EphB/EphrinB Receptors and Wnt Signaling in Colorectal Cancer

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Abstract

Eph receptors and their ephrin ligands mediate cell repulsion during embryonic development. In the intestinal epithelium, EphB receptors are Wnt signaling target genes that control cell compartmentalization along the crypt axis. Recent findings have shown that this family of receptors are key players during colorectal cancer progression. Here, we review the current knowledge of the EphB/ephrinB system in the intestinal epithelium and we discuss their tumor suppressor role in the context of the multistep progression of colorectal cancer. (Cancer Res 2006; 66(1): 2-5)

Genetic Program Driven by β-Catenin and Tcf in Colorectal Cancer

Around 70% of all colorectal cancers show homozygous inactivation of the adenomatous polyposi coli (APC) tumor suppressor gene (1, 2). This genetic alteration results in the activation of the Wnt signaling pathway and the constitutive transcription by the β-catenin/Tcf complex (3, 4). Loss of APC function is present throughout the sequence of intestinal carcinogenesis (i.e., from benign adenomas to fully malignant colorectal cancer and metastasis). Even the earliest precursors of colorectal tumors, the so-called dysplastic crypts, show mutational activation of the Wnt pathway (5). In mice, activating mutations in Wnt signaling pathway components lead to the formation of dysplastic crypts and benign adenomas similar to the preneoplastic lesions developed by humans (6, 7). Together, these observations have led to the notion that constitutive transcription by the β-catenin/Tcf complex is the key step for initiation of colorectal cancer.

About 5 years ago, we undertook the identification of the genetic program driven by β-catenin and Tcf in colorectal cancer. In colorectal cancer cells lines bearing activating mutations of the Wnt pathway, we engineered ways to block the constitutive β-catenin/Tcf-mediated transcription in an inducible manner. Gene expression profile analysis of these tumor cells before and after blockage of β-catenin/Tcf activity revealed a set of target genes consistently expressed in dysplastic crypts and adenomas (8). Strikingly, we observed that virtually all the target genes identified in our screen were also expressed in the progenitor compartment of the normal, nontransformed intestinal epithelium of mice and humans. This observation was initially unexpected because the accepted hypothesis was that the novo activation of the Wnt signaling pathway in the intestinal epithelium initiated colorectal cancer. The explanation for our observations was obvious upon studying β-catenin localization in the intestinal epithelium; whereas most epithelial cells showed only membranous β-catenin, a few cells localized at the bottom of the crypts, presumably the stem cells and early progenitors, accumulated β-catenin in the nucleus. Thus, crypt progenitor cells receive Wnt signals as part of their normal physiology. The similarities between the genetic program driven by β-catenin/Tcf in crypt progenitors and early colorectal cancer lesions lead us to propose that the first step towards malignancy consists in the acquisition of a crypt progenitor-like phenotype.

Modular β-Catenin and Tcf Program Regulates Intestinal Crypt Cell Biology

Given the essential role of Wnt signaling in colorectal cancer, we and others have started to decipher the instructions given by β-catenin/Tcf to intestinal progenitors and colorectal cancer cells. The genetic program driven by β-catenin/Tcf seems to dictate three different sets of instructions that collectively regulate the biology of the crypt cells. The core module enforces the undifferentiated-proliferative phenotype of progenitor crypt cells. Mice genetically manipulated to lack β-catenin/Tcf activity in the intestine lack proliferative progenitors (9-11). Conversely, APC deficiency in the intestinal epithelium leads to an enormous amplification of the progenitor compartment at the expense of the differentiated compartment (12). This core set of instructions also determines the proliferative undifferentiated phenotype of colorectal cancer cells. Blockage of β-catenin/Tcf-mediated transcription in colorectal cancer cell lines results in cell cycle arrest and differentiation even in the presence of multiple alterations in other tumor suppressors and oncogenes (8).

The second module of the β-catenin/Tcf program is necessary for Paneth cell maturation (13, 14). Paneth cells represent a secretory cell type localized close to the bottom of the crypts in the small intestine. These cells display prominent nuclear β-catenin localization owing to the expression of the Wnt receptor Frizzled-5 (Frz5). Several specific Paneth cell differentiation markers, such as cryptdins, are bona fide β-catenin/Tcf target genes, whose expression depends on Frz5 and canonical Wnt signaling. Consequently, constitutive activation of the Wnt signaling pathway in intestinal adenomas and carcinomas results in the expression of some Paneth markers in tumors (14, 15). Although a role in tumorigenesis has been proposed for at least one β-catenin/Tcf Paneth cell target gene (i.e., the matrix metalloproteinase-7/matlrisin; ref. 16), it remains unclear if any other genes within this module might play a role during colorectal cancer progression. Of note, Paneth cells remain postmitotic and differentiated despite the activation of the Wnt signaling pathway, suggesting that they hold mechanisms to switch off the mitogenic instructions provided by β-catenin and Tcf.

The third module of the β-catenin/Tcf program controls the compartmentalization of epithelial cells along the crypt axis and...
regulates their ordered migration (17). The main effectors of this function are the β-catenin/Tcf targets EphB2 and EphB3, two members of the Eph family of receptors. Eph receptors comprise the largest subgroup of receptor tyrosine kinases characterized for binding to membrane-bound ligands known as ephrins (18). With a few exceptions, there is a ligand subclass specificity in the Eph/ephrin family; that is, B-type Eph receptors bind preferentially transmembrane ephrins (B-type ephrins), whereas A-type Eph receptors bind Gpi-membrane anchored ephrins (A-type ephrins). Eph receptors are well-known mediators of cell compartmentalization and ordered migration during embryonic development (18, 19). This function has been associated with their ability to provoke cell repulsion upon activation by ephrin ligands. There are numerous examples in embryonic development where Eph-mediated repulsion plays a key role, including the path finding of some axonal tracts or the formation of boundaries between adjacent cell populations in segmented structures, such as the hindbrain or the somites.

In the small intestine, EphB3 expression is driven by β-catenin and Tcf in epithelial cells that reside at the bottom-most positions of the crypts; that is, Paneth Cells and progenitor cells intermingled between Paneth Cells. EphB2 is expressed by all proliferative crypt cells in a decreasing gradient from the bottom to the top of the crypts. In the large intestine, the expression of both receptors is restricted to progenitor cells localized at the crypt base, but the extent of overlapping between their expression domains has not been clearly characterized yet. The EphB ligands, ephrinB1 and ephrinB2, are highly expressed by differentiating surface and villus epithelial cells. Thus, EphB and EphrinB genes are expressed in counter gradients along the crypt-villus axis.

We described three phenotypes in the small intestine of EphB2/EphB3–deficient mice (17). In adult EphB3−/− mice, Paneth cells are no longer sorted to the crypt base but are rather scattered along the crypt and the villus. Double deficient EphB2/EphB3 mice lose the boundary between the differentiated and proliferative cell compartments, a phenotype that is evident in the intestine of juvenile mice where the crypts are not yet formed. In double EphB3−/−;EphB2−/− adult mice, progenitor cells do not migrate towards the lumen unidirectionally but rather seem to randomly intermingle with their neighbors. Overall, these results indicate that EphB2 and EphB3 control cell positioning and

![Image](https://example.com/image.png)

**Figure 1.** A model for tumor suppression by EphB receptors in the intestinal epithelium. **A,** initial tumor founder cells receive a mutation in APC or β-catenin (blue arrow) that triggers constitutive activation of the β-catenin/Tcf complex. Consequently, the tumor founder cell remains locked into a progenitor phenotype and repopulates the crypt with their mutant descendants (B and C). These initial tumor cells express EphB receptors as a consequence of active Wnt signaling, yet they will only encounter ephrinB bearing cells when they reach the surface epithelium. The activation of EphB receptors in tumor cells, upon interaction with ephrinB–positive surface epithelial cells (D), compartmentalizes the tumor (black arrows) and impairs its ability to repopulate the adjacent epithelium. When tumor cells learn to silence EphB expression (E), they resume their expansion and repopulate the adjacent crypts in a top-down fashion (adapted from ref. 25).
ordered migration in the intestinal epithelium. It is worth noting that we recently found EphB4, a close homologue of EphB2 and EphB3, expressed in the intestinal epithelium with a similar pattern to that of EphB2 (20). Thus, EphB-mediated repulsion in the intestine may not be entirely abolished in EphB2-EphB3 double-mutant mice. Unfortunately, EphB4 knock-out mice die early in embryonic development precluding the analysis of EphB4 function in the intestine (21).

**EphB Receptors and Colorectal Cancer Progression**

The role of EphB receptors as suppressors of colorectal cancer progression was initially suspected after analyzing the β-catenin/Tcf target gene program in a collection of human colorectal cancer samples at different stages of malignancy (20). Dysplastic crypts and small adenomas retained expression of most β-catenin/Tcf targets present in crypt progenitors pinpointing a common tumor initiation mechanism through mutational activation of the Wnt signaling pathway. These initial lesions showed homogenous EphB2, EphB3, and EphB4 expression in all cells at equivalent levels to that of normal crypt progenitors. Strikingly, the majority of colorectal carcinomas contained >50% EphB receptor–negative cells despite evident nuclear β-catenin localization. Although we did not find a correlation between the frequency of EphB positive (EphB+) and EphB negative (EphB−) cells and tumor staging, their distribution was not equivalent. Low- and medium-grade tumor areas were enriched in EphB+ cells, whereas clusters of EphB− cells corresponded mostly to high-grade areas. As adenomas represent the benign precursors of carcinomas and tumors of higher grade often behave more aggressively than low-grade ones, our observations implied that silencing of EphB expression has occurred in a subset of tumor cells concomitantly with the acquisition of malignancy.

Does loss of EphB expression confer any advantages to colorectal cancer cells? The results of our genetic experiments were unequivocal. We engineered mice where the APCmin mutation was placed in a genetic background with low EphB activity. APCmin/+ mice develop benign intestinal lesions, such as dysplastic crypts and adenomas, as a result of constitutive activation of the Wnt signaling pathway (6, 7, 22). In the absence of EphB activity, tumor progression in the large intestine of APCmin/+ mice is strongly accelerated resulting in the development of aggressive colorectal adenocarcinomas. Therefore, whereas constitutive activation of the Wnt signaling pathway is required for the initiation of tumorigenesis (transition from normal epithelium to early adenoma stage), not all the instructions codified within the β-catenin/Tcf crypt progenitor program promote tumorigenesis. Rather, the module that specifies cell positioning seems to block tumor progression beyond the earlier stages.

Our observations have been recently strengthened by two independent studies. Jubb et al. have shown that the extent of EphB2 silencing in colorectal cancer correlates inversely with patient survival (23). Similarly, Lugli et al. also showed that loss of EphB2 expression is a strong indicator of poor overall survival in colorectal cancer (24).

**Tumor Compartmentalization: A New Mechanism of Tumor Suppression?**

At the beginning of tumorigenesis, APC mutant cells are confined to the epithelium within the so-called dysplastic crypts. These tumor founder cells expand laterally and repopulate the surrounding crypts with their mutant descendants (25). It is during this initial phase that EphB receptors most likely suppress tumor growth as EphB+ tumor cells are continuously in contact with normal epithelial cells expressing ephrinB ligands. It is tempting to hypothesize that tumor cells are forced to respect the boundaries imposed by EphB/ephrinB interactions much like normal progenitors and Paneth cells are compartmentalized in the healthy tissue (Fig. 1). Silencing of EphB receptors would generate a subset of tumor cells with unrestricted capacity for repopulating the epithelium. Indeed, the EphB− population is already noticeable in some small adenomas and is present in most large adenomas, suggesting that silencing initiates at early stages. Alternatively, EphB receptors could also suppress tumor progression in more advanced stages, yet it is important to consider that ephrinB ligands are membrane-bound proteins and, therefore, EphB activation can only occur upon interaction of tumor cells with ephrinB bearing cells. Unfortunately, the lack of good anti-ephrinB antibodies has hampered the study of their expression patterns in colorectal cancer.

But how do EphB/ephrinB signals suppress tumor progression at the molecular level? The best understood function of EphB receptors is the control of cell shape by the Rho family of small GTPases. Colorectal cancer cell lines remodel their actin cytoskeleton and contract upon ephrinB stimulation, a process which is dependent on inhibition of rap-1 activity and activation of Rho/ Rock (17, 26, 27). Although a change in cell shape might be necessary to restrict the migration of normal and transformed cells, it remains unclear whether it is sufficient to inhibit tumor progression. The study of the crosstalk between EphB receptors and the signaling pathways that drive intestinal tumorigenesis will be central to understand the tumor suppressor role of EphB receptors in colorectal cancer.

Finally, the mechanism of silencing of EphB expression in colorectal cancer also remains uncharacterized. Point mutations in EphB2 have been identified in a small fraction of prostate cancers (28). EphB2 is localized in 1p35-p36.1, a region of frequent allelic loss in colorectal cancer. A study of 50 colorectal cancer samples identified loss of heterozygosity (LOH) of EphB2 locus in 33% of samples, yet no mutations in the remaining allele were found in any tumor (29). Assessment of the methylation status of the wild-type allele in tumors with EphB2 LOH would be required to complement this study. Although genetic alterations or epigenetic silencing in individual EphB genes will be probably identified in a fraction of colorectal cancers, our observations indicate that EphB2, EphB3, and EphB4 are coordinately silenced in the majority of colorectal cancer samples (20). Thus, it is unlikely that mutations, LOH, or methylation account for the coordinated silencing of all three EphB genes during colorectal cancer progression. In the great majority of cancers, down-regulation of EphB receptors occurs at the mRNA level (20, 23). EphB expression is fully dependent on β-catenin/Tcf activity, yet many colorectal cancers and cell lines down-regulate EphB levels despite constitutive activation of the Wnt signaling pathway. Together, these observations point to a common mechanism of transcriptional silencing of EphB genes that acts in a dominant fashion over β-catenin/Tcf activation.

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