Role of Smad7 in inflammatory bowel diseases

Giovanni Monteleone, Roberta Caruso, Francesco Pallone

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC), the major forms of inflammatory bowel diseases (IBD) in humans, are chronic inflammatory disorders of the gastrointestinal tract. In CD, the inflammation is typically transmural and can affect the gastrointestinal tract anywhere from the mouth to the anus. In contrast, the inflammatory lesion in UC is restricted to the mucosa and extends proximally from the rectum to involve all or part of the colon[1]. The cause of both IBD remains unknown even though there is evidence that the pathologic process results from the interaction of genetic, environmental and immunological factors[2-5]. Studies conducted in experimental models of colitis also suggest that IBD-associated tissue damage is due to an excessive immune response directed against to normal constituents of the bacterial microflora, which is inappropriately controlled

Abstract

Crohn's disease and ulcerative colitis, the major forms of inflammatory bowel diseases (IBD) in man, are complex diseases in which genetic and environmental factors interact to promote an excessive mucosal immune response directed against normal components of the bacterial microflora. There is also evidence that the pathologic process is due to defects in counter-regulatory mechanisms, such as those involving the immunosuppressive cytokine transforming growth factor (TGF)-β1. Indeed, studies in human IBD tissues and murine models of colitis have documented a disruption of TGF-β1 signalling marked by a block in the phosphorylation of Smad3, a signalling molecule associated with the activated TGF-β receptor, due to up-regulation of Smad7, an intracellular inhibitor of Smad3 phosphorylation. Knock-down of Smad7 with a specific antisense oligonucleotide restores TGF-β1/Smad3 signalling, thus resulting in a marked suppression of inflammatory cytokine production and attenuation of murine colitis. These findings together with the demonstration that Smad7 antisense oligonucleotide is not toxic when administered in mice have paved the way for the development of a Smad7 antisense oligonucleotide-based pharmaceutical compound that is now ready to enter the clinics. In this article we review the available data supporting the pathogenic role of Smad7 in IBD and discuss whether and how Smad7 antisense therapy could help dampen the ongoing inflammation in IBD.

Key words: Inflammatory bowel diseases; Gut inflammation; Transforming growth factor-β1; Smad7; Antisense oligonucleotides

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC), the major forms of inflammatory bowel diseases (IBD) in humans, are chronic inflammatory disorders of the gastrointestinal tract. In CD, the inflammation is typically transmural and can affect the gastrointestinal tract anywhere from the mouth to the anus. In contrast, the inflammatory lesion in UC is restricted to the mucosa and extends proximally from the rectum to involve all or part of the colon[1]. The cause of both IBD remains unknown even though there is evidence that the pathologic process results from the interaction of genetic, environmental and immunological factors[2-5]. Studies conducted in experimental models of colitis also suggest that IBD-associated tissue damage is due to an excessive immune response directed against to normal constituents of the bacterial microflora, which is inappropriately controlled
by counter-regulatory mechanisms\(^6\). An example of such mechanisms is that involving transforming growth factor (TGF)-β1, a pleiotropic cytokine with potent immune-suppressive activity. Indeed, mice deficient in TGF-β1 or unable to respond to the cytokine develop intestinal inflammation\(^8\)–\(^14\), while induction of TGF-β1 protects or attenuates colitis in some murine models of IBD\(^6\)–\(^14\). TGF-β1 activity is mediated by binding of the cytokine to a heterodimeric transmembrane serine/threonine kinases receptor, consisting of type I (TGF-βR I) and type II (TGF-βR II) subunits\(^15\)–\(^16\). In particular, binding of TGF-β1 to TGF-βR II promotes phosphorylation and activation of TGF-βR I, which in turn phosphorylates/activates Smad2 and Smad3. These two Smad proteins form then a heterocomplex with Smad4, which translocates to the nucleus and regulates the functional activities of many target genes\(^15\)–\(^17\). Smad3-deficient mice exhibit a diminished cell responsiveness to TGF-β1 and spontaneously develop a chronic inflammation in the stomach and colon, supporting the anti-inflammatory role of TGF-β1-associated Smad3 in the gastrointestinal tract\(^18\). In this article we review the current information on the activity of TGF-β1 in IBD and murine models of colitis and discuss data showing that restoring TGF-β1 signaling is sufficient to dampen intestinal inflammation.

**DEFECTIVE TGF-β1 ACTIVITY ASSOCIATES WITH HIGH SMAD7 IN IBD**

Active TGF-β1 is highly produced in the human gastrointestinal tract of healthy individuals and supposed to play a decisive role in negatively controlling inflammatory pathways\(^19\). Lamina propria mononuclear cells (LPMC) isolated from the normal human colon express high levels of phosphorylated (p)-Smad3, and respond to exogenous TGF-β1 with a marked inhibition of inflammatory cytokines\(^20\). On the other hand, neutralization of endogenous TGF-β1 in cultures of normal mucosal explants and LPMC results in enhanced synthesis of inflammatory cytokines\(^20\). A different scenario emerges when p-Smad3 is analyzed in LPMC and tissue samples of patients with IBD. Indeed, in both CD and UC, phosphorylation of Smad3 is markedly reduced as compared to normal controls, despite TGF-β1 is produced at high level in these patients\(^20\)–\(^23\). Moreover, IBD LPMC do not phosphorylate Smad3 and produce huge amounts of inflammatory cytokines when stimulated with exogenous TGF-β1\(^20\)–\(^23\).

The Smad family also includes two Smad inhibitors, namely Smad6 and Smad7. Smad7 associates stably with the ligand-activated TGF-βR I complex and blocks phosphorylation of Smad2/Smad3\(^23\)–\(^25\). Attenuation of TGF-β1 signaling by Smad7 is also dependent on the ability of Smad7 to induce degradation of TGF-βRII after recruitment of E3 ubiquitin ligases and to recruit protein phosphatase 1 to the receptor complex via interaction with growth arrest and DNA damage protein 34\(^24\). Since up-regulation of Smad7 associates with inhibition of TGF-β1-induced Smad2/3 activation, we explored the possibility that the diminished activation of TGF-β1-associated Smad signaling in IBD could be related to high Smad7. To this end, we examined Smad7 in the same intestinal samples analyzed for Smad3 phosphorylation. Smad7 was over-expressed in whole mucosal and LPMC samples of CD patients and UC patients as compared to controls\(^25\). Interestingly, silencing of Smad7 with an anti-sense oligonucleotide restored the responsiveness of IBD LPMC to exogenous TGF-β1, as indicated by the enhanced phosphorylation of Smad3 and diminished synthesis of inflammatory cytokines\(^25\). Similar results were seen in co-culture organ cultures of CD mucosal explants, in which inhibition of Smad7 with antisense oligonucleotide associated with high Smad3 phosphorylation and decreased inflammatory cytokine production. These effects were blocked by a neutralizing TGF-β1 antibody, clearly indicating that Smad7 knockdown allows endogenous TGF-β1 to act and suppress inflammatory signals\(^26\).

High Smad7 expression and defective TGF-β-associated Smad3 activation are not however specific hallmark of IBD, because similar alterations were documented in the stomach of patients with Helicobacter pylori-related gastritis\(^27\). By contrast, Smad7 expression is not up-regulated in the duodenum of patients with active celiac disease\(^28\), raising the possibility that induction of Smad7 in the gut is not an epiphenomenon of the ongoing inflammation but rather can reflect changes in the local inflammatory milieu.

**SMAD7 EXPRESSION IS POST-TRANSCRIPTIONALLY REGULATED IN IBD**

Further experimentation was performed to understand how Smad7 expression is regulated in IBD. Since experiments with cell lines have shown that Smad7 can be induced by activators of nuclear factor-κB \(\text{e.g., interferon (IFN)}-\gamma\) and \(\text{tumor necrosis factor (TNF)}-\alpha\) and interleukin (IL)-1 \(\text{β}\) and STAT-1 \(\text{[e.g., interferon (IFN)}-\gamma\) and IL-7\] and these transcription factors are hyper-activated in IBD\(^29\)–\(^34\), we initially hypothesized that Smad7 in CD and UC tissue was regulated by these signaling pathways. However, functional studies showed that Smad7 protein expression remained unchanged in IBD LPMC following treatment with IFN-γ/Stat1 or TNF-α/NF-kB inhibitors\(^29\)–\(^31\). Smad7 is also rapidly induced by TGF-β1-driven Smad3 signaling, thus representing an important effector in the feedback loop that restrains TGF-β1 activity\(^31\). We feel however it is unlikely that high Smad7 is induced by TGF-β1 in IBD, because p-Smad3 was reduced in samples exhibiting enhanced Smad7 expression\(^30\). Analysis of Smad7 RNA expression revealed that IBD and control samples contained similar levels of transcripts, raising the possibility that, in IBD, Smad7 is post-transcriptionally regulated\(^34\). Indeed, Smad7 was found to be ubiquitinated and targeted for proteasome
degradation in control but not IBD samples. Difference in the level of ubiquitination of Smad7 between IBD and controls seems to be secondary to the different status of acetylation of the protein, because acetylation and ubiquitination compete for the same lysine residues of Smad7[29,30]. Thus, acetylation prevents ubiquitination and protects Smad7 protein against proteosomal degradation.[27] Smad7 was highly acetylated in vitro in IBD but not control samples.[29] We have also shown that in IBD, the transcriptional coactivator p300 interacted with and promoted Smad7 acetylation, and that silencing of p300 diminished acetylation and expression of Smad7[29].

INHIBITION OF SMAD7 ATTENUATES GUT INFLAMMATION IN MICE

The mouse models of Th1 or Th2-mediated colitis induced by intra-rectal administration of trinitrobenzene sulfonic acid (TNBS) or oxazolone and showing immunological similarities with CD or UC, respectively,[13,31,32] were used to confirm the inflammatory role of Smad7. In both these models, colitis was marked by enhanced production of TGF-β1, reduced phosphorylation of Smad3 and high expression of Smad7[31]. Oral Smad7 antisense oligonucleotide was taken-up by epithelial cells and LPMC in the small intestine and colon and induced no toxicity. This treatment reduced Smad7 and restored TGF-β1-associated p-Smad3 expression and ameliorated both forms of colitis[31]. Analysis of inflammatory cytokines in the colon of treated mice revealed that restoration of TGF-β1 signaling by inhibition of Smad7 resulted in a significant down-regulation of IL-12 and IFN-γ and reduced expression of Th1-associated transcription factors (i.e., T-bet and Stat1) in TNBS-colitis, and reduced production of IL-4 in mice with oxazolone-induced colitis.[31]. These studies suggest that resolution of gut inflammation may be accomplished by down-regulating Smad7 and allowing endogenous TGF-β1 to inhibit inflammatory pathways which promote tissue injury.

SMAD7 ANTISENSE IS SAFE AND TOLERATED IN PATIENTS WITH CD

Altogether the above data indicate that Smad7 is a molecular target for direct therapeutic interventions in IBD. To this end, we have recently developed a pharmaceutical compound containing the Smad7 antisense oligonucleotide and formulated it as a solid oral dosage form. The formulation is protected by an external coat, which allows the antisense oligonucleotide to transit through the stomach and proximal small intestine and reach the terminal ileum and right colon where the active compound is released[31-34]. To examine whether this drug was safe and tolerated, we conducted a phase 1, open-label study in patients with active, steroid-dependent/resistant CD. Fifteen patients were allocated to three treatment groups receiving oral Smad7 antisense oligonucleotide, once daily for 7 d at doses of 40, 80 or 160 mg. The drug was well-tolerated and no patient experienced serious adverse event (AE). Twenty-five AEs were registered in 11 patients, but the majority of AEs was mild and considered unrelated to treatment. Interestingly, treatment associated with a significant reduction in the fraction of circulating CCR9-positive T cells secreting IFN-γ and IL-17A[34]. This finding could be therapeutically relevant as CCR9-positive T cells represent a subset of inflammatory T cells with gut homing properties, which are increased in the active phases of CD[30]. Moreover, administration of Smad7 antisense oligonucleotide to patients resulted in a significant clinical benefit[16].

CONCLUSION

TGF-β1 plays a critical role in the control of gut immune homeostasis and therefore defects in the production and/or activity of the cytokine inevitably associate with pathological responses[8,9,36]. This is for example seen in the inflamed gut of IBD patients and mice with experimental colitis, where TGF-β1 is unable to signal and dampen inflammatory signals due to high Smad7, a protein which binds to TGF-βR and prevents TGF-β1/Smad3 signaling[20,21,33]. Such an alteration is not however irreversible, as knockdown of Smad7 with a specific antisense oligonucleotide restores TGF-β1 activity, inhibits inflammatory cytokine production and attenuates colitis in mice[20,33]. In line with this, results of an exploratory phase 1 trial, testing the Smad7 antisense oligonucleotide in active CD patients, have recently shown that the compound is safe and tolerable and that such treatment attenuates signs and symptoms of CD, supporting the pathogenic role of Smad7 in CD[34]. Appropriately designed placebo-controlled clinical trials will however be necessary to confirm the safety profile of this approach, show if Smad7 is a valid therapeutic target in CD, and examine the long-term risks of Smad7 knockdown. In particular, it would be relevant to exclude the possibility that restoring TGF-β1 activity favors the development of strictures[37], given that TGF-β1 is a powerful inducer of fibrogenesis in many organs[38-40]. TGF-β1 can also exert various effects on the cellular and molecular mechanisms involved in tumor development. In particular TGF-β1 can act as a tumor suppressor in early stages of tumorigenesis and promote advanced tumor cell invasiveness and metastasis[41-44]. Similarly, Smad7 may regulate differently tumorigenesis depending on the cell context analyzed. For example, over-expression of Smad7 promotes initiation of pancreatic cancer, skin cancer, sclerodermia and lung cancer, associates with a worse prognosis in sporadic colorectal cancer (CRC)[40] and enhances liver metastasis in a murine model of CRC[40]. Moreover, genome-wide association studies have shown that common alleles of Smad7 can influence the risk of CRC[40] and that patients carrying on the rs12953717 single nucleotide polymorphism in the SMAD7 gene
have increased risk of CRC. In contrast, Smad7 inhibits metastasis of human melanoma cells to bone and me-
tastasis of human breast cancers to the lung or liver.\cite{47,48} We have recently shown that over-expression of Smad7 in T cells and natural killer cells makes worse the course of experimental colitis yet protects from the develop-
ment of colitis-associated colon-cancer\cite{51}. Therefore, further experimentation will be also needed to ascertain if inhibition of Smad7 in the inflamed gut can enhance the risk of colitis-associated CRC.

REFERENCES

1. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med 2009; 361: 206-2078.
2. Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002; 347: 417-429.
3. Xavier RJ, Podolsky DK. Unraveling the pathogenesis of inflammatory bowel disease. Nature 2007; 448: 427-434.
4. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Ann Rev Immunol 2010; 28: 573-621.
5. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. Nat Rev Immunol 2008; 8: 438-466.
6. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003; 3: 521-533.
7. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. J Clin Invest 2007; 117: 514-521.
8. Kulkarni AB, Karlsson S. Transforming growth factor-beta 1 knockout mice. A mutation in one cytokine gene causes a dramatic inflammatory disease. Am J Pathol 1995; 143: 3-9.
9. Gorenlik L, Flavell RA. Transforming growth factor-beta in T-cell biology. Nat Rev Immunol 2002; 2: 46-53.
10. Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci USA 1993; 90: 770-774.
11. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzl G, Calvin D. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature 1992; 359: 693-699.
12. Neurath MF, Fuss I, Kelsall BL, Presky DH, Waegell W, Strober W. Experimental granulomatous colitis in mice is abrogated by induction of TGF-beta-mediated oral tolerance. J Exp Med 1996; 183: 2605-2616.
13. Boirivant M, Fuss I, Chu A, Strober W. Oxazolone colitis: A murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. J Exp Med 1998; 188: 1292-1393.
14. Powrie F, Carlino J, Leach MW, Mauze S, Coffman RL. A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) CD4+ T cells. J Exp Med 1996; 183: 2669-2674.
15. Heldin CH, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature 1997; 390: 465-471.
16. Franzen P, ten Dijke P, Ichijo H, Yamashita H, Schulz P, Heldin CH, Miyazono K. Cloning of a TGF beta type I receptor that forms a heteromeric complex with the TGF alpha beta receptor type II receptor. Cell 1993; 75: 681-692.
17. Nakao A, Inamurai T, Souchelnytskyi S, Kawabata M, Ishi-
saki A, Oeda E, Tamaki K, Hanai J, Heldin CH, Miyazono K, ten Dijke P. TGF-beta receptor-mediated signalling through Smad2, Smad3 and Smad4. EMBO J 1997; 16: 5353-5362.
18. Yang X, Letterio JI, Lechleider RJ, Chen L, Hayman R, Gu H, Roberts AB, Deng C. Targeted disruption of Smad3 results in impaired mucosal immunity and diminished T cell re-
sponsiveness to TGF-beta. EMBO J 1999; 18: 1280-1291.
19. Di Sabatino A, Pickard KM, Rampton D, Kruidenier L, Ro-
vadati L, Leakey NA, Corazza GR, Monteleone G, MacDo-
nald TT. Blockade of transforming growth factor beta 1 regu-
lates T-box transcription factor T-bet, and increases T helper cell type 1 cytokine and matrix metalloproteinase 3 production in the human gut mucosa. Gut 2008; 57: 605-612.
20. Monteleone G, Kumberova A, Croft NM, McKenzie C, Steer HW, MacDonald TT. Blocking Smad7 restores TGF-

beta1 signaling in chronic inflammatory bowel disease. J Clin Invest 2001; 108: 601-609.
21. Babylatsky MW, Rossiter G, Podolsky DK. Expression of transforming growth factors alpha and beta in colon mu-
cosa in inflammatory bowel disease. Gastroenterology 1996; 110: 975-984.
22. Monteleone G, Mann J, Monteleone I, Vavassori P, Bremner R, Fantini M, Del Vecchio Blanco G, Tersigni R, Alessandro-
ni L, Mann D, Pallone F, MacDonald TT. A failure of transform-
growth factor beta 1 negative regulation maintains sustained NF-kappaB activation in gut inflammation. J Biol Chem 2004; 279: 3925-3932.
23. Nakao A, Arafah M, Morán A, Nakayama T, Christian J L, Heuchel R, Itoh S, Kawabata M, Heldin NE, Heldin CH, ten Dijke P. Identification of Smad7, a TGF-beta-inducible anti-
tagonist of TGF-beta signalling. Nature 1997; 390: 631-635.
24. Shi W, Sun C, He B, Xiong W, Shi X, Yao D, Cao X. GADDS4-PTPc recruited by Smad7 dephosphorylates TGF-

beta type I receptor. J Cell Biol 2004; 164: 291-300.
25. Monteleone G, Del Vecchio Blanco G, Palmieri G, Vavas-
sori P, Monteleone I, Colantoni A, Battista S, Spagnoli LG, Romano M, Borrelli M, MacDonald TT, Pallone F. Induction and regulation of Smad7 in the gastric mucosa of patients with Helicobacter pylori infection. Gastroenterology 2004; 126: 674-682.
26. Benhamed M, Meresse B, Arnulf B, Barbe U, Mention J J, Verkarre V, Allez M, Cellier C, Hermine O, Cerf-Bensussan N. Inhibition of TGF-beta signalling by IL-15: a new role for IL-15 in the loss of immune homeostasis in celiac disease. Gastroenterology 2007; 132: 994-1008.
27. Monteleone G, Pallone F, MacDonald TT. Smad7 in TGF-

beta-mediated negative regulation of gut inflammation. Trends Immunol 2004; 25: 513-517.
28. Monteleone G, Del Vecchio Blanco G, Monteleone I, Fina D, Caruso R, Gioia V, Ballerini S, Federici G, Bernardini S, Pallone F, MacDonald TT. Post-transcriptional regulation of Smad7 in the gut of patients with inflammatory bowel dis-
ease. Gastroenterology 2005; 129: 1420-1429.
29. Simonsson M, Heldin CH, Ericsson J, Grönroos E. The bal-
ance between acetylation and deacetylation controls Smad7 stability. J Biol Chem 2005; 280: 21797-21803.
30. Grönroos E, Hellman U, Heldin CH, Ericsson J. Control of Smad7 stability by competition between acetylation and ubiquitination. Mol Cell 2002; 10: 483-493.
31. Neurath MF, Fuss I, Kelsall BL, Stübner E, Strober W. Anti-
body-mediated interleukin 12 abrogates established experimental colitis in mice. J Exp Med 1995; 182: 1281-1290.
32. Strober W, Fuss IJ, Blumberg RS. The immunology of mu-
cosal models of inflammation. Annu Rev Immunol 2002; 20: 495-549.
33. Boirivant M, Pallone F, Di Giacinto C, Fina D, Monteleone I, Marinaro M, Caruso R, Colantoni A, Palmieri G, Sanchez M, Strober W, MacDonald TT, Monteleone G. Inhibition of Smad7 with a specific antisense oligonucleotide facilitates TGF-beta-mediated suppression of colitis. Gastroenterology 2006; 131: 1786-1798.
34. Monteleone G, Fantini MC, Onali S, Zorzzi F, Sancesario G, Bernardini S, Calabrese E, Viti F, Monteleone I, Biancone L, Pallone F. Phase 1 clinical trial of Smad7 knockdown using antisense oligonucleotide in patients with active Crohn’s disease.
disease. *Mol Ther* 2012; 20: 870-876

35 Saruta M, Yu QT, Avanesyan A, Fleshner PR, Targan SR, Papadakis KA. Phenotype and effector function of CC chemokine receptor 9-expressing lymphocytes in small intestinal Crohn’s disease. *J Immunol* 2007; 178: 3293-3300

36 Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. *Ann Rev Immunol* 1998; 16: 137-161

37 Burke JP, Mulsow JJ, O’Keane C, Docherty NG, Watson RW, O’Connell PR. Fibrogenesis in Crohn’s disease. *Am J Gastroenterol* 2007; 102: 439-448

38 Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. *FASEB J* 2004; 18: 816-827

39 Verrecchia F, Mauviel A. Transforming growth factor-beta and fibrosis. *World J Gastroenterol* 2007; 13: 3056-3062

40 Vallance BA, Gunawan MI, Hewlett B, Bercik P, Van Kampen C, Galeazzi F, Sime PJ, Gauldie J, Collins SM. TGF-beta1 gene transfer to the mouse colon leads to intestinal fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2005; 289: G116-G128

41 Akhurst RJ, Derynck R. TGF-beta signaling in cancer—a double-edged sword. *Trends Cell Biol* 2001; 11: 544-551

42 Roberts AB, Wakefield LM. The two faces of transforming growth factor beta in carcinogenesis. *Proc Natl Acad Sci USA* 2003; 100: 8621-8623

43 Yang L, Pang Y, Moses HL. TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. *Trends Immunol* 2010; 31: 220-227

44 Boulay JL, Mild G, Lowy A, Reuter J, Lagrange M, Terracciano L, Lafer U, Herrmann R, Rochlitz C. SMAD7 is a prognostic marker in patients with colorectal cancer. *Int J Cancer* 2003; 104: 446-449

45 Halder SK, Rachakonda G, Deane NG, Datta PK. Smad7 induces hepatic metastasis in colorectal cancer. *Br J Cancer* 2008; 99: 957-965

46 Slattery ML, Herrick J, Curtin K, Samowitz W, Wolff RK, Caan BJ, Duggan D, Potter JD, Peters U. Increased risk of colon cancer associated with a genetic polymorphism of SMAD7. *Cancer Res* 2010; 70: 1479-1485

47 Briones-Orta MA, Tecalco-Cruz AC, Sosa-Carrocho M, Carligaris C, Macias-Silva M. Inhibitory Smad7: emerging roles in health and disease. *Curr Mol Pharmacol* 2011; 4: 141-153

48 Kuang C, Xiao Y, Liu X, Stringfield TM, Zhang S, Wang Z, Chen Y. In vivo disruption of TGF-beta signaling by Smad7 leads to premalignant ductal lesions in the pancreas. *Proc Natl Acad Sci USA* 2006; 103: 1858-1863

49 Halder SK, Beauchamp RD, Datta PK. Smad7 induces tumorigenicity by blocking TGF-beta-induced growth inhibition and apoptosis. *Exp Cell Res* 2005; 307: 231-246

50 Bornstein S, Hoot K, Han GW, Lu SL, Wang XJ. Distinct roles of individual Smads in skin carcinogenesis. *Mol Carcinog* 2007; 46: 660-664

51 Rizzo A, Waldner MJ, Stolfi C, Sarra M, Fina D, Becker C, Neurath MF, Macdonald TT, Pallone F, Monteleone G, Fantini MC. Smad7 expression in T cells prevents colitis-associated cancer. *Cancer Res* 2011; 71: 7423-7432

S- Editor Gou SX L- Editor A E- Editor Xiong L