EphB1 Is Underexpressed in Poorly Differentiated Colorectal Cancers

Zhen Shenga, Jiandong Wanga, b, Yingchun Donga, Henghui Maa, Hangbo Zhoua, Haruhiko Sugimurac, Guangming Lub, Xiaojun Zhoua

a Department of Pathology, Clinical School of Medical College of Nanjing University/Nanjing Jinling Hospital, and b Center for Molecular Imaging Research, Department of Radiology, Nanjing Jinling Hospital, Nanjing, PR China; c Department of Pathology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Key Words
EphB1 · Colorectal cancer · Quantitative real-time RT-PCR · Immunohistochemical staining

Abstract

Introduction

The Eph family, named for its expression in an erythropoietin-producing human hepatocellular carcinoma cell line [1], is the largest subfamily of the receptor tyrosine kinases and includes at least 14 distinct receptors and 8 distinct ligands. Both the Ephs (receptors) and ephrins (ligands) are divided into 2 groups, the A and B subfamilies [2]. The interactions between Eph receptors and ephrin ligands play important roles in vascular development, tissue border formation, cell migration, axon guidance and angiogenesis [3–7]. Unlike other families of the receptor tyrosine kinases, which bind to soluble ligands, Eph receptors interact with cell membrane-bound ephrin ligands. Moreover, these receptor-ligand interactions activate a bidirectional signaling pathway through both the Eph receptors and ephrin ligands. Some receptors of the Eph gene have been found overexpressed in human tumors, including neuroblastoma, lung, gastric, esophageal, breast and colorectal cancer. Overexpression of Eph receptors could be correlated to altered tumor behaviors, such as increased metastatic potential and poor patient outcome. But more recently, Eph receptors and ephrins have been recognized as being differentially expressed in various human tumors. Our previous studies showed reduced expression of EphB1 in colorectal cancers more often occurred in poorly differentiated and mucinous adenocarcinomas than in well- and moderately differentiated adenocarcinomas. Further, cancer cells with a low level of EphB1 protein showed more invasive power. Conclusion: Our data indicate that EphB1 may have roles in the pathogenesis and development of colorectal cancer.
that EphA7 was underexpressed in colorectal cancers, and we proved that aberrant methylation of the 5’CpG islands is the main mechanism that leads to the down-regulation of EphA7 [8]. We also found that EphA7 receptor is differentially expressed in gastric carcinoma [9].

Colorectal cancer is the second most common type of cancer in the Western world and its incidence has recently also markedly increased in other countries such as China. EphB/ephrinB signaling is essential for the correct formation of crypts and villi in the intestinal epithelium [10–12]. Increasing data have shown that the EphB subfamily is involved in the carcinogenesis of colorectal cancer. Among the Eph family genes, relatively little attention has been directed toward EphB1 in human colorectal cancer, and the potential role of EphB1 in human colorectal cancer has not been addressed. EphB1 was first identified in a rat brain cDNA expression library. In human, EphB1 was preferentially highly expressed in normal brain, testis and colon. Expression of EphB1 in certain human tumors has been investigated. However, the results were inconsistent. In order to investigate the role of EphB1 in tumorigenesis of colorectal cancer, quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry were used to determine expression of EphB1 in human colon cancer cell lines, colorectal adenomas and colorectal cancer tissues. Our data show that underexpression of EphB1 often occurs in colorectal cancer patients with poorly differentiated tumor. And cancer cells with low level of EphB1 protein show more invasive power. The results indicate that EphB1 plays a role in the development and prognosis of colorectal cancer.

**Materials and Methods**

**Colon Cancer Cell Lines and Tissue Specimens**

The colon cancer cell lines SW480, DLD1, HT29, HCT116 and SW620 were used in the present study. The cells were routinely maintained in Dulbecco’s modified Eagle medium (NISSUI Pharmaceutical Co.) supplemented with 1 mmol/l L-glutamine, 10% fetal bovine serum (Life Technologies Inc.), 100 U/ml of penicillin G and 100 mg/ml of streptomycin. The cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO2.

A total of 15 colorectal adenomas, including 6 of low and 9 of high grade, 78 colorectal carcinoma and uninvolved normal mucosa specimens were obtained from surgical resections performed at the Nanjing Jinling Hospital between 2005 and 2006, as part of a study approved by the Research Ethics Board of the Nanjing Jinling Hospital. The distribution of the tumors by sites of origin was as follows: cecum and ascending colon, 14 tumors; sigmoid colon, 9 tumors; rectum, 51 tumors; others, 4 tumors. Formalin-fixed and paraffin-embedded tumor tissue sections were stained with hematoxylin and eosin and examined histologically. The clinicopathological characteristics of the 78 colorectal patients are shown in Table 1. The tissue samples were immediately frozen in liquid nitrogen and stored at ~80°C for the extraction of total RNA and DNA. All tissue specimens were evaluated pathologically. No patients had received irradiation or cancer chemotherapy prior to resection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:female</td>
<td>47:31</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>≤55 years</td>
<td>38</td>
</tr>
<tr>
<td>&gt;55 years</td>
<td>40</td>
</tr>
<tr>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>Rectum and sigmoid colon</td>
<td>60</td>
</tr>
<tr>
<td>Others</td>
<td>18</td>
</tr>
<tr>
<td>Depth of wall invasion</td>
<td></td>
</tr>
<tr>
<td>Mucosa and submucosa</td>
<td>4</td>
</tr>
<tr>
<td>Muscularis propria</td>
<td>22</td>
</tr>
<tr>
<td>Subserosa and serosa</td>
<td>52</td>
</tr>
<tr>
<td>Tumor differentiation</td>
<td></td>
</tr>
<tr>
<td>Well-differentiated adenocarcinoma</td>
<td>12</td>
</tr>
<tr>
<td>Moderately differentiated adenocarcinoma</td>
<td>47</td>
</tr>
<tr>
<td>Poorly differentiated adenocarcinoma</td>
<td>7</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>12</td>
</tr>
<tr>
<td>Stage (TNM)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>23</td>
</tr>
<tr>
<td>II</td>
<td>26</td>
</tr>
<tr>
<td>III+IV</td>
<td>29</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>49</td>
</tr>
<tr>
<td>Present</td>
<td>29</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of 78 patients with colorectal carcinoma

To detect the expression of the EphB1 transcript in carcinoma samples, a quantitative real-time RT-PCR was subjected to the cDNAs. The reactions were performed in triplicate. The sense and antisense primers and TaqMan probe for EphB1 were designed according to the mRNA sequence (GenBank accession No. NM_002046). Amplification of PCR fragments spanning different exons was used to prevent contamination of genomic DNA. The sense primer was 5’-GCGATGGGCCCTGATTATCTAC-3’ and antisense primer was 5’-CGATAGCGCTTTGTTGTC-3’.

The PCR products were 92 bp long. The TaqMan probe was 5’-(FAM) TCTCTGGGATCAGTGCCTG (Eclipse)-3’. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (GenBank accession No. NM_004441). Amplification of PCR fragments spanning different exons was used to prevent contamination of genomic DNA. The sense primer was 5’-GCGATGGGCCCTGATTATCTAC-3’ and antisense primer was 5’-CGATAGCGCTTTGTTGTC-3’.

The PCR products were 130 bp long. The TaqMan probe was 5’-(FAM) TCTCTGGGATCAGTGCCTG (Eclipse)-3’. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (GenBank accession No. NM_004441). Amplification of PCR fragments spanning different exons was used to prevent contamination of genomic DNA. The sense primer was 5’-GCGATGGGCCCTGATTATCTAC-3’ and antisense primer was 5’-CGATAGCGCTTTGTTGTC-3’.

Table 1. Characteristics of 78 patients with colorectal carcinoma

The PCR products were 92 bp long. The TaqMan probe was 5’-(FAM) TCTCTGGGATCAGTGCCTG (Eclipse)-3’. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (GenBank accession No. NM_004441). Amplification of PCR fragments spanning different exons was used to prevent contamination of genomic DNA. The sense primer was 5’-GCGATGGGCCCTGATTATCTAC-3’ and antisense primer was 5’-CGATAGCGCTTTGTTGTC-3’.

The PCR products were 130 bp long. The TaqMan probe was 5’-(FAM) TCTCTGGGATCAGTGCCTG (Eclipse)-3’. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (GenBank accession No. NM_004441). Amplification of PCR fragments spanning different exons was used to prevent contamination of genomic DNA. The sense primer was 5’-GCGATGGGCCCTGATTATCTAC-3’ and antisense primer was 5’-CGATAGCGCTTTGTTGTC-3’.

The PCR products were 92 bp long. The TaqMan probe was 5’-(FAM) TCTCTGGGATCAGTGCCTG (Eclipse)-3’. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (GenBank accession No. NM_004441). Amplification of PCR fragments spanning different exons was used to prevent contamination of genomic DNA. The sense primer was 5’-GCGATGGGCCCTGATTATCTAC-3’ and antisense primer was 5’-CGATAGCGCTTTGTTGTC-3’.

The PCR products were 130 bp long. The TaqMan probe was 5’-(FAM) TCTCTGGGATCAGTGCCTG (Eclipse)-3’. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (GenBank accession No. NM_004441). Amplification of PCR fragments spanning different exons was used to prevent contamination of genomic DNA. The sense primer was 5’-GCGATGGGCCCTGATTATCTAC-3’ and antisense primer was 5’-CGATAGCGCTTTGTTGTC-3’.

The PCR products were 92 bp long. The TaqMan probe was 5’-(FAM) TCTCTGGGATCAGTGCCTG (Eclipse)-3’. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (GenBank accession No. NM_004441). Amplification of PCR fragments spanning different exons was used to prevent contamination of genomic DNA. The sense primer was 5’-GCGATGGGCCCTGATTATCTAC-3’ and antisense primer was 5’-CGATAGCGCTTTGTTGTC-3’.

The PCR products were 130 bp long. The TaqMan probe was 5’-(FAM) TCTCTGGGATCAGTGCCTG (Eclipse)-3’. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (GenBank accession No. NM_004441). Amplification of PCR fragments spanning different exons was used to prevent contamination of genomic DNA. The sense primer was 5’-GCGATGGGCCCTGATTATCTAC-3’ and antisense primer was 5’-CGATAGCGCTTTGTTGTC-3’.
Expression of EphB1 in colon cancer cell lines and representative examples of colorectal cancer.

**Statistical Analysis**

The statistical significance of intergroup differences was analyzed using the χ² test. All statistical analyses were performed using SPSS 11.5 software (SPSS Inc.). For all statistical tests, two-sided p < 0.05 was considered statistically significant.

**Results**

**Expression of the EphB1 Transcript in Colon Cancer Cell Lines**

Expression of EphB1 in the colon cancer cell lines SW480, SW620, DLD1, HT29 and HCT116 was assessed using quantitative real-time RT-PCR. The EphB1 transcript was differentially expressed in colon cancer cell lines, EphB1 expression is high in SW480 and low in HT29 (Fig. 1).

**Expression of the EphB1 Transcript in Colorectal Carcinoma Specimens**

Expression of the EphB1 transcription was detected using quantitative real-time RT-PCR in 78 colorectal carcinoma specimens that contained paired uninvolved normal mucosa and tumor tissue. Colorectal carcinoma samples showed marked interspecimen variability in their levels of EphB1 expression. The expression level of EphB1 in colorectal carcinoma tissues was compared with that in paired uninvolved normal mucosa tissues and classified as A, B or C according to the ratio of the 2: A = uninvolved normal mucosa-to-tumor ratio greater than 2 (N/T >2); B = uninvolved normal mucosa-to-tumor ratio less than 0.5 (N/T <0.5); C = normal mucosa-to-tumor ratio between 0.5 and 2 (N/T 0.5–2; table 2). Downregulation of EphB1 (class A) was observed in 27 (34.6%) colorectal carcinoma specimens, while overexpression of EphB1 (class B) was observed in 36 (46.2%) samples.
EphB1 Is Underexpressed in Poorly Differentiated Colorectal Cancers

Correlation between EphB1 Transcript Expression and Clinicopathological Parameters

Table 2 shows the correlation between clinical variables and the expression of the EphB1 transcript. The transcription level of EphB1 was significantly related to the differentiation of the patients (p = 0.037). The EphB1 transcript is more often reduced in poorly differentiated carcinomas. There was no significant association between EphB1 transcript expression and sex, age, tumor location, clinical stage, depth of wall invasion and lymph node metastasis.

Expression of EphB1 Protein in Colorectal Adenoma and Carcinoma Specimens

A total of 15 colorectal adenoma, 69 colorectal carcinoma and normal adjacent uninvolved mucosa specimens were immunohistochemically stained with a specific polyclonal EphB1 antibody. Five samples of low-grade and 8 samples of high-grade adenomas were positively stained; 1 sample of low-grade and 1 sample of high-grade carcinoma were negative. In all samples of normal colon, EphB1 protein expression was most intense at the base of the crypt with expression declining to the luminal epithelium (fig. 2a). The expression level of EphB1 protein differed between colorectal cancer cells, and heterogeneous staining in the same slide was observed (fig. 2c). However, the colorectal adenoma cells were stained homogenously (fig. 2b). The EphB1 immunoreactivity was observed mainly in golgiosome, cytoplasm and rarely in membrane. Of 69 colorectal carcinoma tissue samples, the EphB1 protein expression was downregulated in 39 (56.5%) and upregulated in 15 (21.7%) tumor samples.

Association of the EphB1 Protein Expression with Clinicopathological Parameters

Table 3 shows the correlation between EphB1 protein expression and clinicopathological characteristics. The

\[ \begin{array}{cccccc}
\text{Table 2. Correlation between EphB1 transcript expression and clinicopathologic parameters} \\
& \text{N/T} \geq 2 & \text{N/T} 2–0.5 & \text{N/T} \leq 0.5 & \text{p} \\
\hline
\text{Overall} & 27 & 15 & 36 \\
\text{Sex} & & & & \\
\text{Male} & 18 & 10 & 19 \\
\text{Female} & 9 & 5 & 17 & 0.458 \\
\text{Age} & & & & \\
\leq 55 \text{ years} & 13 & 9 & 16 & \\
>55 \text{ years} & 14 & 6 & 20 & 0.597 \\
\text{Location} & & & & \\
\text{Rectum and sigmoid colon} & 23 & 11 & 26 & \\
\text{Others} & 4 & 4 & 10 & 0.45 \\
\text{Depth of wall invasion} & & & & \\
\text{Mucosae and submucosa} & 2 & 1 & 1 & \\
\text{Muscularis propria} & 8 & 3 & 11 & \\
\text{Subserosa and serosa} & 17 & 11 & 24 & 0.751 \\
\text{Pathological classification} & & & & \\
\text{Well and moderate} & 18 & 9 & 32 & \\
\text{Poor and mucinous} & 9 & 6 & 4 & 0.037 \\
\text{Clinical stage (TNM)} & & & & \\
\text{I} & 9 & 4 & 10 & \\
\text{II} & 7 & 6 & 13 & \\
\text{III+IV} & 11 & 5 & 13 & 0.894 \\
\text{Lymphatic metastases} & & & & \\
\text{Negative} & 17 & 10 & 22 & \\
\text{Positive} & 10 & 5 & 14 & 0.932 \\
\text{Dukes} & & & & \\
\text{A+B} & 16 & 10 & 22 & \\
\text{C} & 11 & 5 & 14 & 0.892 \\
\end{array} \]

\[ \begin{array}{cccccc}
\text{Table 3. Correlation between EphB1 protein expression and clinicopathologic parameters} \\
& \text{Down-regulation} & \text{No difference} & \text{Up-regulation} & \text{p} \\
\hline
\text{Overall} & 39 & 15 & 15 \\
\text{Sex} & & & & \\
\text{Male} & 29 & 10 & 5 \\
\text{Female} & 10 & 5 & 10 & 0.019 \\
\text{Age} & & & & \\
\leq 55 \text{ years} & 20 & 9 & 4 & \\
>55 \text{ years} & 19 & 6 & 11 & 0.152 \\
\text{Location} & & & & \\
\text{Rectum and sigmoid colon} & 27 & 13 & 14 & \\
\text{Others} & 12 & 2 & 1 & 0.106 \\
\text{Depth of wall invasion} & & & & \\
\text{Mucosae and submucosa} & 2 & 2 & 0 & \\
\text{Muscularis propria} & 8 & 3 & 9 & \\
\text{Subserosa and serosa} & 29 & 10 & 6 & 0.02 \\
\text{Tumor differentiation} & & & & \\
\text{Well and moderate} & 25 & 13 & 14 & \\
\text{Poor and mucinous} & 14 & 2 & 1 & 0.043 \\
\text{Clinical stage (TNM)} & & & & \\
\text{I+II} & 27 & 8 & 9 & \\
\text{III+IV} & 12 & 7 & 6 & 0.521 \\
\text{Lymphatic metastases} & & & & \\
\text{Absent} & 27 & 8 & 9 & \\
\text{Present} & 12 & 7 & 6 & 0.521 \\
\end{array} \]

<table>
<thead>
<tr>
<th>Down-regulation</th>
<th>No difference</th>
<th>Up-regulation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>39</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤55 years</td>
<td>20</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>&gt;55 years</td>
<td>19</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum and sigmoid colon</td>
<td>27</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Others</td>
<td>12</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Depth of wall invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosae and submucosa</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Muscularis propria</td>
<td>8</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Subserosa and serosa</td>
<td>29</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Tumor differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well and moderate</td>
<td>25</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Poor and mucinous</td>
<td>14</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Clinical stage (TNM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I+II</td>
<td>27</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>III+IV</td>
<td>12</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Lymphatic metastases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>27</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Present</td>
<td>12</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

1 The number was not used because it was too small.
EphB1 protein was significantly reduced in male patients (p = 0.019) and in those with poor differentiation and mucinous adenocarcinomas or tumors (p = 0.043). The colorectal cancer cells with low levels of EphB1 protein more often invaded to serosa and subserosa (p = 0.020). Expression of EphB1 protein was not related to other clinicopathological characteristics.

**No Methylation of EphB1 in Colorectal Carcinoma Specimens**

The methylation status of the EphB1 promoter-associated 5'CpG island was assessed by methylation-specific PCR. Unmethylated DNA was detected in all 35 sodium bisulfite-treated DNA samples used in this study, however, no methylated DNA of EphB1 was found.

**Discussion**

The Eph receptors are the largest family of receptor tyrosine kinases, which are involved in cell proliferation, differentiation, migration and other functions. The Eph genes also have important physiologic roles in the intestinal epithelium. In the intestine, epithelia stem

---

**Fig. 2.** Expression of EphB1 protein was analyzed by specific polyclonal anti-EphB1 antibody. **a** EphB1 protein expression was most intense at the base of the crypt with expression declining to the luminal epithelium. **b** EphB1 was homogenously stained in colorectal adenomas. **c** EphB1 protein was not uniformly expressed in colorectal cancer cells. **d** Downregulation of EphB1 in colorectal cancer.
cells reside at the bottom of crypts that are formed by the convolution of the epithelial sheet. Wnt proteins are present at the bottom of crypts and interact with Wnt receptors in epithelial cells. Cytoplasmic β-catenin levels are normally kept low through continuous proteasome-mediated degradation. When epithelial cells receive Wnt signals, the degradation pathway is inhibited, and consequently β-catenin accumulates in the cytoplasm and nucleus. Nuclear β-catenin interacts with transcription factors such as lymphoid enhancer-binding factor 1/T cell-specific transcription factor to affect transcription. As the direct transcriptional target of the β-catenin/T cell-specific transcription factor complex, expression of EphB2 and EphB3 genes is inversely controlled along the crypt-villus axis [12]. EphB receptors have important roles in directing intestinal epithelium cell migration and regulating proliferation as well [11].

Most sporadic colorectal cancers are initiated by activating Wnt pathway mutations and characterized by the stabilization of β-catenin. Although Wnt signaling remains constitutively active, most human colorectal cancers lose expression of EphB at the adenoma-carcinoma transition. Loss of EphB expression strongly correlates with degree of malignancy [15]. EphB4 is frequently downregulated in colorectal tumors through the aberrant hypermethylation of its promoter; patients with low EphB4 tumor levels had significantly shorter survival than patients in the high EphB4 group [16].

In the present study, we analyzed expression levels of EphB1 transcript and protein in a series of colorectal cancer and matched normal mucosa. Our data showed that expression of EphB1 was markedly varied among the colorectal cancer specimens. Downregulation of EphB1 transcript and protein was found in 34.6% (27/78) and 56.5% (39/69) of patients with colorectal cancer, respectively. Although expression of the EphB1 transcript was not completely consistent with expression of the EphB1 protein, either expression of EphB1 transcript or protein is significantly correlated with tumor pathological classification. EphB1 underexpression is often found in colorectal patients with poor differentiation and mucinous tumors. Our results indicate that EphB1 may have roles in differentiation of colorectal cells. Furthermore, cancer cells with low level of EphB1 protein show more invasive power. This can be interpreted as follows: by interacting with ligands of ephrins, EphB suppresses colorectal cancer progression by compartmentalization of tumor cells [10]. Loss of EphB1 protein expression can include EphB1 mutation, increased protein degradation,
a defect in the translation or chromosomal deletions. We are currently investigating these possibilities.

The expression pattern of immunoreactivity of EphB1 in normal mucosa is very similar to that of staining of EphB2 receptor in normal intestinal crypts, in which EphB2 could be observed in the progenitor cells at the bottom of crypts and in a decreasing gradient from the bottom to the luminal epithelium [17]. We postulate that EphB1 has the same roles that EphB3 and EphB4 play in colorectal cancer. The functional study of EphB1 in colorectal cancer will be involved in our next project. Immunostaining of EphB1 was observed in the cytoplasm or both the cell membrane and cytoplasm, and particularly in the golgiosome (fig. 2, 3a). The pattern of EphB1 expression in colorectal cancer cells was focal with considerable variation in the intensity of staining throughout the neoplastic cell population (fig. 2c). However, all colorectal adenomas displayed evidence for homogeneous expression of EphB1 (fig. 2b). This suggests that loss of EphB1 by a proportion of cells in the neoplastic population occurs at the transition from adenoma to cancer.

Hypermethylation of CpG island at promoter region is an important epigenetic mechanism for downregulation of a gene. The promoter region of EphB1 contains a dense 5′CpG island. Promoter methylation analysis was performed. However, methylated DNA was not found in any sample. Our data suggest that there are other mechanisms for the loss of EphB1 expression or promoter methylation in other regulatory regions.

In summary, EphB1 expression is lost in colorectal tumors with poor differentiation and cancer cells with low level of EphB1 protein show more invasive power. Our data show that EphB1 may have important physiologic roles in the intestinal epithelium and may also have roles in the development and prognosis of colorectal cancer.

Acknowledgements

This work was in part supported by the National Basic Research Priorities Program 973 Project (2006, CB 705707) from the Ministry of Science and Technology of China, the China Postdoctoral Science Foundation (No. 2005038578) and the China Nanjing Medicine Science and Technology Research Project (No. 06Z37).

References


Pathobiology 2008;75:274–280

280