Basic Fibroblast Growth Factor and Fibroblast Growth Factor Receptor-1 in Human Meningiomas

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Summary: The expression of basic fibroblast growth factor (bFGF) and fibroblast growth factor receptor-1 (FGFR-1) in human meningiomas and the relationships between their expression and the tumors' histological features and angiogenesis were investigated by means of immunohistochemical technique. The expression of bFGF and FGFR-1 was detected by antibody of bFGF or FGFR-1. The tumors' angiogenesis was evaluated by microvascular density (MVD) and, which was observed by use of CD34-antibody immunohistochemically. The results showed that there were varied degrees of the expression of bFGF and FGFR-1 proteins in meningiomas. The expression was correlated with the tumors' histological characters and angiogenesis. It was concluded that bFGF and FGFR-1 might play important roles in meningiomas' angiogenesis and proliferation. The expression positive rate of bFGF and FGFR-1 may provide an indication of evaluating the histological and malignant degree of the tumor.

Key words: meningiomas; basic fibroblast growth factor; fibroblast growth factor receptor-1; microvascular density; immunohistochemistry

Basic fibroblast growth factor (bFGF) is a factor with the functions of angiogenesis and proliferation. The biological response of bFGF is mediated by its binding to a specific cell surface receptor, fibroblast growth factor receptor (FGFR). Research has shown that bFGF plays crucial roles in tumor-associated angiogenesis, invasion, and proliferation. The expression of bFGF protein was detected in almost all kinds of human extracranial tumors and some of human intracranial gliomas. In this study, the expression of bFGF and FGFR-1 proteins and the intratumoral microvascular density (MVD) in human meningiomas were investigated by immunohistochemistry in order to analyze the significance of bFGF in the tumors' angiogenesis and proliferation.

1 MATERIALS AND METHODS

1.1 Cases and Specimens

Forty-six human meningioma specimens were obtained from the resected meningiomas in Department of Neurosurgery, Tongji Hospital, Huazhong University of Science and Technology. The diagnosis of each case was confirmed by two experienced pathologists. Of the 46 patients, there were 21 males and 25 females with the age ranging from 14 to 75 (mean 44 years). The meningiomas were classified according to the WHO (1999) classification of meningiomas into benign meningiomas (n=25), atypical meningiomas (n=13) and malignant meningiomas (n=8). Histological variations included meningothelial types (n=12), fibrous types (n=9), angiomatous types (n=3), psammomatous types (n=6), transitionals types (n=8) and anaplasia types (n=8). The cases were followed up for 1-3 years (mean 26 months). Eight matched control specimens were normal brain constitutions that were obtained in decompressions of brain due to acute brain trauma.

1.2 Immunohistochemistry

All of the specimens were fixed in 10 % buffered formalin, embedded in paraffin, and cut into 4 μm-thick sections. Using SABC Kit (Boster, China), immunohistochemical procedures were performed according to the instructions of the manufacturers producing primary antibodies to: bFGF (polyclonal, Maixin Company, China), FGFR-1 (monoclonal, Santa Craz, USA), and CD34 (polyclonal, Boster, China). For negative controls, the primary antibodies were replaced by phosphate buffer solution (PBS).

1.3 Observation of the Results

1.3.1 The protein expression of bFGF and FGFR-1

Ten views were randomly selected with 400 powers microscope after the specimens were stained immunohistochemically. All the tumor cells and bFGF or FGFR-1 positive cells in the views were counted. The percentage of positive cells was calculated. The percentage of positive cells was calculated. The bFGF positive cells were stained to be brown-yellow among cytoplasm and nuclei, particularly in cytoplasm. The FGFR-1 positive cells were also stained to be brown-yellow, mainly on cell membrane and in cytoplasm. All the negative cells were stained to be blue.

1.3.2 Microvascular Density (MVD) By using immunohistochemical staining technique, vascular endothelium was stained to be brown-yellow with CD34-antibody. Microvascular "hot spot" was found out first under low power

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microscopy. Then the microvessels of the tumor were counted under a 200 powers microscope. The MVD of each specimen was presented by the mean intratumoral value of the microvessels of at least 5 five 200 powers views. A brown-yellow cell cluster was only counted to one vessel. The mono vascular endothelial cell was also counted to one vessel. Occasionally, there were some macrophages and plasmocytes in the views that were stained to be brown-yellow color because of their response to CD34-antibody. They should be eliminated through their different morphology from endothelial cells[6,7].

### 1.4 Statistical Analysis

T test, variance analysis, regression analysis, and Bartlett’s Chi-square test were used to the attained data by SAS software (6.12).

### 2 RESULTS

#### 2.1 Protein Expression of bFGF and FGFR-1 in Human Meningiomas

There were varied degrees of the expression of bFGF and FGFR-1 proteins in meningiomas. There was no significant difference among the different histological types of human meningiomas except that of anaplasia meningiomas was higher than other types. However, the expression was closely correlated with the tumors pathological grades. The expression in malignant and atypical meningiomas was higher than that of benign meningiomas and control specimen ($P<0.01$). But there was no obvious difference in the expression between malignant and atypical meningiomas ($P>0.05$, table 1). The bFGF expression in meningiomas was correlated with that of FGFR-1 ($r=0.68$, $P<0.01$).

#### 2.2 Relationship between the Protein Expression of bFGF and MVD

There was a positive correlation between the protein expression of bFGF and MVD ($r=0.71$, $P<0.01$). The MVD of the menningiomas whose bFGF expression was above 71.73 % was more than that with the bFGF expression being below 71.73 % ($P<0.01$).

### Table 1 The protein expression of bFGF in human meningiomas (x±s, %)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>bFGF</th>
<th>FGFR-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningothelial</td>
<td>12</td>
<td>65.68±8.63</td>
<td>60.70±14.87</td>
</tr>
<tr>
<td>Fibrous types</td>
<td>9</td>
<td>64.41±11.34</td>
<td>60.57±13.45</td>
</tr>
<tr>
<td>Hemangioblastic</td>
<td>3</td>
<td>67.30±15.08</td>
<td>64.32±10.10</td>
</tr>
<tr>
<td>Microcystic</td>
<td>6</td>
<td>63.54±13.21</td>
<td>59.29±11.22</td>
</tr>
<tr>
<td>Transitionals</td>
<td>8</td>
<td>66.95±13.16</td>
<td>57.24±12.61</td>
</tr>
<tr>
<td>Anaplasia types</td>
<td>8</td>
<td>85.34±8.42*</td>
<td>77.58±8.25*</td>
</tr>
<tr>
<td>Pathology Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control specimens</td>
<td>8</td>
<td>7.72±5.49</td>
<td>7.75±2.41</td>
</tr>
<tr>
<td>Benign meningiomas</td>
<td>25</td>
<td>63.13±13.97*</td>
<td>52.71±13.65*</td>
</tr>
<tr>
<td>Atypical meningiomas</td>
<td>13</td>
<td>79.87±9.50*</td>
<td>72.15±7.95*</td>
</tr>
<tr>
<td>Malignance meningiomas</td>
<td>8</td>
<td>85.34±8.42*</td>
<td>77.58±8.25*</td>
</tr>
</tbody>
</table>

*P<0.01 as compared with other histological types, *P<0.01 as compared with control specimen, *P<0.01 as compared with benign meningiomas

### 3 DISCUSSION

In 1984, bFGF was first found in cattle pituitary gland by Gospodarowicz et al[6]. There are several FGF receptors. FGFR-1 is the receptor with the highest affinity to bFGF. In the past, it was reported that the protein expression of bFGF was detected in many human neoplasm such gliomas, which correlated with the tumors' malignant grades and angiogenesis[1-3].

In the present research, it was demonstrated that there was positive expression of bFGF and FGFR-1 in human meningiomas, their expression rate was not related to the tumors'histological types but to the tumors' pathological grades, and the expression of bFGF was positively correlated with that of FGFR-1. It was suggested that bFGF might play important roles in growth, proliferation or infiltration of tumors through the pathway of autocrine or paracrine.

At the same time, it was found that the expression rate of bFGF protein in meningioma cells was positively correlated with the MVD of the tumor. This indicated that bFGF expressed in meningioma cells could also promote the angiogenesis, which was also very important for the tumor's growth, proliferation or infiltration. These bioactivities might also be completed through the pathway of autocrine or paracrine as mentioned above.

In conclusion, we found that bFGF and FGFR-1 played important roles in the carcinogenesis of human meningiomas. They can form ligand-receptor-compound, directly accelerating the growth, proliferation, or infiltration of the tumor, or indirectly playing above roles through accelerating angiogenesis of the tumor. Thus, anti-bFGF or -FGFR therapy may put off the period