Expression of Betacellulin, Heparin-binding Epidermal Growth Factor and Epiregulin in Human Malignant Fibrous Histiocytoma

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Abstract. Background: Heparin-binding epidermal growth factor (HB-EGF), betacellulin (BTC) and epiregulin (EPR) are members of the EGF system and involved in the cell growth of various epithelial malignancies. There have been no reports on the HB-EGF, BTC and EPR expression in mesenchymal malignancies of fibrohistiocytic origin including malignant fibrous histiocytoma (MFH). Materials and Methods: We investigated the expression of HB-EGF, BTC, EPR and EGF-receptor (EGF-R) in 43 human MFH tissue samples using immunohistochemical techniques. Results: Positive immuno-reactivity for HB-EGF, BTC, EPR and EGF-R was identified in 28 (65%), 7 (16%), 43 (100%) and 36 (84%) out of the 43 MFH cases analyzed, respectively. Coexpression of HB-EGF/BTC, BTC/EPR and HB-EGF/EPR was observed in 6 (14%), 7 (16%) and 28 (65%) of the MFHs, respectively. Coexpression of HB-EGF/EGF-R, BTC/EGF-R and EPR/EGF-R was observed in 25 (58%), 6 (14%) and 36 (84%) of the MFHs, respectively. Conclusion: These results revealed that HB-EGF, BTC and EPR are expressed not only by epithelial tumor cells, but also by MFH cells. It is suggested that HB-EGF and EPR might be more important tumor growth regulators of MFH through autocrine or paracrine pathways, when compared with BTC.

The epidermal growth factor (EGF) signaling system consists of at least seven ligands including EGF, amphiregulin (AR), transforming growth factor-α (TGF-α), heparin-binding EGF (HB-EGF), betacellulin (BTC), epiregulin (EPR) and epigen, which is the newest member of the EGF family ligands (1,2). All these ligands are structurally and functionally related proteins and bind and activate the EGF-receptor (EGF-R) that possesses intrinsic tyrosine kinase activity (1). The EGF signaling system plays a key role in regulating the proliferation, survival, migration and differentiation of various cancer cells through autocrine or paracrine pathways. Of the EGF family ligands, HB-EGF, BTC and EPR are relatively newly identified polypeptides. HB-EGF was originally purified from the conditioned medium of macrophage-like U937 cells (3). HB-EGF was found to be overexpressed in pancreatic (4) and hepatocellular carcinomas (5), indicating that it might play a role in the cell growth of various gastrointestinal malignancies. BTC was identified and purified from the conditioned medium of mouse pancreatic β-cell tumors (6). BTC is a potent mitogen for vascular smooth muscle cells and retinal pigment epithelial cells (6,7). EPR was initially isolated from the conditioned medium of mouse tumorigenic fibroblasts NIH3T3/clone T7 cells established from NIH3T3 cells (8). There are very low expression levels of EPR transcript in most normal tissues except peripheral blood monocytes and the uterus (9,10), but several cancer cells and cytokine-induced smooth muscle cells overexpress EPR mRNA (9). It is known that EPR is more mitogenic than EGF for several types of normal cells.

Many investigators demonstrated that these three ligands of the EGF family are involved in the cell growth of epithelial neoplastic cells in vivo and in vitro (11-15). However, little information is currently available on the prevalence and distribution of HB-EGF, BTC and EPR in mesenchymal malignant tumors including malignant fibrous histiocytoma (MFH). The role of these three ligands in malignant mesenchymal neoplasms has not been

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delineated. MFH is one of the most common soft tissue sarcomas in adults with an aggressive behavior and a high metastatic potential (16).

The aims of the current study were to investigate the endogenous expression of HB-EGF, BTC, EPR and EGF-R in human soft tissue MFH tissues using immunohistochemical techniques.

Materials and Methods

Tissue samples. Tissue samples from 43 cases of soft tissue MFH were selected from the files of the pathology departments in the current authors’ institutions. All the specimens were fixed in 10% neutral buffered formalin and embedded in paraffin blocks. There were 21 female and 22 male patients. The patients ranged in age from 17 to 83 years (mean, 61 years). The tumors were located in the upper extremity (n=10), lower extremity (n=28) and in the trunk (n=5). The specimens were obtained from biopsies or resections prior to chemotherapy. Four-micrometer serial sections were prepared for hematoxylin and eosin staining and for immunohistochemical studies. All cases conformed to the diagnostic histological criteria for MFH proposed by Enzinger and Weiss (16). The specimens were classified, based on their histology, into 31 spindle-pleomorphic, 8 myxoid, 2 inflammatory and 2 giant cell MFHs according to Enzinger and Weiss’s classification.

Immunohistochemistry. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded sections by the indirect immuno-peroxidase method. Briefly, the sections were deparaffinized with xylene and routinely dehydrated through a series of graded alcohols. For HB-EGF immunostaining, antigen retrieval in the sections was performed by autoclaving pretreatment for 15 min. For BTC immunostaining, the sections were pretreated by incubating with proteinase K (Dako Japan, Kyoto, Japan) at room temperature for 10 min. Following elimination of endogenous peroxidase activity with a 10-min incubation in 3% H2O2, the sections were incubated at room temperature for one hour with primary antibodies against BTC (monoclonal, 10 µg/ml, Genzyme-Techne), or at 4°C overnight with a primary antibody against HB-EGF (polyclonal, 10 µg/ml, Genzyme-Techne), or at 4°C overnight with a primary antibody against HB-EGF (monoclonal, 250 µg/ml, Genzyme-Techne), or at 4°C overnight with a primary antibody against BTC (monoclonal, 100 µg/ml, Genzyme-Techne), or at 4°C overnight with a primary antibody against HB-EGFR (polyclonal, 100 µg/ml, Genzyme-Techne). For BTC and EPR immunostaining, the sections were incubated at room temperature for 40 min with goat anti-mouse immunoglobulins conjugated to peroxidase-labeled dextran polymer (EnVision™, HRP™, Dako). For HB-EGF immunostaining, the sections were developed using the streptavidine-biotin-peroxidase complex technique (Dako LASB+ kit / HRP, Dako). 3,3-diaminobenzidine was used for color development and the sections were counterstained with hematoxylin.

For EGF-R immunostaining, after the sections had been deparaffinized and washed, they were incubated at room temperature for one hour with a primary antibody against EGF-R (10 µg/ml, monoclonal, Uptstate Biotechnology, Lake Placid, NY, USA). The sections were then incubated at room temperature for one hour with fluorescein iso-thiocyanate (FITC)-conjugated rabbit anti-sheep antibody (Dako). The slides were mounted with Vectorshield (Vector Laboratories, Burlingame, CA, USA) and examined by fluorescence microscopy. In each immunostaining, negative controls were obtained by omission of the primary antibodies.

Table I. Expression of HB-EGF, BTC, EPR and their receptor EGF-R in MFH.

<table>
<thead>
<tr>
<th></th>
<th>HB-EGF</th>
<th>BTC</th>
<th>EPR</th>
<th>EGF-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>(0)</td>
<td>15 (35%)</td>
<td>36 (84%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Focal</td>
<td>(+1)</td>
<td>10 (23%)</td>
<td>2 (5%)</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>(+2)</td>
<td>9 (21%)</td>
<td>4 (9%)</td>
<td>7 (16%)</td>
</tr>
<tr>
<td>Diffuse</td>
<td>(+3)</td>
<td>9 (21%)</td>
<td>1 (2%)</td>
<td>30 (70%)</td>
</tr>
</tbody>
</table>

Total number: n=43
HB-EGF: heparin-binding epidermal growth factor, BTC: betacellulin, EPR: epiregulin, EGF-R: epidermal growth factor receptor, MFH: malignant fibrous histiocytoma

Evaluation of immunohistochemistry. A semi-quantitative system was employed to evaluate the level of antigen expression: immunoreactivity was scored as negative, focal (less than 10% of positive tumor cells), moderate (10-50% of positive tumor cells), or diffuse (more than 50% of positive tumor cells).

Results

Immunohistochemical results of expression of the EGF family ligands and their receptor EGF-R are shown in Table I. Positive cytoplasmic immunoreactivity for HB-EGF, BTC, EPR and EGF-R was identified in tumor cells of 28 (65%), 7 (16%), 43 (100%) and 36 (84%) out of the 43 MFH cases analyzed, respectively (Figure 1). High expression (2+, 3+) of HB-EGF, BTC, EPR and EGF-R was observed in 18 (42%), 5 (11%), 37 (86%) and 31 (72%) of the MFHs, respectively. Coexpression of HB-EGF/BTC, BTC/EPR and HB-EGF/EPR was observed in 6 (14%), 7 (16%) and 28 (65%) of the MFHs, respectively. Six MFHs (14%) coexpressed all three, HB-EGF, BTC and EPR. Coexpression of HB-EGF/EGF-R, BTC/EGF-R and EPR/EGF-R was observed in 25 (58%), 6 (14%) and 36 (84%) of the MFHs, respectively.

Discussion

There have been many reports on the role of the EGF family ligands for normal and neoplastic epithelial cells (11-15), while little information is currently available on the effects of the ligands on mesenchymal tumor cells.

Previous reports showed increased expression of HB-EGF, BTC and EPR in a variety of epithelial malignant tumor cell lines and tissues. Naef et al. (11) found that gastric cancer tissues expressed 3- to 5-fold increased HB-EGF and EGF-R mRNA levels, as compared with normal gastric tissues.
Torrington et al. (12) demonstrated that the mRNA expression for TGF-α, AR, HB-EGF and EPR was increased 10- to 100-fold in androgen-independent prostate cancer cells, as compared with normal prostate epithelial cells. Thogersen et al. (13) reported a significantly higher expression of HB-EGF, TGF-α and EPR in invasive bladder tumors as compared with superficial bladder tumors. Zhu et al. (14) reported that EPR mRNA levels were increased 2-fold in pancreatic ductal adenocarcinoma tissues as compared with normal pancreatic tissues. O-Charoenrat et al. (15) demonstrated that EGF, TGF-α, AR, HB-EGF and BTC were abundantly expressed and produced by squamous cell carcinoma cell lines. These investigators believed that HB-EGF, BTC and EPR play an important role in the development and progression of epithelial malignancies in an autocrine/paracrine manner. In addition, the expression of these ligands is clinically associated with a poor prognosis for patients with lung and bladder cancer (13,17).

In contrast, the involvement of HB-EGF, BTC and EPR in the cell growth and differentiation of mesenchymal neoplasms is poorly understood. A review of the literature yielded only a little data on the expression and production of the ligands in normal and neoplastic mesenchymal tissues. Mioh and Chen (18) showed that the growth of the MG63 osteosarcoma cell line was stimulated by adding exogenous HB-EGF. Yamanaka et al. (19) demonstrated that, in a primary culture system of differentiated smooth muscle cells, EGF-R was activated by HB-EGF, EPR and BTC. To the best of our knowledge, there have been no reports investigating the expression of HB-EGF, BTC and EPR in mesenchymal malignant tumors of a fibrohistiocytic origin, including MFH. In the present study, we immunohistochemically investigated the expression of HB-EGF, BTC, EPR and their receptor EGF-R in 43 human MFH tissues. We demonstrated that positive cytoplasmic immunoreactivity for HB-EGF and EPR was identified in 65% and 100% of the 43 MFHs analyzed, respectively. The expression levels of HB-EGF and EPR were high (2+, 3+) in 42% and 84% of the MFHs analyzed, respectively. However, the number of BTC-positive MFHs was limited (16%), with high expression levels of BTC being observed in only 11% of the MFHs. As EPR was expressed in all MFHs analyzed, all the MFHs expressed one or more of the EGF family ligands and various coexpression patterns of the ligands were observed. HB-EGF/EPR coexpression was the most frequent (65%) and all three ligands were coexpressed in 14% of cases.

It is known that all three ligands bind and activate EGF-R (1). In the present study, EGF-R was positive in 84% of the MFHs analyzed. Coexpression of HB-EGF/EGF-R and EPR/EGF-R was observed in 58% and 84%, respectively. Gusterson et al. (20) reported that approximately 60% of MFHs studied showed strong cytoplasmic immunoreactivity for EGF-R. Perosio and Brooks (21) immunohistochemically demonstrated that 50% of soft tissue sarcomas exhibited positive immunoreactivity for EGF-R. Our data suggest that HB-EGF and EPR might be more important tumor growth regulators of MFH through autocrine or paracrine pathways, when compared with BTC. These results revealed that HB-EGF, BTC and EPR are expressed not only by epithelial tumor cells but also by mesenchymal tumor cells.

Figure 1. Tumor cells of many MFHs show positive immunoreactivity for HB-EGF (A) and EPR (B), while most MFHs show negative or focal immunoreactivity for BTC (C). More than 70% of MFHs studied show high expression of EGF-R (D). (A-C: immunostaining, original magnification, x400, D: FITC-labeled immunostaining, original magnification, x400).
In addition to the expression of the HB-EGF, BTC and EPR, we have previously found that many human MFH tissues overexpress various growth factors including AR (22), stem cell factor (23), platelet-derived growth factor (24), insulin-like growth factors (25) and TGF-β (unpublished data). It is speculated that the proliferation activity of MFH cells is controlled by a complex interaction between these various growth factors and their receptors. These observations provide oncologists with a better understanding of the tumor growth mechanism of MFH.

References


