EphB2 Expression across 138 Human Tumor Types in a Tissue Microarray: High Levels of Expression in Gastrointestinal Cancers

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Abstract

Purpose: To comprehensively evaluate ephrin receptor B2 (EphB2) expression in normal and neoplastic tissues. EphB2 is a tyrosine kinase recently implicated in the deregulation of cell-to-cell communication in many tumors.

Experimental Design: EphB2 protein expression was analyzed by immunohistochemistry on tissue microarrays that included 76 different normal tissues, >4,000 samples from 138 different cancer types, and 1,476 samples of colon cancer with clinical follow-up data.

Results: We found most prominent EphB2 expression in the intestinal epithelium (colonic crypts) with cancer of the colorectum displaying the highest EphB2 positivity of all tumors. Positivity was found in 100% of 118 colon adenomas but in 33.3% of 45 colon carcinomas. EphB2 expression was also observed in 75 tumor categories, including serous carcinoma of the endometrium (34.8%), adenocarcinoma of the esophagus (33.3%), intestinal adenocarcinoma of the stomach (30.2%), and adenocarcinoma of the small intestine (70%). The occasional finding of strong EphB2 positivity in tumors without EphB2 positivity in the corresponding normal cells [adenocarcinoma of the lung (4%) and pancreas (2.2%)] suggests that deregulation of EphB2 signaling may involve up-regulation of the protein expression. In colon carcinoma, loss of EphB2 expression was associated with advanced stage ($P < 0.0001$) and was an indicator of poor overall survival ($P = 0.0098$).

Conclusions: Our results provide an overview on the EphB2 protein expression in normal and neoplastic tissues. Deregulated EphB2 expression may play a role in several cancer types with loss of EphB2 expression serving as an indicator of the possible pathogenetic role of EphB2 signaling in the maintenance of tissue architecture of colon epithelium.

Ephrin receptors represent a family of receptor tyrosine kinase comprising two subfamilies: EphA and EphB (1–3). They are located on the cell surface and transduce signals in a bidirectional manner when they bind with their ligands, the ephrins A and B, which are typically located on the surface of neighboring cells (1–3). Binding of ephrin to ephrin receptors leads not only to their activation but also to the transduction of a reverse signal toward the ephrin-expressing cell (4). Ephrin receptors were first described as important regulators in axon pathfinding (5) and in the development of nervous system. Later, it was shown that ephrin receptors are also involved in the control of various other cell functions, including vascular interactions, angiogenesis, integrin activity, and specific epithelial functions (6, 7).

Several studies provide insight regarding the role of EphB2 in normal and neoplastic colon. In normal colon, an important role of EphB in the control of positioning of intestinal epithelial cells via interaction with β-catenin and T-cell factor was recently described (6). In colorectal cancer, overexpression of both of EphB2 and ephrinB2 was described in cell lines and clinical cancer specimen (8–10). Studies in a mouse xenograft model have linked high levels of EphB2 expression with reduced tumor growth (9). Batlle et al. (6) identified EphB2 as a target of the wnt-signaling pathway, which is activated by genetic defects associated with the majority of colorectal cancers. Mao et al. (10) suggested that EphB2 expression is largely restricted to colorectal tumors. However, EphB2 was subsequently found at high levels in cancer cell lines derived from breast, stomach, esophagus, colon, and kidney cancer (11, 12) and in primary carcinomas derived from stomach, colon, lung, and endometrium as well as in neuroblastomas (2, 11, 13). We recently reported mutational inactivation of the EphB2 in prostate cancer cell lines and samples from advanced metastatic tumors. This

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suggests a putative role of EphB2 in the metastatic dissemination of human prostate cancer (14).

In this study, we screened the expression of EphB2 both in normal tissues and in different types of cancers to explore the biological significance of the protein as well as its involvement in cancer progression. For this purpose, we utilized tissue microarrays, including >6,000 different normal tissue and cancer sample types from 138 different tumor categories (methylation target array and colon cancer array). Our results suggest that EphB2 expression is predominantly found in intestinal epithelium and that loss of EphB2 may be associated with unfavorable tumor phenotype and poor survival in colorectal cancer.

Materials and Methods

Tissue microarray construction. Tissue microarray was constructed as described (15). Briefly, tissue cylinders with a diameter of 0.6 mm were punched from morphologically representative tissue areas of each "donor" tissue block and brought into one recipient paraffin block (3 × 2.5 cm) using a homemade semiautomated tissue arrayer.

Tumors. Three different sets of tissue microarrays were utilized for this study. (*a*) A normal tissue microarray composed of eight samples each of 76 different normal tissue types (n = 608). (*b*) A multitumor tissue microarray composed of up to 50 different samples from 138 different tumor types and subtypes. The composition of this tissue microarray is described in detail in Results. (*c*) A colorectal cancer tissue microarray composed of 1,476 cancers (1,414 with survival data). The median follow-up time of these patients was 46 months (range 0-152). All these tumors had been systematically reevaluated by one pathologist (L. Terracciano) for pT, pN, histologic subtype, grade, lymphocytic infiltration, and infiltration type (pushing versus infiltrative) according to criteria previously defined (16). All tumors were formalin fixed, paraffin embedded.

Immunohistochemistry. Four-micrometer sections of tissue microarray blocks were transferred to an adhesive-coated slide system (Instrumedics, Inc., Hackensack, NJ) to facilitate the transfer of tissue microarray sections on the slide and to minimize tissue loss, thereby increasing the number of sections that can be taken from each tissue microarray block. Standard indirect immunoperoxidase procedures were used for immunohistochemistry (ABC-Elite, Vector Laboratories, Burlingame, CA). A monoclonal mouse antibody was used for EphB2 detection (1:200; R&D Systems, Minneapolis, MN). Optimal staining could be achieved after steam cooker pretreatment (5 minutes, 120°C) in target retrieval solution (DAKO, Glostrup, Denmark; pH 9) for antigen retrieval. A 3,3′-diaminobenzidine chromogen (liquid DAB DAKO) was used. Nuclei were counterstained with hematoxylin. Normal colon epithelium was used as a positive control. The primary antibody was omitted as a negative control.

In normal tissues, the staining intensity was estimated for each cell type on a four-step scale (0-3+). For tumors, the staining intensity (0-3+) and the percentage of positive tumor cells was estimated. Tumors were then grouped into four categories according to staining intensity and percentage of positive cells. For statistical analyses, the staining results were categorized into four groups. Tumors without any staining were considered negative. Tumors with 1+ staining intensity in <80% of cells and 2+ intensity in <30% of cells were considered weakly positive.

Tumors with 1+ staining intensity in ≥80% of cells, 2+ intensity in 30% to 79% or 3+ intensity in <30% were considered moderately positive. Tumors with 2+ intensity in ≥80% or 3+ intensity in ≥30% of cells were considered strongly positive.

Despite thorough optimization of the staining protocol for formalin-fixed tissues, some diffuse cytoplasmic background staining could not be avoided especially in glandular and brain tissues. As such stainings were considered most likely to be nonspecific, the analysis was strictly limited to membranous staining.

Statistical analysis. Contingency table analysis and χ^2 tests were used to study the relationship between EphB2 expression, histologic tumor types and subtypes, grade, stage, and nodal status. Survival curves were plotted according to Kaplan-Meier. A log-rank test was applied to examine the relationship between molecular or histologic data and raw survival. Cox proportional hazard model with stepwise selection of the covariates was used to determine the parameters with greatest influence on patient survival.

Results

Normal tissues. Normal organs and cell types with a detectable membranous EphB2 expression are shown in Table 1. Unequivocal membranous EphB2 protein was only detected in gastrointestinal epithelial cells with strongest positivity in absorptive and crypt cells in appendix and colorectum. Examples of positive EphB2 immunohistochemistry in normal cells are shown in Fig. 1A and B. No membranous EphB2 positivity was observed in the following organs: aorta, heart, nose, lung, breast, ovary, fallopian tube, uterus, placenta, kidney, urinary bladder, penis, scrotum, prostate, seminal vesicle, epididymis, testis, skin, lip, oral cavity, tongue, parotic, submandibular and sublingual gland, small salivary gland, gall bladder, liver, pancreas, tonsil, lymph node, spleen, thymus, fat tissue, adrenal, parathyroid and thyroid gland, cerebrum, cerebellum, and pituitary gland.

Tumor screening. The results of our comparative tumor analyses are shown in Table 2. Membranous EphB2 positivity was most frequently seen in colon adenomas and carcinomas. There was a significant decrease in frequency and intensity of staining from colon adenoma to carcinoma (P < 0.0001). Whereas all 118 analyzed adenomas were positive, 67% of 45 analyzed colon adenocarcinomas were negative (Fig. 2A and B). Some level of EphB2 positivity in at least one tumor was seen in 75 additional tumor types and subtypes. In 22 of 138 tumor categories, a strong positivity was detected at least in one case.

Colorectal carcinomas. A colon cancer tissue microarray containing 1,476 cancers with follow-up data was analyzed for EphB2 expression based on the suggestion that the multitumor tissue microarray data suggested a role for EphB2 protein down-regulation in colon cancer progression. Of the 1,414 colon carcinomas, 1,176 (82.9%) were evaluable. Among these, 593 (50.4%) tumors were negative, 264 (22.4%) were weakly, 173 (14.7%) moderately, and 146 (12.4%) strongly positive. Loss of EphB2 expression was significantly associated with high pT (P < 0.0001), nodal positivity (P < 0.0001), and infiltrative tumor margin (P = 0.0023; Table 3). Also, loss of EphB2 expression showed a strong, inverse association with patient survival (P = 0.0098; Fig. 3). However, in a multivariate analysis, including pT (P < 0.0001) and pN (P < 0.0001), EphB2 was not an independent predictor of poor prognosis (P = 0.9). When the series was stratified by pT and pN categories, pooling together normal and weak positivity and moderate and strong positivity, a significant difference was observed in pT_2 and pT_4 groups (Fig. 4).

Discussion

The availability of large tissue microarrays allowed the rapid analysis of EphB2 protein expression in >6,000 specimens resulting in a comprehensive "pathomics" overview of the

Table 1. EphB2 expression in normal tissues

Organ	Cell type	EphB2 intensity
Stomach, fundus, and corpus	Columnar, mucous neck, parietal and chief cell	++
Stomach, antrum	Columnar and mucosa-secreting cell	++
Small intestine, duodenum	Absorptive and crypt cell	++
Small intestine, ileum	Absorptive and crypt cell	++
Colon	Absorptive and crypt cell	+++
Appendix	Absorptive and crypt cell	+++
Rectum	Absorptive and crypt cell	+++

EphB2 expression in normal and neoplastic tissues. The results confirm the previously described high-level of EphB2 expression in normal colonic mucosa and expand these observations to show a significant association between loss of EphB2 expression and colorectal tumor progression. Furthermore, we also found evidence of the deregulation EphB2 expression in other intestinal tissues as well as in several intestinal and nonintestinal tumor types.

All 118 colonic adenomas were positive for EphB2 protein, which is consistent with an important role of EphB2 in the cell biology of colonic epithelium. The markedly lower frequency of EphB2 positivity in the 45 colonic carcinomas of our multitumor tissue microarray suggested that loss of EphB2 expression accompanies progression of colonic neoplasms. To further validate this hypothesis, we expanded our study to an organ-specific tissue microarray composed of >1,400 colon carcinomas with full pathology data and clinical follow-up information. A significant association was seen between the loss of EphB2 expression and advanced tumor stage, high grade, presence of vascular invasion, infiltrative tumor growth, and poor survival. This provided significant new clinical evidence for a link between EphB2 inactivation in colon cancer progression. These results are particularly intriguing when viewed in light of recent evidence concerning the role of EphB2 in the normal development of colonic epithelium. EphB2 knockout mice displayed defects in the compartmentalization between stem cells and differentiated colonic epithelial cells (6). The correct positioning of cells and maintenance of cellular architecture in the intestinal crypt depends upon intact EphB2 signaling. Down-regulation of such signaling in human tumor tissues may, therefore, be a direct pathogenetic mechanism at least in a subset of colorectal tumors.

EphB2 signaling is mediated through its interactions with a number of scaffolding and adaptor proteins, such as p120 Ras GTPase-activating protein (RasGAP), Nck, and Dok-1 (1). EphB2 negatively regulates a number of these signaling mediators. For example, adhesion-dependent activation of the Ras–mitogen-activated protein kinase pathway, Rac, and focal adhesion kinase are abrogated by EphB2 ligation (6, 17, 18). Additionally, disruption of the intracellular domain of EphB2 has been shown to allow for the invasion of cells possessing this truncated receptor into areas of cells expressing the ephrin B ligands (19). Given the fact that ephrin receptor/ephrin system is important in controlling tissue architecture, it is not surprising that EphB2 is down-regulated in colon cancer cells. It is possible that loss of EphB2 function facilitates deregulation of the normal compartmentalization of the colon stem cells, loss of tissue architecture, perhaps even invasion and metastatic spread of the tumor cells.

Other tumors of the gastrointestinal tract that were EphB2 positive in a fraction of cases and for which the corresponding normal tissues showed EphB2 positivity included carcinomas of the small intestine and the stomach. It is possible that also in these tumor types, EphB2 down-regulation/inactivation may be relevant for tumor progression. In a recent study, we were able to identify potentially inactivating EphB2 mutations in metastatic prostate cancer (14). EphB2 inactivation in gastrointestinal tumors could, therefore, also be due to gene mutation. However, Oba et al. (20) did not find EphB2 mutations in 50 colon carcinomas, suggesting that the inactivation of this pathway may happen upstream of the EphB2 or via other mechanisms, including epigenetic inactivation.

EphB2 was recently even proposed as a possible target for antibody drug therapy in colon cancer (10). The fact that we

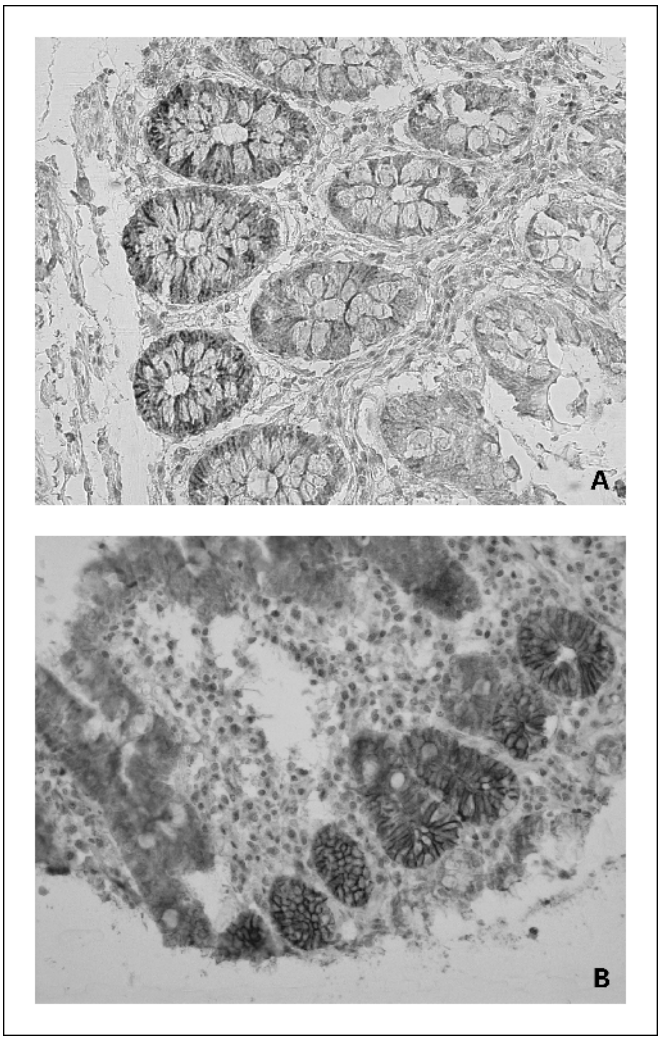

Fig. 1. EphB2 weakly positive cryptal cells of normal colon (original magnification, ×20; *A*) and moderately positive normal small intestinal mucosa (original magnification, ×20; *B*).

Table 2. EphB2 expression in human tumors (percentage values)

Tumor entity	Ephrin receptor immunostaining			
	n	Weak (%)	Moderate (%)	Strong (%)
Skin tumors				
Skin, basalioma	44	6.8	0.0	0.0
Skin, benign appendix tumor	31	3.2	0.0	0.0
Skin, benign nevus	47	0.0	0.0	0.0
Skin, Merkel cell cancer	5	20.0	0.0	0.0
Skin, malignant melanoma	49	12.2	0.0	0.0
Skin, squamous cell cancer	45	0.0	0.0	0.0
Respiratory tract tumors				
Oral cavity, squamous cell carcinoma	42	4.8	0.0	0.0
Larynx, squamous cell carcinoma	30	10.0	0.0	0.0
Lung, squamous cell carcinoma	50	0.0	2.0	0.0
Lung, adenocarcinoma	50	0.0	2.0	4.0
Lung, large cell cancer	49	2.0	2.0	0.0
Lung, small-cell cancer	48	2.1	0.0	0.0
Malignant mesothelioma	15	13.3	13.3	0.0
Pharynx, lymphoepithelial carcinoma	5	0.0	0.0	0.0
Gynecologic tumors				
Breast, apocrine cancer	3	0.0	0.0	0.0
Breast, cribriform cancer	6	0.0	0.0	0.0
Breast, ductal cancer	49	0.0	0.0	0.0
Breast, lobular cancer	43	0.0	0.0	0.0
Breast, medullary cancer	29	3.4	0.0	0.0
Breast, mucinous cancer	27	3.7	0.0	0.0
Breast, Phylloides tumor	10	10.0	0.0	0.0
Breast, tubular cancer	25	0.0	0.0	0.0
Ovary, dysgerminoma	2	0.0	0.0	0.0
Ovary, gonadoblastoma	1	0.0	0.0	0.0
Ovary, yolk sack tumor	1	0.0	0.0	0.0
Ovary, undifferentiated carcinoma	1	0.0	0.0	0.0
Ovary, Brenner tumor	1	0.0	0.0	0.0
Ovary, serous cancer	49	10.2	6.1	2.0
Ovary, mucinous cancer	21	4.8	0.0	0.0
Ovary, endometroid cancer	50	10.0	2.0	2.0
Vagina, squamous cell cancer	5	40.0	0.0	0.0
Vulva, squamous cell cancer	43	0.0	0.0	0.0
Uterus cervix, squamous cell carcinoma	32	0.0	0.0	3.1
Uterus, cervix, CIN III	17	0.0	0.0	0.0
Uterus, cervix, adenocarcinoma	3	33.3	0.0	0.0
Uterus, carcinosarcoma	5	0.0	0.0	0.0
Endometrium, endometroid carcinoma	49	6.1	0.0	2.0
Endometrium, serous carcinoma	23	30.4	4.3	0.0
Endometrioid stroma sarcoma	4	25.0	0.0	0.0
Digestive tract tumors				
Salivary gland, pleomorphic adenoma	48	6.3	0.0	0.0
Salivary gland, cylindroma	53	3.8	0.0	0.0
Salivary gland, adenolymphoma	30	0.0	0.0	0.0
Salivary gland, small-cell cancer	1	100.0	0.0	0.0
Salivary gland, acinus cell cancer	7	14.3	0.0	0.0
Salivary gland, squamous cell cancer	2	0.0	0.0	0.0
Salivary gland, unclassified carcinoma	1	0.0	0.0	0.0
Salivary gland, undifferentiated carcinoma	6	0.0	0.0	0.0
Salivary gland, mucoepidermoid cancer	5	0.0	0.0	0.0

(*Continued on the following page*)

Table 2. EphB2 expression in human tumors (percentage values) (Cont'd)

Tumor entity	Ephrin receptor immunostaining			
	n	Weak (%)	Moderate (%)	Strong (%)
Salivary gland, adenocarcinoma	2	0.0	0.0	0.0
Esophagus, adenocarcinoma	6	16.7	0.0	16.7
Esophagus, squamous cell carcinoma	31	3.2	0.0	3.2
Esophagus, small-cell carcinoma	1	0.0	0.0	0.0
Stomach, diffuse adenocarcinoma	21	14.3	4.8	0.0
Stomach, intestinal adenocarcinoma	43	20.9	7.0	2.3
Small intestine, adenocarcinoma	10	40.0	20.0	10.0
Colon adenoma, mild dysplasia	42	21.4	26.2	52.4
Colon adenoma, moderate dysplasia	41	4.9	29.3	65.9
Colon adenoma, severe dysplasia	35	5.7	28.6	65.7
Colon, adenocarcinoma	45	4.4	13.3	15.6
Anus, squamous cell cancer	3	33.3	0.0	0.0
GIST	31	6.5	0.0	0.0
Gall bladder, adenocarcinoma	21	19.0	0.0	0.0
Hepatocellular carcinoma	46	8.7	2.2	0.0
Pancreas, adenocarcinoma	46	21.7	2.2	2.2
Genitourinary tract tumors				
Kidney, clear cell cancer	47	2.1	0.0	0.0
Kidney, chromophobic cancer	12	0.0	0.0	0.0
Kidney, papillary cancer	46	6.5	0.0	2.2
Kidney, oncocytoma	10	10.0	0.0	0.0
Urinary bladder cancer, TCC noninvasive (pT_a)	45	6.7	0.0	0.0
Urinary bladder cancer, cancer, TCC invasive (pT_{2-4})	40	5.0	0.0	0.0
Urinary bladder, squamous cell cancer	8	12.5	0.0	0.0
Urinary bladder, small-cell cancer	4	0.0	0.0	0.0
Urinary bladder, sarcomatoid cancer	8	0.0	0.0	0.0
Urinary bladder, adenocarcinoma	4	0.0	0.0	0.0
Urinary bladder, inverted carcinoma	1	0.0	0.0	0.0
Prostate cancer, untreated	55	0.0	1.8	0.0
Prostate cancer, hormone refractory	31	9.7	0.0	0.0
Testis, mixed cancer	2	0.0	0.0	0.0
Testis, seminoma	50	2.0	0.0	0.0
Testis, nonseminomatous cancer	54	5.6	0.0	0.0
Testis, teratoma	6	0.0	0.0	0.0
Penile cancer	32	0.0	0.0	0.0
Neuroendocrine tumors				
Adrenal gland, adenoma	15	33.3	0.0	0.0
Adrenal gland, cancer	6	0.0	0.0	0.0
Paraganglioma	9	0.0	11.1	0.0
Pheochromocytoma	30	0.0	0.0	0.0
Thyroid, adenoma	44	20.5	0.0	0.0
Thyroid, follicular cancer	48	0.0	0.0	2.1
Thyroid, papillary cancer	37	8.1	0.0	0.0
Thyroid, anaplastic cancer	5	0.0	0.0	0.0
Thyroid, medullary cancer	8	0.0	0.0	0.0
Parathyroid, adenoma	24	0.0	0.0	0.0
Parathyroid, cancer	1	0.0	0.0	0.0
Carcinoid tumor	44	9.1	0.0	0.0
Hematologic neoplasias				
NHL, diffuse large B	22	4.5	4.5	0.0
NHL, others	30	3.3	3.3	0.0
MALT lymphoma	48	0.0	4.2	2.1

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Table 2. EphB2 expression in human tumors (percentage values) (Cont'd)

Tumor entity	Ephrin receptor immunostaining			
	n	Weak (%)	Moderate (%)	Strong (%)
Hodgkin lymphoma, nodular sclerosis	35	5.7	0.0	0.0
Hodgkin lymphoma, mixed cell	19	0.0	0.0	0.0
AML	1	0.0	0.0	0.0
CML	5	0.0	0.0	0.0
Thymoma	24	0.0	0.0	0.0
Brain tumors				
Meningeoma	48	8.3	4.2	0.0
Astrocytoma	40	2.5	2.5	0.0
Glioblastoma multiforme	48	4.2	2.1	4.2
Oligodendroglioma	29	6.9	3.4	0.0
Medulloblastoma	5	0.0	0.0	40.0
Esthesioneuroblastoma	2	50.0	0.0	50.0
Craniopharyngeoma	29	0.0	0.0	0.0
Ependymoma	8	0.0	0.0	0.0
Soft tissue tumors				
Lipoma	11	0.0	0.0	0.0
Liposarcoma	29	10.3	0.0	0.0
Benign histiocytoma	28	0.0	0.0	0.0
Dermatofibroma protuberans	4	25.0	0.0	0.0
Malignant fibrous histiocytoma	29	6.9	3.4	0.0
Leiomyoma	59	0.0	0.0	0.0
Leiomyosarcoma	40	12.5	0.0	0.0
Alveolar sarcoma	1	0.0	0.0	0.0
Rhabdomyosarcoma	14	14.3	0.0	0.0
Fibrosarcoma	9	22.2	0.0	0.0
Tendon sheath, giant cell tumor	35	2.9	0.0	0.0
Synovial sarcoma	3	33.3	33.3	33.3
Epitheloid sarcoma	2	0.0	0.0	0.0
Epitheloid hemangioma	1	0.0	0.0	0.0
Glomus tumor	6	0.0	0.0	0.0
Capillary hemangioma	27	0.0	0.0	0.0
Hemangiopericytoma	9	0.0	11.1	11.1
Angiosarcoma	4	0.0	0.0	0.0
Kaposi sarcoma	26	3.8	0.0	0.0
Neurofibroma	40	0.0	0.0	0.0
Ganglioneuroma	7	0.0	0.0	0.0
Granular cell tumor	8	0.0	0.0	0.0
Schwannoma	49	0.0	0.0	0.0
Malignant Schwannoma	8	0.0	0.0	0.0
Angiomyolipoma	1	0.0	0.0	0.0
Opticus glioma	1	0.0	0.0	0.0
PNET	17	17.6	0.0	0.0
Adenomatoid tumor	10	20.0	0.0	0.0

Abbreviations: CIN, cervical intraepithelial neoplasia; GIST, gastrointestinal stromal tumor; TCC, transitional cell carcinoma; NHL, non-Hodgkin's lymphoma; MALT, mucosa-associated lymphatic tissue lymphoma; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; PNET, primitive neuroectodermal tumor.

did not observe evidence of EphB2 overexpression in colorectal cancer could be due to the fact that, in our study, the antibody staining was titrated for the entire panel of different tumor types, not just to detect overexpression in colorectal cancer. However, we did observe several other tumor types, where EphB2 expression was seen in the tumor tissues, when the adjacent tumor tissue was negative. Tumor entities belonging to this category included carcinomas of the pancreas and lung as well as a serous carcinoma of the endometrium, suggesting that in some tumor types the ephrin receptor/ephrin system may also be deregulated in other ways.

EphB2 expression was predominantly seen in gastrointestinal and neuronal tumors, consistent with the known role of

EphB2 in normal brain and intestine (2, 21, 22). In tumor types that were previously examined for EphB2 expression, especially by tumor cell lines, our data significantly extended the previously published data immunohistochemically. Previous studies had described EphB2 positivity in small-cell lung cancer in 2.7% of 11 cell lines (our data: 2.1%) and in 18.2% of 22 melanoma cell lines (our data: 12%; refs. 23, 24). Our list of strongly EphB2-positive tumors also includes several entities for which an EphB2 expression has never been described. This includes malignant mesothelioma, serous carcinoma of the endometrium (Fig. 3), adenocarcinoma of the esophagus, medulloblastoma (Fig. 4), and esthesioneuroblastoma. Our finding of EphB2 expression in several tumor entities for which the corresponding normal tissues were EphB2 negative would be consistent with a possible oncogenic effect of high epbB2 protein levels at least in some tissues. The causes resulting in fundamentally different roles of EphB2 on facilitating/preventing tumors in different tissue types require further studies. EphB receptors, and in particular EphB2, are involved in the control of several established key pathways for cancer biology. They have a typical receptor tyrosine kinase structure (25), with a fairly long juxtamembrane regulatory domain (4). Mutation in this domain could lead to increased kinase activity and overexpression of the receptor or to constitutive activation of EphB2, as it has been shown in glioblastoma (26).

A recent study in breast cancer suggested a prognostic role of cytoplasmatic EphB2 immunostaining, which was found in 99% of breast cancers (12). Cytoplasmic EphB2 staining was disregarded in our study because normal tissues showed exclusively membranous staining, and also because the cytoplasm is the most frequent cellular compartment showing non specific immunohistochemical reactions. However, some cytoplasmic immunostainings was also seen in some of our tumors, including breast cancer. Although it currently cannot be excluded that cytoplasmic EphB2 staining could arise as a result of a subcellular redistribution of EphB2 protein in tumor

Table 3. Relationship between EphB2 and clinicopathologic parameter

	n	EphB2+ (%)	*P*
pT			
pT_1	49	35 (72.0)	
pT_2	175	100 (57.1)	
pT_3	749	377 (50.4)	<0.0001
pT_4	179	62 (36.5)	
pN			
pN_0	606	340 (56.1)	
pN_1	300	148 (49.3)	<0.0001
pN_2	233	77 (33.1)	
Lymphatic infiltration			
Present	248	127 (51.2)	0.6677
Absent	905	447 (49.4)	
Margin			
Pushing	434	242 (55.8)	0.0023
Infiltrative	718	331 (46.1)	

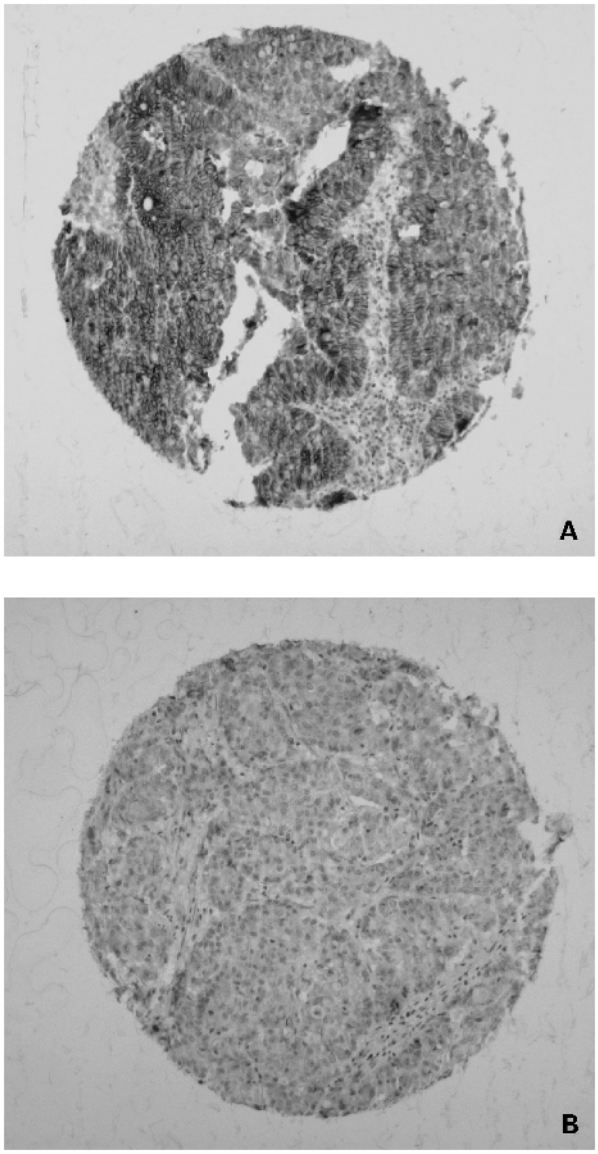

Fig. 2. EphB2 strong positivity (*A*) and negativity (*B*) in colorectal adenocarcinoma (original magnification, ×10).

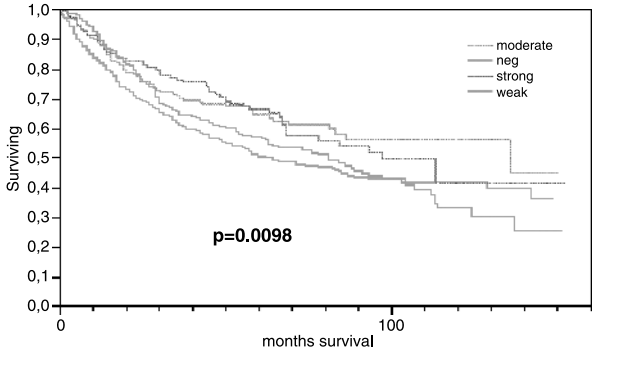

Fig. 3. Kaplan-Meier survival curve in 1,176 colorectal carcinomas. Log-rank test.

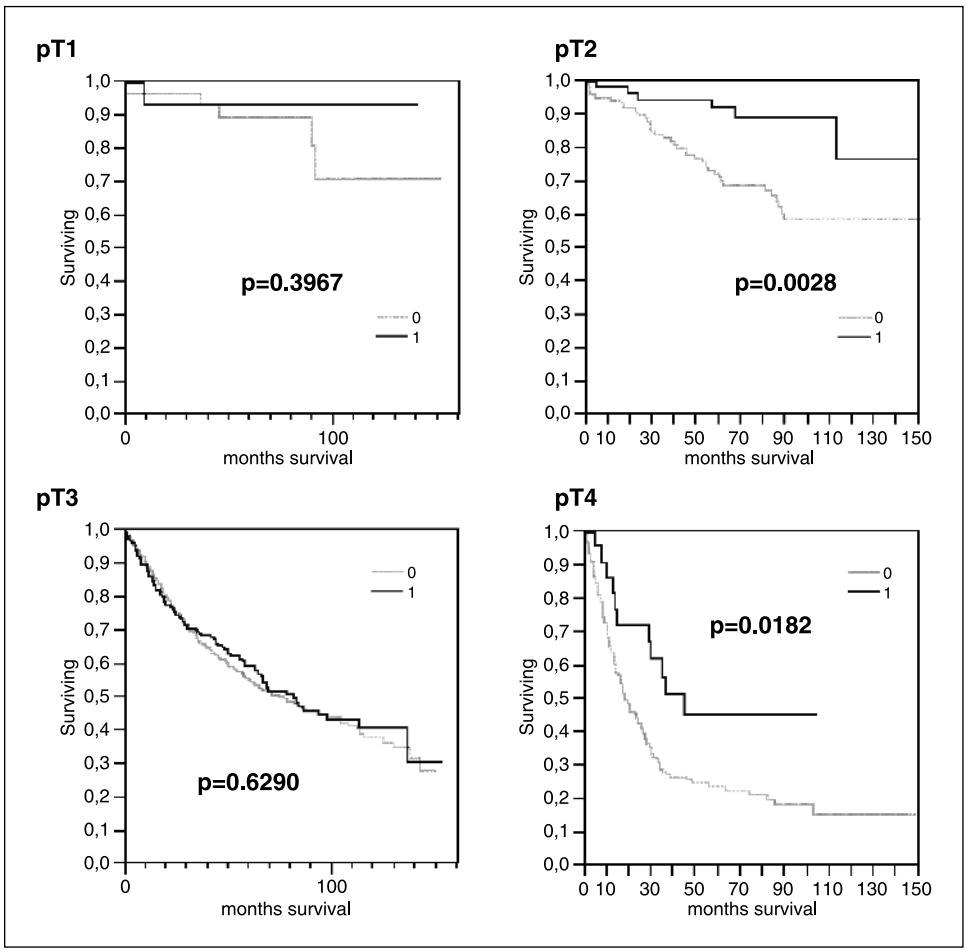

Fig. 4. Kaplan-Meier survival curves stratified by tumor stages (0 = EphB2 negative or weakly positive; 1 = EphB2 moderately or strongly positive). Log-rank test (×10).

cells, it is generally believed that localization on the cell membrane is necessary for the function of the ephrin receptor/ephrin system (8, 9, 27). Finally, we did not see any EphB2 staining in either normal or tumorous human prostate tissue, an observation worth noting in light of our recent findings of inactivating mutations in advanced and metastatic prostate cancers. This may reflect the fact that the normal levels of EphB2 expression in these tissues may be below the detectability limit of the immunohistochemical assay.

In summary, this study provides a comprehensive overview on the expression of EphB2 in normal and cancerous tissues. Among normal tissues, colonic tissue shows the highest levels of expression, which is retained in colonic adenomas. However, we found a consistent, very significant trend for loss of EphB2 expression during colon cancer progression, which suggests a significant role for the deregulation of the EphB2 signaling in human colon cancer progression, consistent with previous mouse model systems functionally linking EphB2 to tumor tissue disorganization and defects in the compartmentalization and differentiation of colorectal epithelial cells. Our observations, therefore, also suggest the possible meaning that manipulation and restoration of EphB2 signaling could have in colorectal cancer development. Most colon cancers show a significant down-regulation of EphB2, which is related to unfavorable tumor phenotype (advanced infiltration of the wall and presence of lymph node metastasis). The importance of EphB2 expression is also stressed by the fact that stratifying the series by stage, we observed significant difference in survival between negative/weakly positive cases compared with strongly positive ones in pT_2 and pT_4 cases.

In other tumor types, such as pancreatic or lung cancer, EphB2 may play an opposite role through overexpression compared with normal tissues, suggesting multiple modes of deregulation of the ephrin receptor/ephrin system in tumorigenesis.

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