Consistent with the mRNA data, the number of IFNγ-expressing CD4+ T cells resulted in about 10-fold higher expression in the Smad7Tg than in the wild-type at both LPMC and TIL level (Fig. 4E). In contrast, the number of IL-17A-expressing cells in the Smad7Tg was higher in the LPMC but lower in the TILs as compared with wild-type. These data indicate that in contrast to the Wt, a prevalent Th1 immune response develops in the tumor microenvironment of Smad7Tg mice.

**A critical role of IFNγ in mediating protection of Smad7Tg against CAC**

Because IFNγ is upregulated in Smad7Tg mice, we next assessed the functional role of this cytokine in tumor protection in these mice. To this end, IFNγ-deficient Smad7Tg (IFNγ−/− Smad7Tg) mice were used in the AOM/DSS model. In the IFNγ−/− Smad7Tg mice, tumor incidence was restored to levels observed in the wild-type (Fig. 5A and B). However, the tumor score was still significantly lower than wild-type (Fig. 5C) thus suggesting that factors other than IFNγ contribute to suppress tumor growth in Smad7Tg mice. Histologic analysis of colonic sections from the 3 groups of mice showed severe inflammation in both Smad7Tg and IFNγ−/− Smad7Tg mice, although in the latter, many dysplastic areas were detected (Fig. 5D, outlined). TNFα, IL-6, and IL-17A were the most expressed cytokines in both LPMCs and TILs isolated from IFNγ−/− Smad7Tg mice (Fig. 5E and F, left). However, levels of TNFα, IL-6, and IL-17A expressed by both LPMCs and TILs were reduced in the IFNγ−/− Smad7Tg as compared with wild-type mice (Fig. 5E and F, right). These data indicate that colitis develops in Smad7Tg mice independently of IFNγ but its
expression is required for protection against CAC in these mice.

Smad7Tg mice are characterized by IFNγ-dependent accumulation of CD8+ and natural killer T cells into the tumors

To investigate how IFNγ regulates tumor growth in Smad7Tg mice, we evaluated the percentages of CD4+, CD8+, CD3+DX5+ natural killer (NK), and CD3+DX5+ NKT cells in LPMCs and TILs isolated from wild-type, Smad7Tg, and IFNγ−/−Smad7Tg at the end of the AOM/DSS protocol. Although not statistically significant, we observed an IFNγ-dependent accumulation of CD8+ and NKT cells in the LPMCs of Smad7Tg mice (Fig. 6A). However, the percentage of intratumoral CD8+ and NKT cells in IFNγ−/−Smad7Tg mice was similar to wild-type.
No significant differences were observed in CD4+ and NK cell accumulation in both LPMCs and TILs of the 3 groups of mice. Similar results on CD4+ and CD8+ T-cell accumulation in the tumor and peritumoral areas of wild-type, Smad7Tg, and IFNγ−/−Smad7Tg mice were obtained by immunofluorescent staining of colonic sections (Supplementary Fig. S4).

**IFNγ increases CD8+ and NK/NKT cell–mediated cytotoxicity in the tumoral and peritumoral areas of Smad7Tg mice**

Th1 cells are required to activate cytotoxic lymphocytes and to generate antitumor immune response in different experimental systems. Accordingly with the dominant Th1-mediated immune response observed in the Smad7Tg mice, the cytotoxicity-related markers Perforin1, Granzyme B, and FasL mRNA were upregulated in the TILs and LPMCs of these mice in an IFNγ-dependent manner (Fig. 7A). To assess whether CD8+ and NK/NKT cells isolated from Smad7Tg mice were more cytotoxic, we cocultured CD8+ and DX5+ cells sorted from mesenteric lymph nodes of wild-type, Smad7Tg, and IFNγ−/−Smad7Tg mice with YAC-1 lymphoma cell line. CD8+ and DX5+ cells isolated from Smad7Tg mice induced more YAC-1 cell death (Fig. 7B). The higher cytotoxic activity of Smad7Tg effector cells was completely reverted by the loss of IFNγ. Consistently, tumors developed in the Smad7Tg mice exhibited more terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL)-positive cells than neoplastic lesions developed in wild-type and IFNγ−/−Smad7Tg mice (Fig. 7C).

**Discussion**

Patients with IBD have an increased risk to develop CAC, which is related to the duration and extent of inflammation. Although circumstantial evidence suggests that severity of colitis could also affect the development of CAC, the role of inflammation in the initiation, promotion, and progression of CAC remains poorly characterized (22–24).

The Smad7-dependent block of the TGFβ signaling is thought to sustain chronic inflammation in IBD (11). Data of
In our mouse model, and similarly to human IBD, Smad7 overexpression in T cells caused more severe colitis as compared with wild-type. However, when we used Smad7Tg mice in the well-established AOM/DSS model of CAC, surprisingly, we observed a significant and highly reproducible reduction of tumor incidence even in the presence of more severe colitis. Analysis of the cytokines and transcription factors showed higher expression of the Th1-related markers IFNγ and Tbet in the peritumoral areas and even more in the tumors of Smad7Tg mice than in the wild-type. In contrast, the Th17-related markers IL-17, IL-6, and RORγt were relatively downregulated in the tumors of Smad7Tg mice thus indicating that a Th1-mediated immune response prevails in the transgenics.

Both Th1 and Th17 cells contribute to intestinal inflammation in different models of colitis and their differentiation is influenced by TGFβ (25–27). TGFβ mediates the differentiation of Th1 cells by directly inhibiting the expression of Tbet and IFNγ (3). In contrast, in the presence of IL-6, TGFβ promotes Th17 cell differentiation (28, 29). Moreover, recent studies indicate that the TGFβ-mediated suppression of Th1 cells and IFNγ expression promotes the generation of Th17 (30). These data fit well with our results showing that Smad7-mediated block of the TGFβ promotes the accumulation in the gut of Th1 cells and increases IFNγ expression while reducing the number of Th17 cells especially in the tumor microenvironment.

TGFβ has been also involved in the differentiation of Tregs, a subset of cells implicated in the suppression of the immune response against cancer. As observed in human IBD, the more severe inflammation observed in the Smad7Tg was associated with accumulation of Tregs in both the tumor and peritumoral areas thus ruling out the possibility that less FOXP3+ cells in the Smad7Tg mice may enhance the immune response against cancer (20).

Loss of IFNγ restored susceptibility to CAC in Smad7Tg. IFNγ has been shown to play a pivotal role in the immune response against cancer. In vivo, IFNγ expression protects from tumor in different tumor models (31–33). In a murine model of CAC, Osawa and colleagues showed that IFNγ-deficient mice are more susceptible to develop CAC (34). IFNγ expression is also essential for CD8-mediated antitumor activity. This effect can be partially explained by the capacity of IFNγ to induce Fas on dysplastic target cells surface thus inducing cell death by Fas ligand–Fas interaction (35, 36). Moreover, IFNγ increases the expression of MHC class I expression on target cells thus promoting recognition of tumor-associated antigens (37). IFNγ might also contribute to NK and NKT cell activation and killing activity. However, it is worth noting that the tumor score in the IFNγ−/− Smad7Tg was still lower than in the wild-type thus indicating that even in the absence of IFNγ, Smad7 overexpression in T cells can restrain tumor growth.

In the attempt of identifying the mechanism by which IFNγ determines protection against tumors in the Smad7Tg, we observed several effects linked to high expression of this cytokine. Indeed, the higher accumulation and cytotoxic activity of CD8+ and NKT cells observed in the Smad7Tg mice was reverted in the IFNγ−/− Smad7Tg mice. The high IFNγ-dependent activation and cytotoxic activity of CD8+ and DX5+ NK/NKT cells in the Smad7Tg might be sustained by the prevalent

![Image](cancerres.aacrjournals.org)
Th1 immune environment operating in these mice. Alternatively, it might be secondary to the Smad7-mediated block of the TGFβ signaling in these subsets of cells as previously reported (38, 39).

Although we were unable to provide direct demonstration of a stronger cytotoxic activity toward dysplastic epithelial cells in vivo, due to the difficulties encountered in culturing these cells ex vivo, these data strongly suggest the central role of IFNγ in the protection from colonic tumor development in our model.

We have previously shown that the block of the TGFβ signaling in mice overexpressing the dominant-negative form of the TGFβ receptor II (dnTGFβRII) in T cells develop more inflammation and more CAC (14). The opposite outcomes obtained in the dnTGFβRII and Smad7 transgenic mice might be explained considering the level at which the block of the TGFβ signaling occurs. Indeed, while the expression of dnTGFβRII blunts all the downstream TGFβ-dependent intracellular signals, the result of the Smad7-mediated block at intracellular level is less clear. In agreement with published data, we have previously shown that Smad7 prevents the activation of Smad2/3 (12). However, TGFβ is known to activate SMAD-independent pathways mediated by mitogen-activated protein kinases (i.e., p38, Akt, extracellular signal-regulated kinase), and the activation of these pathways is not affected by Smad7 expression (40). Accordingly, while dnTGFβRII transgenic mice develop a rapidly fatal multiorgan autoimmune disease, Smad7Tg mice did not (41). Therefore, a possibility is that in primary tumors, Smad7 might modulate, rather than fully inhibit, the TGFβ signaling in T cells and that the net result of Smad7 overexpression is an antitumor immune response instead of the generation of a tumor-promoting environment as in the case of dnTGFβRII transgenic mice. However, it is worth noting that in a different system, the block of TGFβ signaling obtained with the overexpression of the dnTGFβRII in CD4+ T cells protected mice from liver tumor metastasis (15), thus suggesting that the TGFβ intracellular signals required in T cells to favor tumor progression might be tumor stage dependent.

Another possibility to explain the different results obtained with the dnTGFβRII and the Smad7Tg mice relies on the fact that Smad7 overexpression could inhibit other members of the TGFβ superfamily [e.g., activin and bone morphogenetic protein (BMP)] whose role in the tumor immunity is currently poorly understood.

In our system, a persisting Th1-mediated immune response characterized by high expression of IFNγ was protective toward the development of CAC. This observation is in agreement with the emerging concept that an indolent but chronic inflammatory process sustains cancer promotion and progression rather than a highly detrimental acute inflammation. Translating this model into humans, one could envision that while acute flares of colitis are required to generate dysplastic cells, a phase of persisting subacute inflammation, allowed by low Smad7 expression, might be more effective at sustaining CAC growth. It is tempting to speculate that the Smad7 overexpression in T cells might block the evolution of the inflammatory process thus maintaining a condition resembling an acute flare in which emerging dysplastic cells are destroyed by the immune system rather than supported in their expansion.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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