EphB2 Is a Prognostic Factor in Colorectal Cancer

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Abstract

A receptor tyrosine kinase for ephrin ligands, EphB2, is expressed in colorectal cancer and has been proposed as a target for immunoconjugate therapy. The aim of this study was to perform a detailed histologic analysis of EphB2 expression in normal and neoplastic colorectal tissues. In addition, we sought to evaluate EphB2 expression as a prognostic factor in colorectal cancer. Expression of EphB2 was examined in normal colon (n = 28), colorectal cell lines (n = 20), colorectal adenomas (n = 148), primary cancers (n = 28), and metastases (n = 39) using immunohistochemistry. In addition, a series of primary cancers and matched normal (n = 342) with outcome data were profiled in tissue microarrays. The intensity of EphB2 expression was assessed in the entire series by immunohistochemistry, and in a subset by in situ hybridization. Overall survival and recurrence-free survival were correlated with EphB2 protein expression in retrospective subset analyses. Epithelial EphB2 expression was shown at all stages of colorectal tumorigenesis, including the base of all normal crypts, 77% of adenomas, 82% of primary cancers, and 64% of metastases. Although homogeneous expression was observed in adenomas, the pattern of staining was focal (mean 25%) in most malignant lesions. Patients whose tumor stained 2+ for EphB2 expression (versus 0/1+) exhibited significantly prolonged overall survival: mean duration of survival, 2,514 versus 1,044 days; hazard ratio, 0.45; 95% confidence interval, 0.18 to 0.95 (P = 0.035). In summary, EphB2 is expressed in normal crypts, colorectal adenomas, primary cancers, and metastases. High levels of EphB2 expression are associated with a longer mean duration of survival in colorectal cancer.

Eph receptors make up the largest family of receptor tyrosine kinases in the human genome (reviewed in ref. 1). The family is divided by sequence identity into two classes, EphA and EphB, with corresponding transmembrane ligand families, called type A and type B ephrins. Ligand-receptor binding results in forward and reverse signaling, which have been implicated in a range of functions consistent with the cytoskeleton as a downstream effector (1). EphB2 and EphB3 are believed to direct epithelial patterning along the crypt-villus axis in the intestine (2). Moreover, EphB2 has been identified as a target of the Wnt signaling pathway (2), which is frequently hyperactivated early in the colorectal adenoma to adenocarcinoma sequence. Recently, up-regulation of both ligand and receptor family members has been described in a range of human tumors and cell lines, including colorectal cancer (2–11). Nevertheless, it is not clear how Eph activity might make a functional contribution to the malignant phenotype. Indeed, notwithstanding reports implicating members of the EphB and ephrin B families in tumor progression (3, 4, 9), there is growing evidence to suggest that they may function as tumor suppressors (12, 13). The aims of this study were to investigate the prognostic impact of EphB2 in colorectal cancer and to examine the relative levels of EphB2 expression across the adenoma to adenocarcinoma sequence.

Materials and Methods

Tissue samples and tissue microarray construction. Colo206 and CX-1 were obtained from DSMZ (Braunschweig, Germany), KM12 was obtained from the National Cancer Institute (Bethesda, MD), and all other colorectal cancer cell lines were obtained from American Type Culture Collection (Manassas, VA). Cells were cultured and total RNA was harvested and hybridized to Affymetrix HG-U133 GeneChip probe arrays as described previously (14). Cell pellets were also fixed in formalin, embedded in paraffin, and represented in tissue microarrays as previously described (15).

Small intestinal tissues were harvested from two male apcmin/− mice (B6 background) at 6 months of age, fixed in 10% neutral-buffered formalin, and embedded in paraffin. All animal experiments were conducted in accordance with the Institutional Animal Care and Use Committee regulations.

An ethical review committee (University of Leeds, Leeds, United Kingdom) approved the use of all tissues and clinical information. The Leeds General Infirmary (Leeds, United Kingdom) histopathology archive was screened to identify 148 anonymized colorectal adenomas, including 84 tubular adenomas, 27 tubulovillous adenomas, 5 villous adenomas, and 32 flat adenomas. Formalin-fixed paraffin-embedded tissues were biopsied to represent each adenoma in tissue microarrays, as previously described (15).

Formalin-fixed paraffin-embedded tissues from matched normal colon, primary cancers, and metastases from multiple sources were
retrieved from the Genentech archives. The series comprised 28 normal mucosal samples, 28 primary colorectal cancers, and 30 metastases (27 lymph nodes, and 3 hepatic), representing 27 patients. An additional nine unmatched hepatic metastases from nine patients were also included. Whole sections were cut and used for downstream assays.

This study also investigated 342 colorectal cancers from the University of Leeds tissue archive, representing 330 patients who underwent a resection in the Department of Surgery at the Marien-Hospital (Düsseldorf, Germany) between January 1990 and December 1995 (a collaboration between W. Mueller and H. Grabsch). Matched normal mucosa and survival data were available for all patients, including information on recurrence of disease. The median follow-up time was 4.2 years (range, 5 months-11.4 years), and patients were censored in survival analyses according to date last seen. Postoperatively, 14 patients received chemotherapy, 12 patients received radiotherapy, and 7 patients both. One hundred ten tumors (32%) were proximal to the splenic flexure, the mean age at diagnosis was 69 years (range, 28-88 years), and 150 patients (45%) were male. At the end of the follow-up period, local disease recurrence was noted in 25 patients, distant metastasis in 49 patients, and 8 patients showed both.

**In situ hybridization.** ³²P-labeled riboprobes were used to evaluate ephB2 mRNA expression. cDNA templates were amplified by PCR from whole human brain marathon-ready cDNA (BD Clontech, Palo Alto, CA). Forward and reverse primers contained 5′ T7 and T3 RNA polymerase initiation sites, respectively. Riboprobes were designed to complement nucleotides 3,043 to 3,500 of the transcript variant 2 mRNA sequence (Genbank accession no. NM_004442; forward 5′-T7-GCCCTCCTGGTGCTCTATCC-3′, reverse 5′-T3-TCTGTCCATCT-GTCCGTCCT-3′). In vitro transcription of sense and antisense probes, hybridization, and development of sections were carried out as previously described (17). Hybridized tissue sections were reviewed by bright- and dark-field microscopy and scored on an observational scale of 0 to 3 for the maximum intensity of ephB2 expression in >10% of the epithelium. Tumors were scored 0 if <10% of cells expressed any level of ephB2, 1 if >10% of epithelium expressed low levels, 2 if >10% of epithelium expressed moderate levels, and 3 if >10% of epithelium expressed high levels (illustrated in Fig. 1). Matched normal colon tissues were included as internal controls for the intensity of EphB2 hybridization in each cancer. **In situ** hybridization for β-actin was used as a control for mRNA integrity, as previously described (17).

**Immunohistochemistry.** Immunohistochemistry was done as previously described (17). In brief, antigen retrieval was carried out on formalin-fixed paraffin-embedded tissue sections for 20 minutes at 95°C using Target retrieval solution (pH 6.0; DakoCytomation, Carpinteria, CA), according to the manufacturer's instructions. Immunolabeling was done with a goat anti-EphB2 polyclonal antibody (R&D Systems, Minneapolis, MN), a mouse anti-β-catenin monoclonal antibody (clone 14, BD PharMingen, San Diego, CA), or naïve immunoglobulins (R&D Systems) at 1 μg/mL. Immunocomplexes were labeled with a biotinylated anti-goat secondary antibody (Vector Labs, Burlingame, CA), an avidin-biotin-horseradish peroxidase complex (Vectastain Elite, Vector Labs), and the 3,3′-diaminobenzidine chromogen. To prevent cross-reactivity when immunolabeling mouse tissues, anti-β-catenin immunoglobulins were biotinylated and blocked using the animal research kit (DakoCytomation); all other protocol details were identical to those used in the immunolabeling of human tissues.

**Fig. 1.** EphB2 expression by immunohistochemistry (IHC) and in situ hybridization (ISH) in normal colon and colorectal cancers. Shown are representative primary cancers with immunohistochemical scores of 0, 1, and 2. Membranous and cytoplasmic 3,3′-diaminobenzidine chromogen deposition (brown), illustrating EphB2 protein, is observed over normal colon and neoplastic cells. Corresponding dark field in situ hybridization images show an identical pattern of epithelial-restricted ephB2 mRNA (silver grains). In situ hybridization for β-actin was done as a control for RNA integrity. Bar, 100 μm.
Tissues were scored on an observational scale of 0 to 3 for the maximum intensity of EphB2 expression in >10% of the epithelium. Tumors were scored 0 if <10% of cells expressed any level of EphB2, 1 if >10% of epithelium stained weakly, 2 if >10% of epithelium stained moderately, and 3 if >10% of epithelium stained strongly (Fig. 1). In addition, the percentage of tumor cells that scored positive (score ≥1) for EphB2 protein expression was recorded for whole sections. Cell pellet tissue microarrays were included in each staining run to control for variability in EphB2 staining intensity and to assist in the assignment of intensity scores. Matched normal colon tissues were included as internal controls for the intensity of EphB2 staining for each cancer. Nuclear expression of β-catenin was scored on serial sections. Tumors were considered positive if >10% of neoplastic cells showed nuclear β-catenin localization. The presence or absence of an association between nuclear β-catenin localization and EphB2 expression was assessed with direct reference to serial sections stained for EphB2. The two antigens were considered to be associated in an individual case if the neoplastic cells were either uniformly negative for EphB2 and showed no nuclear β-catenin expression, or if the intensity of EphB2 expression was consistently and positively associated with the intensity of nuclear β-catenin localization throughout the neoplastic cell population.

Statistical analysis. The same pathologist (H. Koeppen) scored all cases, blinded to the clinical outcome. In all tissue microarrays, the score from the highest expressing core (n = 3/sample) was used to represent each patient. Mean survival times within each subgroup were estimated from Kaplan-Meier curves and corresponding hazard ratios were estimated from proportional hazards fit modeling using JMP software version 5.1 (SAS Institute, Inc., Cary, NC). Statistical associations were assessed using the χ² test, Student’s t test, or Spearman’s rank correlation as appropriate. Statistical significance was assumed if P < 0.05.

Results

EphB2 localization. The majority of colorectal cancer cell lines (60%) expressed EphB2 protein by immunohistochemistry (score ≥1), which correlated with oligonucleotide microarray analyses of ephb2 mRNA expression (P < 0.0001; Fig. 2). In both normal and neoplastic large intestine, expression of EphB2 mRNA and protein was localized to the epithelium (Fig. 1). EphB2 protein expression was predominantly membranous, with weaker cytoplasmic expression. In all samples of normal colon, both mRNA (n = 47) and protein (n = 370) expression were most intense at the base of the crypt (score = 2/3), with expression declining to the luminal epithelium (score = 0). The maximum intensity of expression in neoplastic cell populations was equivalent to expression at the base of the colonic crypt (Fig. 1). Identical observations were made in the morphologically normal small intestinal crypts of the apc<sup>min/+</sup> mouse tissues (data not shown). Expression of EphB2 in the apc<sup>min/+</sup> mouse villi was low, approximating the level of expression observed at the luminal epithelium of the normal human colonic crypts. Hybridization of sense in situ hybridization probes and staining of naive immunoglobulins did not exceed background, and all cases were positive for β-actin by in situ hybridization (data not shown). Benign stroma, normal lymph node tissue, and normal hepatic tissue were uniformly negative for EphB2. No vascular or neural staining was observed.

EphB2 expression in apc<sup>min/+</sup> mouse tumors and human colorectal adenomas, primary cancers, and metastases. All apc<sup>min/+</sup> mouse tumors observed (n = 30) were homogeneously positive for EphB2 expression, with an equivalent intensity of expression to that at the base of the morphologically normal small intestinal crypts. One hundred fifteen (77%) of 148 human adenomas were positive (score ≥1) for EphB2 expression (Table 1). This was not statistically different from the intensity of expression observed in primary colorectal cancers (P = 0.37). Where present, EphB2 was homogeneously expressed throughout the adenomas. Different histologic subtypes of polyloid adenomas showed no statistically significant differences in the intensity of EphB2 expression (Table 1), although expression was significantly greater in flat adenomas (P = 0.001). There were no significant associations with site, severity of dysplasia, polyp frequency, polyp size, or patient sex (data not shown). Eighty-two percent of primary...
colorectal cancers and 64% of all metastases were positive (score ≥1) for EphB2 expression in whole sections (Fig. 3A). Of primary colorectal cancers, 5 scored 0 (18%), 9 scored 1 (32%), 13 scored 2 (46%), and 1 scored 3 (4%). Among metastases, 14 scored 0 (36%), 8 scored 1 (20%), 13 scored 2 (33%), and 4 scored 3 (10%). EphB2 expression was comparable in primary colorectal cancers and metastases (P = 0.17). The frequency of hepatic metastases that scored positive for EphB2 was higher than the frequency of lymph node metastases scored positive, although this did not reach statistical significance (P = 0.06). Of 12 hepatic metastases, 10 (83%) were positive for EphB2 expression (score ≥1); 2 scored 0 (17%), 1 scored 1 (8%), 7 scored 2 (58%), and 2 scored 3 (17%). Of 27 lymph node metastases, 15 (56%) were positive for EphB2 expression (score ≥1); 12 scored 0 (44%), 7 scored 1 (26%), 5 scored 2 (19%), and 3 scored 3 (11%). When analyzed separately, neither the intensity scores for the liver metastases nor the lymph node metastases were significantly different from the intensity scores for the primary cancers, respectively (P = 0.25 and P = 0.08).

The pattern of EphB2 expression in colorectal cancers was focal, with considerable variation in the intensity of staining throughout the neoplastic cell population. A mean 25% of tumor cells expressed EphB2 protein in primary and secondary malignancies scored positive for EphB2 expression. This was not significantly different between primary cancers (mean 22% of cells expressing in tumors scored positive) and metastases (mean 30%; P = 0.18). However, the percentage of tumor cells positive for EphB2 expression was lower in 50% of metastases compared with their matched primary cancers; the percentage of positive tumor cells was identical in 29% of metastases and was increased in 21% (Fig. 3B).

The dysregulated expression of proteins concerned with invasion and metastasis has been proposed to be most significant at the leading or invasive edge of the primary cancer. EphB2 expression was specifically assessed in this neoplastic cell population, using a scoring system identical to that used in the appraisal of overall expression. Two of 28 primary cancers (all whole sections) were not assessable due to the relatively small size of the tissue sections. At the invasive edge, 8 cancers scored 0 (31%), 7 scored 1 (27%), 10 scored 2 (38%), and 1 scored 3 (4%). Agreement with the overall expression was relatively weak, κ statistic = 0.49. However, there was a significant association between the two scores (P = 0.02). The scores were identical in 17 cancers (65%), higher at the invasive edge in three cancers (12%), and lower at the invasive edge in six cancers (23%).

**EphB2 expression and β-catenin localization.** Nuclear β-catenin localization was observed in the epithelial cells at the base of the morphologically normal apc<sup>min/+</sup> small intestinal crypts and at the base of the human large intestinal crypts, an equivalent pattern to that observed for EphB2. Similar to EphB2 expression, nuclear β-catenin localization was observed throughout the intestinal tumors (n = 30) in both flat versus polypoid adenomas (P = 0.001).

<p>| Table 1. Intensity of EphB2 expression in different histologic subtypes of adenoma |
|--------------------------------|----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Histology</th>
<th>EphB2 immunohistochemistry intensity score, n (%)</th>
<th>P (χ² test vs villous adenomas)</th>
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</thead>
<tbody>
<tr>
<td>Flat adenomas (n = 32)*</td>
<td>2 (6)</td>
<td>13 (41)</td>
</tr>
<tr>
<td>Tubular adenomas (n = 84)</td>
<td>22 (26)</td>
<td>35 (42)</td>
</tr>
<tr>
<td>Tubulovillous adenomas (n = 27)</td>
<td>9 (33)</td>
<td>10 (37)</td>
</tr>
<tr>
<td>Villous adenomas (n = 5)</td>
<td>3 (60)</td>
<td>1 (20)</td>
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*χ² test for flat versus polypoid adenomas (P = 0.001).
apc<sup>min/+</sup> mice assessed (Fig. 4A). Fifteen of 20 human colorectal cancer cell lines (75%) were positive for nuclear β-catenin localization (Fig. 2), which was significantly associated with the EphB2 immunohistochemistry intensity score (P = 0.01) and the EphB2 Affymetrix signal intensity (P = 0.02). β-catenin localization was also assessed in serial sections of primary (n = 28) and secondary (n = 38) colorectal cancers scored for EphB2 expression. One lymph node metastasis was excluded from the analysis due to high background staining, which did not permit a reliable interpretation of the immunolabeling. Fifty-three of 66 cancers (80%) showed nuclear localization of β-catenin. This was not significantly different between primary colorectal cancers (82% positive), lymph node metastases (73% positive, versus primary cancers P = 0.63), and liver metastases (92% positive, versus primary cancers P = 0.77, versus lymph node metastases P = 0.38). Of 66 cancers assessed, 13 (20%) were completely negative for EphB2 and nuclear β-catenin, 24 (36%) showed no association between nuclear β-catenin localization and EphB2 expression, and 29 (43%) showed an association between nuclear β-catenin and EphB2 expression (P = 0.001; Fig. 4B). The lack of an association between nuclear β-catenin and EphB2 protein was due to either expression of the proteins in different cell populations or the absence of EphB2 protein despite nuclear β-catenin localization. The association between nuclear β-catenin and EphB2 expression was not significantly different for individual analyses of primary cancers, lymph node metastases, or liver metastases (data not shown). However, nuclear β-catenin localization was observed more frequently in cancer cells at the invasive edge than in more central tumor regions, in contrast to EphB2 expression.

**EphB2 expression and prognosis.** Three hundred twenty-seven of 330 patients (99%) were informative for EphB2 protein expression: 185 patients scored 0 (57%), 101 patients scored 1 (31%), and 41 patients scored 2 (13%). (No patients scored 3 in this series, and cores were absent from three patients.). In situ hybridization was done on one tissue microarray containing 47 colorectal cancers: 8 patients scored 0 (17%), 19 patients scored 1 (40%), 18 patients scored 2 (38%), and 2 patients scored 3 (4%). Matched normal colonic crypts were positive at the base in all cases. A significant association was observed between EphB2 immunohistochemistry and in situ hybridization scores in 46 cancers informative for both (P < 0.01).

The effects of EphB2 protein expression were included in a proportional hazards fit for overall survival. Patients with an immunohistochemistry score of 2 exhibited a significantly better prognosis than those with a score of 1 or 0 (mean survival, 2,514 versus 1,044 days; hazard ratio, 0.45; 95% confidence interval, 0.18-0.95; P = 0.035; Fig. 5A). Similar results were found in analyses of recurrence-free survival (mean survival, 2,233 versus 795 days; hazard ratio, 0.60; 95% confidence interval, 0.30-1.10; P = 0.103; Fig. 5B). Survival curves for patients scored 0 or 1 were overlapping. The mean age was significantly lower in patients with high EphB2 protein expression (65 versus 69.3 years, P < 0.02), although age was not a prognostic factor in this series (data not shown). No statistically significant associations were observed with stage, grade, lymphatic invasion, vascular invasion, tumor site, or patient sex, and there were no differences in the frequency of adjuvant therapy received by the two groups (data not shown). The survival impact of the clinicopathologic variables has been previously discussed (16). In a multivariate analysis, including
stage, grade, lymphatic invasion, and blood vessel invasion, an EphB2 score of 2 was a significant, independent prognostic factor for overall survival (hazard ratio, 0.47; 95% confidence interval, 0.18-0.99; \( P = 0.047 \)).

Discussion

Using oligonucleotide microarray data and quantitative reverse transcriptase-PCR, Mao et al. (7) have proposed that EphB2 is up-regulated in colorectal tumorigenesis. However, analyses on tissue homogenates do not accurately record expression at the cellular level. Indeed, EphB2 is reported to behave as a tumor suppressor in prostate cancer (12) and the ephrin B2 ligand is reported to inhibit the growth of a human colon tumor xenograft (13). These data seem paradoxical, especially when we consider that there is little evidence to support a functional relationship between EphB2 and the malignant phenotype. To characterize the prevalence and clinical impact of EphB2 expression, we assessed expression across the colorectal adenoma to adenocarcinoma sequence, and investigated EphB2 as a prognostic factor in colorectal cancer using histologic methods.

The epithelial-restricted expression pattern of EphB2 observed was consistent with previous reports of EphB2 localization in the mammalian intestine (2, 7). Eph-ephrin signaling is reported to have a prominent role in vascular and neural development (1). However, this was not the focus of our analyses on colorectal cancer and we found no evidence for EphB2 expression by these two cell populations. In situ techniques showed comparable levels of expression at the base of the colonic crypt and in colorectal tumors (Fig. 1). All subtypes of colorectal adenomas displayed evidence for homogeneous expression of EphB2, with relatively greater levels in flat compared with polypoid lesions (Table 1). The lack of a correlation with tumor size or the severity of dysplasia suggests that expression persists throughout adenomatous growth. Twenty-three percent of all adenomas did not express EphB2, which may reflect tumor initiation by a non-Wnt pathway or, alternatively, top-down tumorigenesis.

Similar to the adenomas, 82% of primary colorectal cancers and 64% of metastases showed some level of EphB2 expression in whole sections. However, in contrast to adenomatous lesions, EphB2 was not homogeneously or uniformly expressed throughout the malignancies. This suggests that loss of EphB2 by a proportion of cells in the neoplastic population occurs at the transition from a large adenoma to a primary cancer. In addition, there was a trend for decreased EphB2 expression in metastatic lesions. Thus, one may speculate that loss of EphB2 also assists in the progression of advanced cancers, but no functional data exists to support such a mechanism in the colon. It is possible that forward or reverse EphB2 signaling contributes to the differentiation of colorectal neoplasms in the same manner that it governs the orderly progression of cells in the normal crypt (2). Nevertheless, a significant association between the expression of EphB2 and advanced tumor grade was not observed, nor was EphB2 expression appreciably different in cells at the invasive edge of primary cancers. Huusko et al. (12) reported that expression of an EphB2 transgene suppressed invasive growth in a prostate cancer cell line as assessed by an in vitro assay. However, Mao et al. (7) reported that blockade of EphB2 signaling using an unconjugated neutralizing antibody did not affect the in vitro or in vivo growth of a colorectal cancer cell line expressing both ligand and receptor. In summary, the role of EphB2 in colorectal neoplasia is unclear and more functional studies are required to explain the observations reported herein.

The mechanism for the variegated loss of EphB2 expression in most colorectal cancers is not yet apparent, but it may involve a defect in the translation of ephB2 message, an epigenetic phenomenon (such as promoter hypermethylation), nonsense mediated decay of mutated mRNA transcripts (12), or chromosomal deletions (18). In support of the first hypothesis, the cell lines LS180 and LS1034 were observed to express very high levels of EphB2 mRNA, but relatively low amounts of
EphB2 protein (Fig. 2). This phenomenon was also apparent in isolated primary cancers, for which in situ hybridization and immunohistochemistry data were available (data not shown). Alternatively, this may represent loss of antigenicity, although this is unlikely as the staining of matched normal colon samples was consistent and identical in pattern and intensity for all cases assessed. Loss of heterozygosity at chromosome 1p35, the ephB2 locus, is a frequent event in colorectal cancer (incidence, 0.33-0.50; refs. 18, 19), and is yet more frequent in hepatic metastases (incidence, ~0.66; ref. 19) and correlates with a higher Dukes’ stage (20). This is not in complete agreement with our data, as the intensity of EphB2 expression was not significantly different at the invasive edge or in hepatic or lymph node metastases compared with primary cancers. In addition, EphB2 expression was not significantly associated with tumor stage in our series. Whereas half of all matched metastatic lesions showed a decrease in the percentage of cells positive for EphB2, compared with the primary cancer, this difference was largely attributable to lymph node metastases and not hepatic metastases. Analysis of EphB2 protein expression and loss of heterozygosity at 1p35 in the same series will be essential to determine whether EphB2 expression is a function of this genomic lesion.

Oba et al. (18) did not find any somatic ephB2 mutations in colorectal cancers with low heterozygosity at 1p35, but a comprehensive study has not been carried out. Interestingly, 1p35 is the site of a putative phenotypic modifier in patients with the autosomal dominant familial adenomatous polyposis disorder (20). Familial adenomatous polyposis results in a heavy burden of colorectal adenomas at a young age due to an inherited mutation in the apc gene, a negative regulator of Wnt signaling. These observations have led to the suggestion that 1p35 probably encodes one or more genes that are important in colorectal neoplasia (20). EphB2 is a known target of Wnt signaling (2), an observation supported by data herein showing the expression of EphB2 in intestinal tumors arising in the apc–/– model (Fig. 4A) and a positive association between EphB2 and nuclear β-catenin in cell lines and primary and secondary human colorectal cancers (Figs. 2 and 4B). However, 36% of colorectal cancers did not show an association between nuclear β-catenin and EphB2 expression. Further work is needed to determine whether this is due to the influence of other regulatory mechanisms, such as promoter methylation, or it is the effect of tumor-associated lesions, such as loss of heterozygosity. Moreover, there are many candidate genes at 1p35, and it remains to be seen what, if any, role EphB2 plays in modifying the phenotype of familial adenomatous polyposis.

To our knowledge, only one study has previously examined the impact of EphB2 expression on patient survival in human cancer. Wu et al. (5) reported that strong EphB2 protein expression was associated with a shorter duration of survival in breast carcinoma. This is contrary to data presented herein, which suggest that EphB2 expression is a good prognostic factor in colorectal cancer. The discrepancy may be explained by methodologic differences, fundamental distinctions in the role of EphB2 in cancers of the large intestine and the breast, or the relatively small size of the breast cancer series (n = 94). Nevertheless, external confirmation of these results, together with a mechanistic explanation, is essential to validate this hypothesis. Our prognostic series of colorectal cancers represented only patients who underwent a potentially curative resection. Therefore, it will be important to assess the impact of EphB2 expression in a more advanced cohort of malignancies. Differences in the frequency of EphB2 expression are apparent between the prognostic series of colorectal cancers, represented in tissue microarrays, and the whole sections of primary and secondary colorectal cancers. This may represent sampling error of the tissue microarrays, however, surveying a large population minimizes individual sampling bias and does not impact the validity of the prognostic findings.

In summary, EphB2 is expressed at all stages of colorectal neoplasia. High levels of EphB2 expression are associated with improved survival in colorectal cancer, and multivariate analyses suggest that EphB2 is an independent prognostic factor.

References
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