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De Novo Crypt Formation and Juvenile Polyposis on BMP Inhibition in Mouse Intestine

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Little is known about the signaling mechanisms that determine the highly regular patterning of the intestinal epithelium into crypts and villi. With the use of mouse models, we show that bone morphogenetic protein (BMP)–4 expression occurs exclusively in the intravillus mesenchyme. Villus epithelial cells respond to the BMP signal. Inhibition of BMP signaling by transgenic expression of noggin results in the formation of numerous ectopic crypt units perpendicular to the crypt-villus axis. These changes phenocopy the intestinal histopathology of patients with the cancer predisposition syndrome juvenile polyposis (JP), including the frequent occurrence of intraepithelial neoplasia. Many JP cases are known to harbor mutations in BMP pathway genes. These data indicate that intestinal BMP signaling represses de novo crypt formation and polyp growth.

Juvenile polyposis (JP) is an autosomal-dominant, gastrointestinal polyposis syndrome (*1*). Germline mutations in *SMAD4* (homolog of the *Drosophila* gene *mothers against decapentaplegic*) have been found in a subset of JP patients (*2*, *3*). Smad4 is an intracellular signal transducing transcription factor shared by the transforming growth factor β, activin and BMP pathways (*4*). More recently, mutations in the gene encoding the BMP receptor type IA (BMPRIA) have been found in some JP patients who were wild-type for *SMAD4* (*5*), implying a role for the BMP pathway in the pathogenesis of JP and, by inference, in the physiology of the intestine. Targeted disruption of BMP2, BMP4, and BMPR types IA and II in mice all lead to embryonic lethality (*6*–*9*). Similarly, mice deficient for the soluble BMP inhibitors noggin and gremlin die at birth (*10*–*12*).

To address the role of BMP signaling during intestine development and function, we first determined expression of *bmp4* message and protein during development. It has been reported that, in the early developing mouse intestine, *bmp4* message is expressed uniformly in the mesenchymal layer before folding of the epithelium (*13*). We examined *bmp4* expression at embryonic days (E15.5 to E18.5) when villus formation is underway and found that *bmp4* message is expressed at high levels in the intravillus mesenchyme of nascent villi but not in the mesenchyme underlying the proliferative intervillus epithelial pockets, which are the precursors of the crypts of Lieberkühn (*14*). At postnatal day 28 (P28) when the crypts have reached their mature form, BMP4 protein expression was strong in the intravillus mesenchyme (Fig. 1A). Phosphorylation and nuclear translocation of BMP-associated Smads 1, 5, and 8 [the hallmark of active BMP signaling (*15*, *16*)] was observed predominantly in differentiated villus epithelial cells (Fig. 1C), indicating that paracrine BMP signaling occurs specifically in the villus from the mesenchyme to the adjacent epithelium.

To investigate the role of BMP signaling in intestinal homeostasis, we generated transgenic mice expressing a *Xenopus* cDNA encoding the BMP inhibitor noggin (*X-noggin*) (*17*) under control of the mouse villin gene promoter. This promoter drives expression throughout the intestinal epithelium and becomes fully active during late gestation (*18*). In situ hybridization with a transgene-specific riboprobe revealed that *X-noggin* (*19*) was indeed expressed in the intestinal epithelium of adult transgenic mice (Fig. 1B). Immunoreactivity to phosphorylated Smad 1, 5, and 8 was almost entirely absent in *X-noggin*–transgenic intestines (Fig. 1D), demonstrating the effectiveness of the inhibition of BMP signaling by exogenous noggin.

At 3 months of age, transgenic animals of all three generated lines displayed identical architectural abnormalities throughout the small intestine with complete penetrance. One line was further characterized. No morphological alterations were detected in the intestines of newborns and animals up to 3 weeks old (*14*). We first noticed subtle abnormalities in the small intestine of 4-week-old animals, when the shallow proliferative intervillus pockets are being replaced by adult crypts of Lieberkühn, which extend downward toward the muscularis mucosae of the intestine. The villi in the transgenics were broad-based with blunted tips. Abnormal epithelial invaginations were seen alongside the villi that contained proliferative Ki-67-positive cells (Fig. 2B) and expressed crypt-specific genes such as the Wnt signaling targets

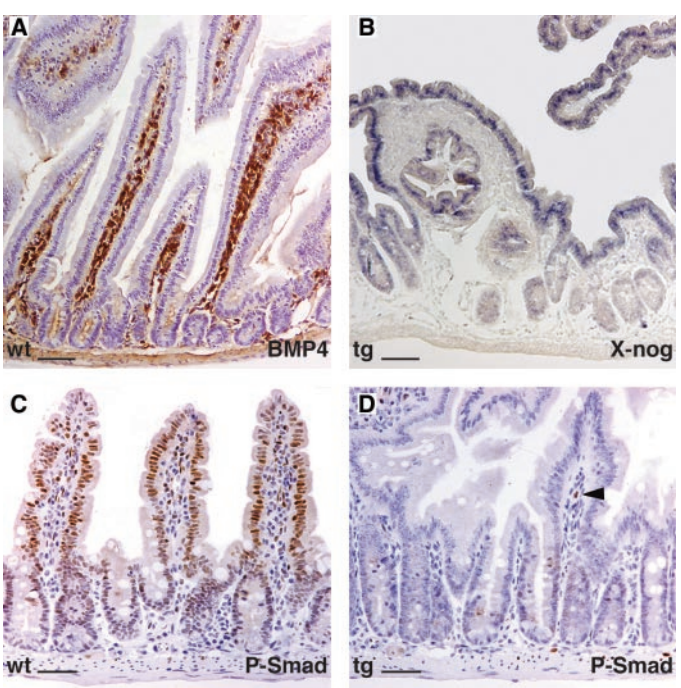

Fig. 1. Villus epithelial cells in the mouse intestine respond to mesenchymal BMP signaling. (**A**) Expression of BMP4 protein is detected in the intravillus mesenchyme of adult wild-type (wt) mice. (**B**) Transgenic expression of *Xenopus* noggin (X-nog) inhibits BMP signaling. In situ hybridization with a transgene-specific riboprobe reveals that the *Xenopus* noggin mRNA is expressed by intestinal epithelial cells in adult mice. (**C**) Immunoreactivity to phosphorylated Smad1, 5, and 8 proteins (P-Smad) reveals nuclear staining in wt villus epithelial cells. (**D**) In noggin-transgenic intestines, immunoreactivity to phospho-Smad1, 5, and 8 is almost absent in epithelial cells. Arrowhead indicates a positive mesenchymal cell. tg, noggin-transgenic. Scale bars indicate 0.1 mm in (A) and (B) and 25 μm in (C) and (D).

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c-myc (*20*), (fig. S1B) and EphB3 (see below). These invaginations appeared to be capable of developing into complete ectopic crypt-villus units that established polarity and a new crypt-villus axis, which was often perpendicular to the original axis. The aberrant units comprised a defined proliferative compartment as revealed by Ki67 immunostaining (Fig. 2E) and contained differentiated Paneth cells at their base as evidenced by staining for the receptor tyrosine kinase EphB3 (*21*) and lysozyme (fig. S1, C to F). Epithelial differentiation in the ectopic crypt-villus units appeared grossly normal, because goblet cells, enterocytes, and enteroendocrine cells were present in near-normal numbers (*14*).

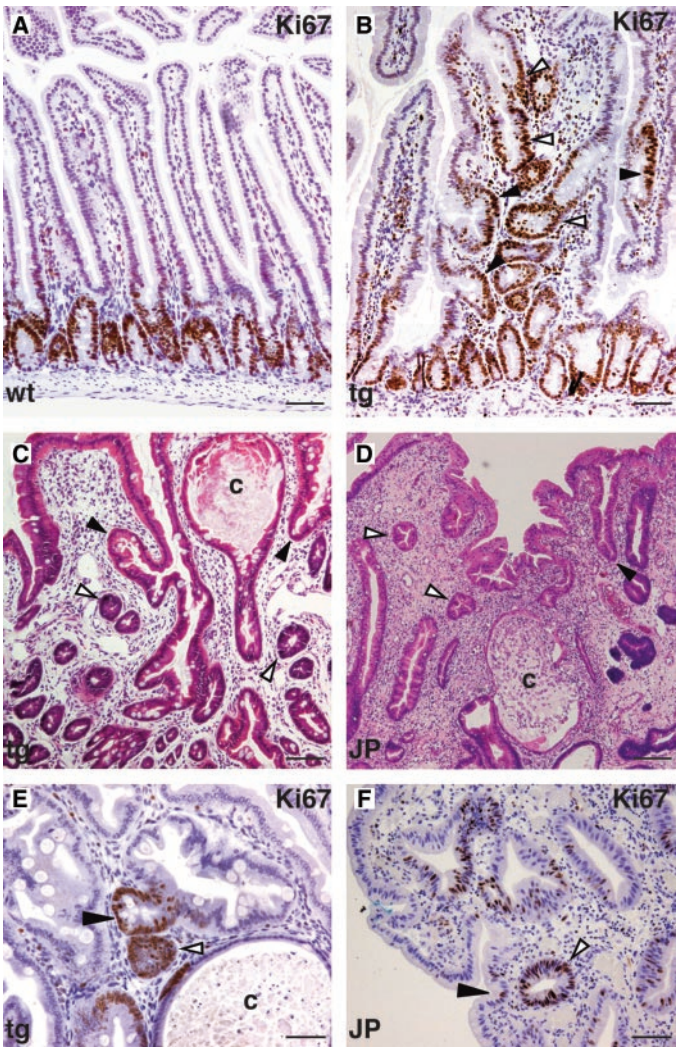

Fig. 2. The intestinal histopathology of noggin-transgenic mice resembles that of humans with JP. (**A**) Section of wt mouse intestine stained with Ki67. Ki67-positive epithelial cells are confined to the proliferative compartment of the crypts. (**B**) Section of noggin-transgenic intestine stained with Ki67. Ectopic epithelial invaginations contain proliferative cells and give rise to ectopic crypt villus units. (**C**) Section of the small intestine of a noggin-transgenic mouse stained with hematoxylin and eosin (H&E), showing the presence of abnormal crypt-villus units, cystic dilated crypts (c), and numerous crypts dispersed in the stroma. (**D**) Excision of an intestinal polyp from a human JP patient stained with H&E, showing the branching and budding of the intestinal epithelium and the dilated cysts (c). (**E**) Ki67 staining of a noggin-transgenic intestine showing an ectopic crypt-villus unit with a proliferative compartment. The epithelium lining the cyst contains proliferating cells. (**F**) Ki67 staining of a human JP polyp. Abnormally positioned crypts contain proliferating cells. Black arrowheads in (B) to (F) indicate ectopic epithelial invaginations; white arrowheads indicate dispersed crypts in the stroma. Scale bar, 0.1 mm in (A) to (D) and 25 μm in (E) and (F).

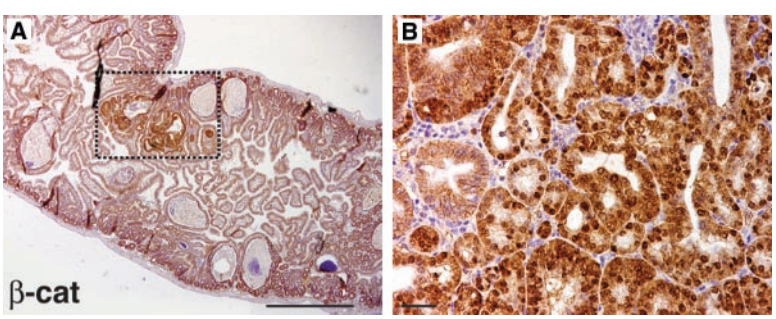

Fig. 3. Foci of dysplastic epithelium and adenomatous changes in polyps of 7-month-old noggin-transgenic mice. (**A**) β-catenin (β-cat) staining of an intestinal adenoma of a 7-month-old transgenic mouse. (**B**) Enlargement of the boxed area in (A). High levels of cytoplasmic and nuclear β-catenin are observed in the adenoma. Scale bar, 1 mm in (A) and 20 μm in (B).

At later stages, invaginations and ramifications of the intestinal epithelium extended from these proliferative foci deep into the villi, resulting in a complex architecture of branching villi with dilated cysts as the characteristic feature of the polyps observed in 3-month-old mice. The cystically dilated crypts were often filled with inspissated mucin and remnants of shed cells and lined by flattened epithelium. Lymphoid aggregates were sometimes present inside villi, and an increase in inflammatory cells was observed (Fig. 2C). These features are also seen in humans with JP (Fig. 2, D and F). The branching and budding of the intestinal epithelium, combined with the dilated cysts and reactive inflammatory changes, are particularly typical of the polyps in JP and discriminate them from the histopathological changes seen in familial adenomatous polyposis (FAP) and Peutz-Jeghers syndrome (*22*).

JP patients have an elevated risk of developing gastrointestinal cancers later in life (*23*). To study neoplastic changes in our mouse model, we examined the intestines of 7- and 8-month-old transgenic mice. In four of seven aged mice analyzed, we observed one or more foci of dysplastic epithelium and adenomatous change, in which the cells were abnormally arranged with an increased nuclear cytoplasmic ratio and pseudostratification of the cells. Most cells in these adenomatous foci were proliferating, as assessed by Ki67 staining (fig. S2, A and B). The overwhelming majority of colorectal cancers in humans initiate with activating mutations of the Wnt signaling cascade, typically targeting the tumor suppressor protein adenomatous polyposis coli (APC) (*24*). The hallmark of this mutational transformation is the stabilization and accumulation of β-catenin. Indeed, all adenomatous foci in aged *X-noggin* transgenics displayed high levels of cytoplasmic and nuclear β-catenin (Fig. 3). The cells in the adenomas also expressed CD44 (fig. S2, C and D), a target gene of the Wnt signaling cascade that is up-regulated in intestinal adenomas upon loss of APC (*25*).

Thus, the *X-noggin*–transgenic mice displayed a JP-like phenotype at a relatively young age (2 to 3 months), whereas adenomatous polyps frequently developed at a later stage (6 to 8 months), recapitulating the syndrome in humans. *Smad4*$^{+/-}$ mice were previously reported to develop polypous lesions, though not as juveniles and without some of the pathologic features described above (*26*).

It was originally proposed that JP polyps develop because of defects in the stroma ("landscaper" defects) (*27*). An early study of

juvenile polyps gave support to this notion, because deletions of the tumor suppressor locus 10q22-24 were found in the lamina propria and not the epithelium (*28*). However, in a more recent study, homozygous *SMAD4* deletions were found specifically in the epithelium of JP polyps (*29*). Similarly, loss of the wild-type *Smad4* allele was observed in the epithelium of the inflammatory polyps in *Smad4*$^{+/-}$ mice (*26*). In contrast to these studies, no consistent loss of heterezygosity of the *BMPRIA* gene was observed in the stroma or epithelial cells of the JP polyps (*5*), which would argue against a tumor-suppressor mechanism.

We propose that inactivating mutations in BMP pathway components primarily affect the perception of mesenchymal BMP signals by epithelial cells. BMP signaling delimits ectopic crypt induction once the orthotopic structures have been specified. With loss of these inhibitory signals, the epithelial cells proceed to establish ectopic crypts, ultimately leading to the growth of juvenile polyps and to neoplasia. Thus, the "landscaper" defect would reflect the disruption of mesenchymal-epithelial communication. It is likely that JP patients with wild-type *SMAD4* and *BMPRIA* genes (*2*, *30*) harbor mutations in other components of the BMP pathway.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/303/5664/1684/DC1
Materials and Methods
Figs. S1 and S2

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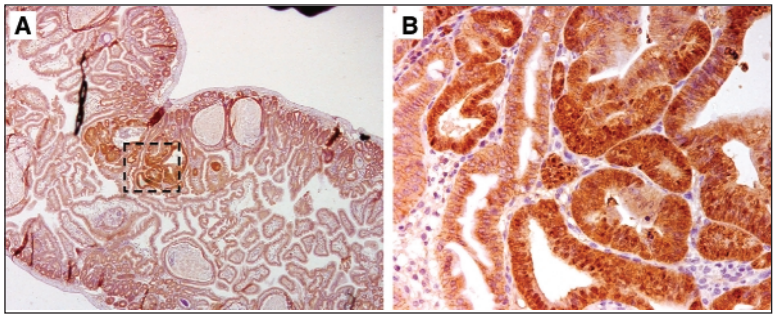

REPORTS: "De novo crypt formation and juvenile polyposis on BMP inhibition in mouse intestine" by A.-P. G. Haramis *et al.* (12 Mar. 2004, p. 1684). There was an error in Fig. 3. Fig. 3B should be an enlargement of the area boxed in Fig. 3A. The corrected figure is shown here.

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