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COMMENTARY

Tales from the Crypt: β-Catenin in the Development of Juvenile Polyps

Commentary on the article by Iwamoto *et al.* on page 4

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Normal colonic epithelium transforms into polyps and eventually cancer through a series of coordinated mutations in several genes, including tumor suppressors, proto-oncogenes, and stability genes. Loss of tumor suppression by adenomatous polyposis coli (APC) is an early and important mutation in this hierarchy (1). The APC gene, originally implicated in the pathogenesis of familial adenomatous polyposis, is also mutated in a majority of sporadic human colorectal cancers (2). Intense study of APC and its co-conspirators, including β-catenin, has led to a "gatekeeper" hypothesis in which APC attenuates proliferative signals in the Wnt pathway (3). Normally, APC serves as a scaffold for the phosphorylation and degradation of β-catenin (Fig. 1). In the face of Wnt signaling, the APC interaction is interrupted and β-catenin is stabilized. As the cytoplasmic pool of β-catenin accumulates, the equilibrium shifts to favor translocation into the nucleus with members of the T cell factor (TCF) family. The β-catenin/TCF complex then alters transcription at appropriately responsive genes. In this model, mutations of APC may be critical for tumor initiation—by taking the "brake" off β-catenin, cells are able to proliferate inappropriately.

Two landmark studies from The Netherlands and Canada have expanded on this model and established a role for β-catenin in early polyp formation as well as the positioning of cells along the intestinal villus (4,5). In an experimental *tour de force*, the investigators sifted through >24,000 RNA transcripts to find a differential gene expression profile when β-catenin/TCF signaling is interrupted in colonic epithelium. Only a modest list of ~230 target genes filtered out, but a strikingly impressive pattern emerges. β-Catenin/TCF signaling up-regulates proliferative genes, including the proto-oncogene c-myc and the developmental signal bone morphogenic protein 4 (BMP4), while simultaneously repressing genes that herald terminal epithelial differentiation (*e.g.* the cell-cycle arrest factor p21, as well as multiple markers of typical absorptive epithelium). The β-catenin/TCF transcriptional program also controls cellular migration along the intestinal villus through the expression of the cell–cell repulsion factor ephrin B1 and its receptors (Fig. 2) (5). These results suggest that tonic Wnt signaling might maintain crypt cells in a proliferative and undifferentiated state. As cells migrate up the villus and away from the presumed Wnt source, the effect of Wnt diminishes and β-catenin/TCF signaling is silenced. The sequelae include proliferative arrest (*via* p21) and completion of terminal epithelial differentiation. Despite being a reasonable hypothesis, no one has yet identified a reliable source of Wnt factors in normal intestine, polyps, or colorectal cancer.

The role of APC and β-catenin in the development of juvenile polyps (JPs) and juvenile polyposis coli (JPC) has, until recently, been relatively unexplored. Isolated JPs are thought to be nonmalignant hamartomas, but JPC carries an increased risk for malignant transformation (6,7). JPC is an autosomal dominant disorder, and 40% of kindreds have loss of function mutations in genes involved in the TGF-β signaling pathway, including MADH4 and BMP receptor 1A (8,9). In this issue of *Pediatric Research*, Hoffenberg *et al.* evaluate the expression of β-catenin and APC in polyps from 45 children with either sporadic JP or the JPC syndrome. They find a ubiquitous nuclear accumulation of β-catenin in the JPs but not in the normal epithelium. This finding recapitulates the results of the Dutch investigators who showed nuclear accumulation of β-catenin in aberrant crypt foci (early adult colonic lesions) but not in surrounding normal tissue (4). The authors also address whether nuclear β-catenin in JPs could be due to inactivation of APC. In adult neoplasia, β-catenin/TCF signaling is constitutively active in an APC null (−/−) background or when there are inactivating truncations of the APC protein (10,11), but in the Hoffenberg study, there is no difference in

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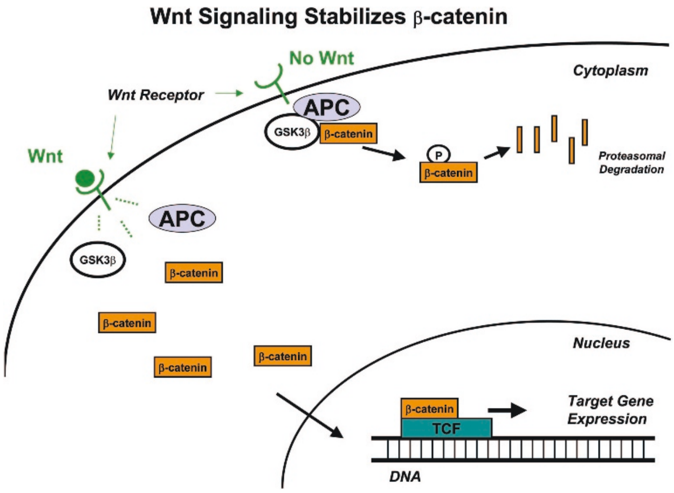

Figure 1. In the absence of extracellular Wnt signaling, APC facilitates the degradation of β-catenin through an interaction with the intracellular kinase GSK3β as well as other supporting proteins (data not shown). Phosphorylation of β-catenin by GSK3β serves as a signal for ubiquitinylation and degradation by the proteasome. Wnt signals, affected through a transmembrane receptor, disrupt the interaction of APC with GSK3β and β-catenin. This results in relative hypophosphorylation and stabilization of β-catenin.

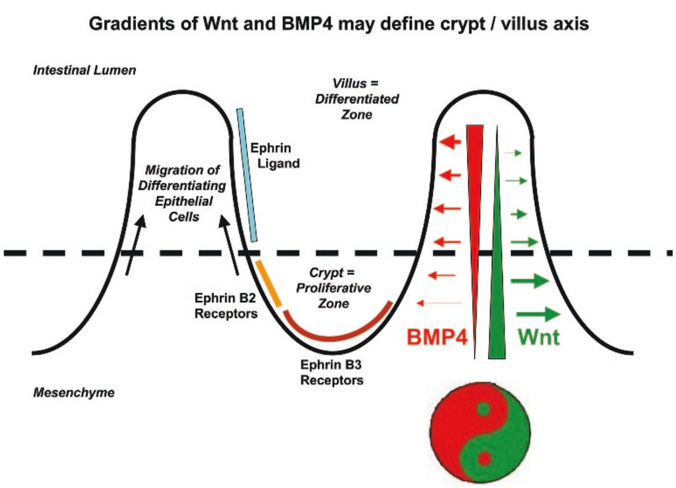

Figure 2. A presumed source of Wnt signaling from the mesenchyme stabilizes β-catenin and enables its nuclear localization in colonic crypt cells, resulting in up-regulation of c-myc and ongoing cellular proliferation. The heavy dotted line divides the proliferating from the differentiated compartment. Recent work suggests that BMP4 may be more important for terminal differentiation in the villous tips (13). In normal intestine, either from downstream effects of BMP4 signaling or directly from the lack of Wnt signaling, there is β-catenin repression, cell-cycle arrest, and terminal differentiation. The crypt-villus junction is maintained partially by the mutually repulsive interaction of Ephrin ligand and receptors, which are also under the control of β-catenin signaling.

APC expression between JPs and normal neighboring tissue. There is also no loss of heterozygosity at the APC locus and no apparent APC truncating mutations in these polyps. This report refutes the idea that APC inactivation has an important role in initiating JP formation.

An APC-independent explanation may lie just below the surface. . .literally. On the basis of embryologic studies, it is well known that signals from the mesenchyme influence the fate of an overlying epithelium. Wnt signaling *via* β-catenin and other diffusible factors, including BMP4, have already been established as major contributors to normal lung development through epithelial–mesenchymal interaction (12). Specific overexpression of β-catenin in the endoderm of the developing lung yields a surprising result. The transgenic lungs seem normal, but the epithelial lining betrays a different career trajectory—it makes a dramatic switch in lineage commitment to a secretory, intestinal phenotype (13). Microscopically, the epithelium appears cuboidal with microvilli and stored glycogen. By microarray analysis and correlation with *in situ* hybridization, specific Paneth and goblet cell markers are up-regulated, whereas normal lung developmental markers are switched off (13).

Borrowing on the lessons of lung development, BMP4 is now joining Wnt/β-catenin as a major actor in JP formation. BMP4 is a member of the TGF-β superfamily and exclusively expressed in the mesenchyme of developing intestinal villi. Inhibition of BMP4 signaling yields ectopic, dilated crypts; cystic change; and an inflammatory pattern that is histologically identical to that seen in JPs (14). The polyps also express other crypt-specific Wnt signaling targets such as c-myc and Ephrin B3. Most important, the dysplastic epithelium shows a high level of nuclear β-catenin accumulation (14). Perhaps this explains why Hoffenberg *et al.* find nuclear β-catenin but no APC mutations in their series of JPs. It would be interesting to re-explore them for BMP4 expression.

These findings suggest that BMP4 is important for maintaining differentiated intestinal epithelium and that loss of this stimulus results in JP formation. Wnt signaling tracks with the crypts and BMP4 with the villi—but how are the two interrelated? Because BMP4 is itself a downstream target of Wnt signaling within epithelial cells (4), there is likely to be significant cross-talk between these signaling pathways. Undoubtedly, these two systems are closely intertwined. Like the Taoist symbol of balance, in which Yin simultaneously leads and follows Yang, BMP4 and Wnt signaling seem to struggle together to create balance between intestinal proliferation and differentiation (Fig. 2). Polyp formation and, by extension, cancer follows when the balance is perturbed.

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