Published in final edited form as:
Gastroenterology. 2009 July ; 137(1): 33–36. doi:10.1053/j.gastro.2009.05.011.

Intersection of Transforming Growth Factor-β and *Wnt* Signaling Pathways in Colorectal Cancer and Metastasis

JOHN M. CARETHERS
Department of Medicine and Rebecca and John Moores Comprehensive Cancer Center University of California, San Diego and VA San Diego Healthcare System San Diego, California

Colorectal cancers develop, mature, and metastasize when a compendium of genetic and epigenetic lesions in a colonic stem cell accumulate to cause dysregulation of a variety of cellular processes, including multiple growth regulatory signaling pathways.[1] Genomic instability seems to drive the process of formation of colorectal cancers from normal colonic epithelium to adenoma to carcinoma, and perhaps through metastasis. This genomic instability—either chromosomal instability, microsatellite instability, or forms of epigenetic instability—may seem to damage the genome randomly, but ultimately accrues a nonrandom aggregation of very specific targets that are deregulated and allow the establishment of an initial colorectal neoplasm, or further drive an early neoplasm to malignant transformation and eventual distal spread.[1]

The initiation of colorectal neoplasms is believed to occur when there is dysregulation of Wnt signaling, a critical pathway in embryogenesis and colonic homeostasis in the adult.[1,2] The critical effectors of Wnt signaling include the *adenomatous polyposis coli* (*APC*) tumor suppressor gene product, and the proto-oncogene *β-catenin.* APC regulates the cytoplasmic and eventual nuclear concentration of *β-catenin* by promoting its proteosome mediated degradation, thus preventing *β-catenin's* ability to combine with specific T-cell factor transcription factors to promote growth proliferation.[3] In early colorectal neoplasms, Wnt signaling is deregulated often by acquired mutation and loss of heterozygosity (LOH) of *APC*, thus removing the colonocyte's capability to regulate intracellular compartmental *β-catenin* concentration. The phenotype of colorectal neoplasia initiation is highlighted in the extreme case with familial adenomatous polyposis, in which patients harbor a germline mutation in *APC* and develop multiple adenomas by their teenage years. Both sporadic and familial adenomatous polyposis-associated adenomas show high nuclear concentrations of *β-catenin*, consistent with complete inactivation of APC function.[1]

A key signaling cascade that seems to be involved during adenoma progression and metastasis is that of transforming growth factor (TGF)-*β*.[1] TGF-*β*, belonging to a ligand-receptor family that also includes bone morphogenetic protein and activin,[4-6] is often excessively produced in colorectal cancers, presumably owing to loss of feedback inhibition with disruption of its intracellular SMAD signaling pathway.[1] There seems to be phased-in mechanisms during neoplastic progression to disrupt TGF-*β* tumor suppressor signaling down its canonical receptor-SMAD cascade. In early adenomas, activated mutant *K-RAS* that is observed in ~50% of adenomas and colorectal cancers slows TGF-*β* signaling by phosphorylating a linker domain within SMAD2 and SMAD3, braking SMAD translocation

Address requests for reprints to: John M. Carethers, MD, University of California, San Diego, Division of Gastroenterology, UC303, MC 0063, 9500 Gilman Drive, La Jolla, California 92093-0063. jcarethers@ucsd.edu; fax: 858-534-3337..

Conflicts of interest
The author declares no conflicts of interest.

to the nucleus to execute growth suppression. [1,7] Later adenomas acquire LOH of chromosome 18q, the genetic location of SMAD2 and SMAD4, which then completely disrupts the TGF-β-SMAD suppression cascade.[1] In liver metastasis from colorectal cancer patients, there is increased LOH of chromosomal 18q, suggesting the selection for cells that abate these TGF-β pathway components and which portends a poorer prognosis for patients.[8,9] The autocrine activity from elevated secretion of TGF-β ligand has further consequences in that signaling through SMAD-independent pathways, unmasked with interruption of SMAD-dependent signaling, enhance cell proliferation and cell motility, 2 phenotypes consistent with metastatic behavior.[10]

Do the Wnt and TGF-β pathways cooperate for colorectal tumor progression? The answer is yes, with murine intestinal tumors that are initiated by *Apc* mutation showing a more aggressive phenotype when *Tgfbr2* is conditionally knocked out in intestinal epithelial cells.[11] Another mechanism to silence TGF-β signaling, in addition to genetic ablation of its receptor, is the deployment of the BMP and activin membrane-bound inhibitor (BAMBI), a pseudoreceptor that is related to TGF-β receptor type I, but lacks an intracellular kinase domain that is important in activating intracellular SMADs.[12] BAMBI resembles the homodimerization domain of TGF-β receptor type I to act as a decoy to prevent the formation of receptor complexes between the type I and type II receptors after TGF-β ligand binding.[12] BAMBI is aberrantly elevated in most colorectal cancers when compared with matched normal colon tissue from the same patient, and Wnt signaling, as evidenced by experimental interruption of β-*catenin* or T-cell factor nuclear transcriptional activity, induces the expression of BAMBI.[3,13] Thus, Wnt signaling through β-*catenin* activation transcriptionally activates BAMBI as a mechanism to block TGF-β signaling. Additionally, there is evidence for positive feedback regulation by BAMBI on Wnt signaling.[3] BAMBI interacts with the Wnt receptor Frizzled5 and its co-receptor LRP6, promotes the nuclear localization of β-catenin, and overexpression of BAMBI promotes Wnt/β-catenin transcriptional activity, including the expression of *c-myc* and *cyclin D1*, 2 of Wnt/β-catenin's transcriptional targets.[2] Last, perhaps as a countermeasure to negatively regulate its own pathway, TGF-β SMAD signaling can also induce *BAMBI* expression, because the *BAMBI* gene contains SMAD-binding elements in its promoter.[13] Thus, BAMBI can be induced by both Wnt signaling and TGF-β-SMAD signaling to positively regulate Wnt signaling and negatively regulate TGF-β signaling. The overall effect of BAMBI would be to increase cellular growth through enhancement of Wnt proliferative signaling and inhibition of TGF-β-SMAD suppressive signaling. These pathways are highlighted in Figure 1.

With BAMBI predicted to enhance cell growth, a larger question is it involved in metastatic behavior? The answer was initialized through utilization of gene microarray techniques. In this issue of Gastroenterology, Fritzmann et al[14] examined and compared gene expression patterns from 41 metastatic and nonmetastatic primary tumors (patients who survived ≥ 5 years after diagnosis). At their initial look, colorectal metastases were not very different from primary tumors on a global scale; however, approximately 115 genes were identified that could separate metastatic from nonmetastatic primary tumors, and this “metastatic genetic signature” was present in the primary colorectal cancer and in 50 metastases to lymph nodes, liver, and lung,[14] suggesting a predestined state or acquirement of metastatic capabilities at the primary tumor location that is similar to its eventual metastases. One of the elevated genes in the metastatic signature profile was *BAMBI*, which the authors found elevated expression in about half of primary metastatic colorectal tumors as well as in subgroups of metastases to lymph node and liver. In generating Kaplan-Meier survival curves based on BAMBI expression, a significant survival disadvantage ($P = .02$) became apparent for patients whose tumors had high BAMBI expression.

To further understand the mechanism of elevated BAMBI expression and its poorer prognosis, Fritzmann et al teased apart Wnt and TGF-β signaling, and studied a human cell injection murine model for metastasis. Wnt signaling through β-*catenin* up-regulates *BAMBI* expression, and this upregulation by β-catenin requires the coactivator BCL9-2, as siRNA knockdown of BCL9-2 (and not BCL9) caused significant down-regulation of *BAMBI* mRNA.[14] In turn, siRNA knockdown of BCL9-2 (or knockdown of *BAMBI*) increased TGF-β signaling responses, consistent with endogenous BAMBI (and BCL9-2) inhibiting TGF-β signaling. BAMBI overexpression increased colorectal cancer cell motility 4-fold over controls, and injecting BAMBI-overexpressing cells into spleens of nude mice produced liver metastases in about 50% of mice, compared with 0% liver metastases by control cells. All in all, Fritzmann et al make a convincing argument for the involvement of BAMBI in metastasis, through human patient survival curves, cell motility measurements, and a model of murine metastasis, as well as confirmed and enhanced the regulatory understanding of Wnt/β-*catenin* activation of BAMBI (with BCL9-2 as a required co-activator) and its subsequent negative regulation of TGF-β signaling.

The context for these observations lies in how TGF-β signaling can in 1 instance be a growth suppressor, and in another instance be a metastasis enhancer. There is growing evidence for more complex and divergent signaling pathways as a consequence of TGF-β binding to its receptors. At 1 level of regulation, TGF-β-SMAD signaling is growth suppressive and its cells are anti-migratory; this pathway remains intact until mechanisms evolve during colorectal tumorigenesis that mute or abate TGF-β-SMAD signaling.[1,7,10] Subsequently, normally less dominant TGF-β-SMAD-independent pathways become apparent, and these pathways are very growth proliferative and enhance cell migration.[1,10] This can translate into differences in patient survival. For instance, patients with microsatellite-unstable colon cancers that had intact *TGFBR2* expression (but presumed SMAD-dependent signaling interruption) showed a poorer survival than patients whose tumors acquired mutations in *TGFBR2*, which would abrogate SMAD-independent signaling as well as SMAD-dependent signaling.[9] BAMBI is the ideal molecule to mimic loss of *TGFBR2*, but carries the opposite prognosis for patients in 2 recent studies.[14,15] How can this be? Some questions need to be answered. First, is there any TGF-β signaling activity in the absence of the type I and type II receptors? A finding confirming this would suggest even a more complex and comprehensive effect of the TGF-β ligand. Second, does BAMBI's mimicry of the type I receptor completely abrogate TGF-β's ability to signal through its type II receptor? Are there any extensive cross-talk capabilities between the type I and type II receptors of TGF-β, activin and BMPs? Alternatively, are the survival effects of BAMBI different than that of *TGFBR2* mutation because of its positive regulation of Wnt signaling? These questions require more study to approach these pathways as therapeutic targets for improving patient survival.

The study by Fritzmann et al as well as others highlight how 1 molecule, BAMBI, can affect 1 key colonic signaling pathway in opposition to another key colonic signaling pathway to drive colorectal cancer progression and metastasis. The intersection of these key pathways with shared components may afford clever attempts to regulate the effects on both pathways. It is precisely these studies that will allow the investigation of scientific approaches that ultimately may improve outcomes for patients with metastatic colorectal cancer.

Acknowledgments

Funding

Supported by the U.S. Public Health Service (DK067287), the UCSD Digestive Diseases Research Development Center (DK080506), and the Veterans Administration Research Service.

References

1. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology. 2008; 135:1079–1099. [PubMed: 18773902]
2. Lin Z, Gao C, Ning Y, et al. The pseudoreceptor BMP and activin membrane-bound inhibitor positively modulates Wnt/beta-catenin signaling. J Biol Chem. 2008; 283:33053–33058. [PubMed: 18838381]
3. Sekiya T, Adachi S, Kohu K, et al. Identification of BMP and activin membrane-bound inhibitor (BAMBI), an inhibitor of transforming growth factor-beta signaling, as a target of the beta-catenin pathway in colorectal tumor cells. J Biol Chem. 2004; 279:6840–6846. [PubMed: 14660579]
4. Beck SE, Jung BH, Fiorino A, et al. Bone morphogenetic protein signaling and growth suppression in colon cancer. Am J Physiol Gastrointest Liver Physiol. 2006; 291:G135–G145. [PubMed: 16769811]
5. Jung B, Doctolero RT, Tajima A, et al. Loss of activin receptor type 2 protein expression in microsatellite unstable colon cancers. Gastroenterology. 2004; 126:654–659. [PubMed: 14988818]
6. Jung BH, Beck SE, Cabral J, et al. Activin type 2 receptor restoration in MSI-H colon cancer suppresses growth and enhances migration with activin. Gastroenterology. 2007; 132:633–644. [PubMed: 17258738]
7. Kretzschmar M, Doody J, Timokhina I, et al. A mechanism of repression of TGFbeta/ Smad signaling by oncogenic Ras. Genes Dev. 1999; 13:804–816. [PubMed: 10197981]
8. Carethers JM, Hawn MT, Greenson JK, et al. Prognostic significance of allelic loss at chromosome 18q21 for stage II colorectal cancer. Gastroenterology. 1998; 114:1188–1195. [PubMed: 9609755]
9. Watanabe T, Wu TT, Catalano PJ, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med. 2001; 344:1196–1206. [PubMed: 11309634]
10. Chow JYC, Cabral JA, Chang J, et al. TGF-beta modulates PTEN expression independently of SMAD signaling for growth proliferation in colon cancer cells. Cancer Biol Ther. 2008; 7:1694–1699. [PubMed: 18769113]
11. Munoz NM, Upton M, Rojas A, et al. Transforming growth factor beta receptor II inactivation induces the malignant transformation of intestinal neoplasms initiated by Apc mutation. Cancer Res. 2006; 66:9837–9844. [PubMed: 17047044]
12. Onichtchouk D, Chen YG, Dosch R, et al. Silencing of TGF-beta signalling by the pseudoreceptor BAMBI. Nature. 1999; 401:480–485. [PubMed: 10519551]
13. Sekiya T, Oda T, Matsuura K, et al. Transcriptional regulation of the TGF-beta pseudoreceptor BAMBI by TGF-beta signaling. Biochem Biophys Res Commun. 2004; 320:680–684. [PubMed: 15240101]
14. Fritzmann J, Morkel M, Besser D, et al. Colorectal cancer expression profile that includes transforming growth factor ss inhibitor BAMBI predicts metastatic potential. Gastroenterology. 2009; 137:165–175. [PubMed: 19328798]
15. Togo N, Ohwada S, Sakurai S, et al. Prognostic significance of BMP and activin membrane-bound inhibitor in colorectal cancer. World J Gastroenterol. 2008; 14:4880–4888. [PubMed: 18756595]

See “A colorectal cancer expression profile that includes transforming growth factor β inhibitor BAMBI predicts metastatic potential,” by Fritzmann J, Morkel M, Besser D, et al, on page 165.

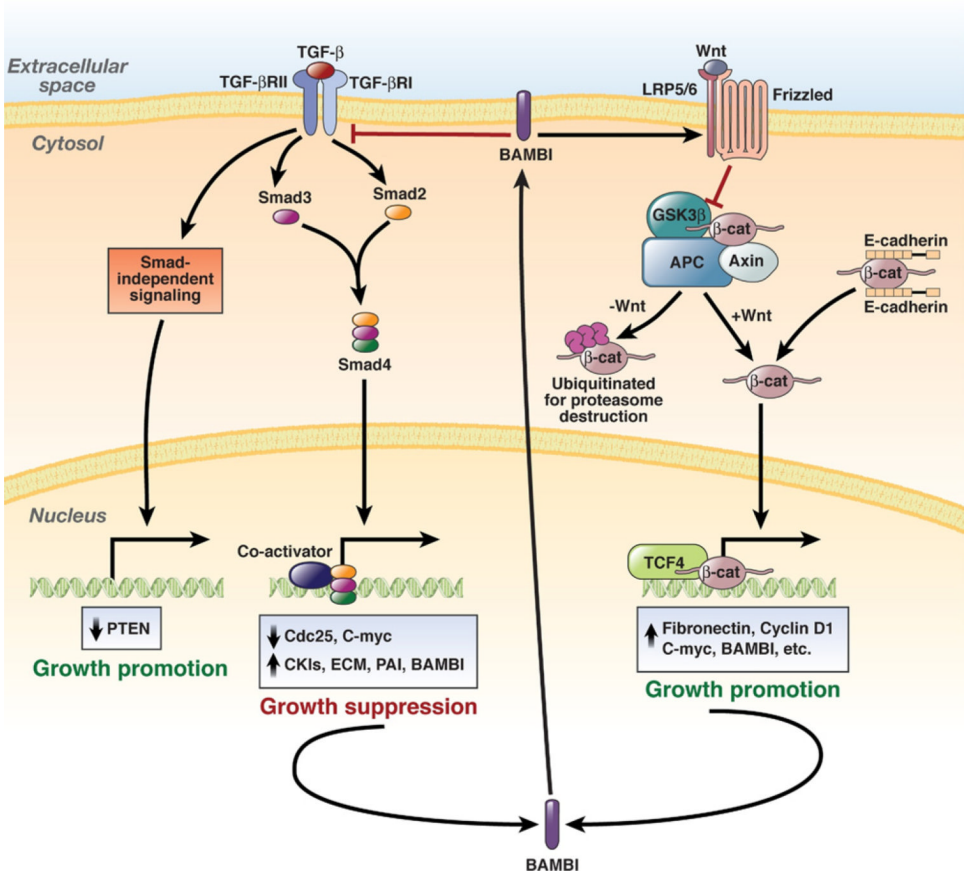

Figure 1.
Schematic diagram of the Wnt and TGF-β signaling pathways, and their common transcriptional target and mediator, BAMBI.