Expression of monocyte chemoattractant protein-1 in meningioma

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Monocyte chemoattractant protein-1 (MCP-1), purified from glioma cell line (U-105MG) culture fluid, attracts monocytes but not neutrophils. Macrophage accumulation is one of the pathological features of meningioma. To investigate the mechanism of macrophage infiltration into meningioma, the expression and localization of MCP-1 in 16 cases of meningioma were studied using Northern blot analysis and immunohistochemistry. Seven of 16 meningiomas expressed MCP-1 messenger ribonucleic acid and protein, and some degree of macrophage infiltration was seen in all 16 meningiomas.

There was a relationship between MCP-1 expression and the degree of macrophage infiltration; MCP-1 was strongly expressed in meningiomas with a high degree of macrophage infiltration. Sometimes the meningioma was accompanied by perifocal edema; a correlation between macrophage infiltration into brain tumors and perifocal edema has already been reported. It was found that the degree of MCP-1 expression is not correlated with the extent of perifocal edema.

The authors’ findings suggest that MCP-1 plays an important role in macrophage infiltration into meningioma.

KEY WORDS • monocyte chemoattractant protein-1 • meningioma • macrophage infiltration • mRNA • immunohistochemistry

MENINGIOMA, a common primary human intracranial tumor, accounts for 15% of all brain tumors.\textsuperscript{15} Previous reports demonstrated that meningiomas, like glioblastomas multiforme, contain high numbers of infiltrating host cells, primarily lymphocytes and macrophages.\textsuperscript{10,11} Wood, et al.\textsuperscript{22} reported that the mean macrophage content of meningiomas was 42\% (range 5\% to 80\%) and that of glioblastomas was 41\% (range 5\% to 78\%). Although the mechanism and significance of macrophage infiltration into brain tumors remain to be clarified, macrophages may suppress or enhance tumor growth.\textsuperscript{9} Shinonaga, et al.\textsuperscript{16} noted an excellent correlation between the degree of macrophage infiltration and peritumoral edema, especially in meningiomas.

In a previous study, we detected monocyte chemotactic activity in the culture supernatants of several culture supernatants of several human malignant glioma cell lines;\textsuperscript{5} monocyte chemoattractant protein-1 (MCP-1) was purified, sequenced, and cloned from one of these cell lines (U-105MG).\textsuperscript{12,25} The MCP-1 is an attractant for monocytes, but not for neutrophils or lymphocytes.\textsuperscript{9,13} In this study, the expression and localization of MCP-1 in meningiomas were examined, using Northern blot analysis and immunohistochemistry, and the correlation between MCP-1 expression, the degree of macrophage infiltration, and the extent of perifocal edema was also determined.

Materials and Methods

Tissue Sample Study

Sixteen fresh meningioma specimens were obtained at surgery, frozen immediately in liquid nitrogen, and stored at \(-80\)^\circ C. Of these 16, five were meningotheliomatous, nine were transitional, one was fibrous, and one was microcystic. All were primary tumors.

Northern Blot Analysis

Total cellular ribonucleic acid (RNA) was prepared from the 16 meningiomas by guanidium isothiocyanate lysis followed by CsCl gradient ultracentrifugation.\textsuperscript{3} Ribonucleic acid prepared from U-105MG cells was used as the positive control. For Northern blot analysis, equal amounts of RNA (10 \(\mu\)g) were denatured, subjected to electrophoresis on 1\% formaldehyde–agarose gels, and blotted by capillary transfer onto nitrocellulose filters. The membranes were then baked at 80\^\circ C for 2 hours in a vacuum and prehybridized for 3 hours at 42\^\circ C. The prehybridization solution contained 6 \(\times\)
MCP-1 in meningioma

**Fig. 1.** Northern blot analysis of monocyte chemoattractant protein-1 (MCP-1) and 28S ribosomal ribonucleic acid (rRNA) in meningiomas. Seven cases with positive expression of MCP-1 are shown. The U-105MG cells were used as a positive control. Arrows show the position of rRNA in 28S and 18S. The degree of MCP-1 messenger RNA expression is summarized in Table 1.

Endogenous peroxidase activity was blocked by incubating the slides for 30 minutes in 0.3% H₂O₂ in absolute methanol. The tissue samples were exposed for 20 minutes to 3% bovine serum albumin (BSA) and incubated for 1 hour with optimally diluted primary antibodies. To stain MCP-1, anti–human MCP-1 polyclonal antibody was used as the primary antibody. To detect macrophages, anti–human macrophage monoclonal antibody, PM-2K, was used. After washing with phosphate-buffered saline, the samples were exposed for 1 hour to biotinylated anti–rabbit immunoglobulin G or anti–mouse immunoglobulin G. The slides were then incubated for 1 hour in avidin–biotin peroxidase complex. Peroxidase activity was visualized by incubating the sections with 0.5 mg/ml 3,3′-diaminobenzidine tetrahydrochloride and 0.01% H₂O₂ for 10 to 15 minutes. The sections were counterstained with hematoxylin. As a control for nonspecific staining, primary antibody was omitted. All other steps in the staining sequence were as described previously.

**Statistical Analysis**

Statistical analysis was performed using Student’s t-test. Statistical significance was determined at the p less than 0.05 level.

**Results**

**Expression of MCP-1 mRNA in Meningioma Specimens**

Sixteen meningioma specimens obtained at surgery were studied using Northern blot analysis. In seven of these, MCP-1 mRNA was detected (Fig. 1). There were differences in the mRNA expression levels for MCP-1 among the tumor specimens. The levels of MCP-1 mRNA in Cases 2 and 5 were the same as in U-105MG; in all other cases the levels were lower. The expression of MCP-1 mRNA could not be detected in the other nine meningiomas. The results of Northern blot analysis are summarized in Table 1.

**Expression and Localization of MCP-1 in Meningioma**

Monocyte chemoattractant protein-1 was detected by immunohistochemistry in the same seven cases that were positive by Northern blot analysis. Expression of MCP-1 was diffuse in the cytoplasm of meningioma cells (Fig. 2 left).
Macrophage Infiltration Into Meningioma

The degree of macrophage infiltration into meningioma was determined based on the percentage of PM-2K–positive cells in the cells around the perivascular region within a microscopic field (× 200). There was some degree of macrophage infiltration in all meningiomas (mean 20.3%; range 1% to 39.6%) (Fig. 2 right). Immunohistochemical data for the degree of macrophage infiltration are summarized in Table 1.

Relationship Between MCP-1 Expression and Degree of Macrophage Infiltration

Figure 3 shows the relationship between the MCP-1 mRNA expression level and the degree of macrophage infiltration. Compared with cases that lacked expression of MCP-1 mRNA (mean macrophage infiltration 13.8% ± 8.4%; mean ± standard deviation), the two groups positive for the expression of MCP-1 mRNA (26.1% ± 12.5% and 34.9% ± 3.3%) showed significantly higher macrophage infiltration (p < 0.05 and p < 0.01, respectively).

Relationship Between MCP-1 Expression and Clinical Features

There is no statistically significant association between MCP-1 expression and the tumor size, location, or age of the patient.

Relationship Between MCP-1 Expression and Perifocal Edema

The extent of peritumoral edema was evaluated on contrast-enhanced computerized tomography (CT) as the ratio of the low-density area around the tumor to the enhanced high-density area and on T₂-weighted magnetic resonance (MR) imaging as the ratio of the high-intensity area around the tumor to the gadolinium-enhanced high-intensity area in T₁-weighted images (Fig. 4). As shown in Fig. 5, there was no significant correlation between the MCP-1 mRNA expression level and the extent of perifocal edema.

Discussion

This study uses Northern blot analysis and immunohistochemistry to confirm the expression of MCP-1 in meningiomas. Based on the positive correlation between

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**Table 1**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex, Age (yrs)</th>
<th>Histopathology</th>
<th>MCP-1 Expression mRNA</th>
<th>MCP-1 Expression Protein</th>
<th>Macrophage Infiltration (%)</th>
<th>Edema/Tumor Ratio</th>
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<td>1</td>
<td>F, 47</td>
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</table>

*Histopathological diagnosis is classified according to the World Health Organization classification system. Expression of MCP-1 mRNA is graded as follows: − = negative; + = thinner than the U-105MG band; ++ = same density as the U-105MG band. Expression of MCP-1 protein is classified as follows: − = negative; + = positive; ++ = strongly positive. Edema/tumor ratio is shown in Fig. 5. Abbreviations: MCP-1 = monocyte chemoattractant protein-1; mRNA = messenger ribonucleic acid.
the MCP-1 expression level and the degree of macrophage infiltration (Fig. 3), we believe that MCP-1 plays an important role in macrophage infiltration into meningiomas.

Tumor cells of mesenchymal origin, that is, fibrosarcomas and malignant fibrous histiocytomas, as well as gliomas and melanomas have been shown to produce MCP-1,4,9,21,23 although little is known about its biological function in various tumors. Take-shima, et al.,17 found an excellent correlation between the level of MCP-1 expression and the degree of macrophage infiltration in malignant gliomas. In intra- and extramedullary brain tumors, MCP-1 contributes to macrophage infiltration.

Platelet-derived growth factor (PDGF) enhances MCP-1 expression.14 Meningioma cells secrete PDGF-like molecules that stimulate their own growth in an autocrine manner.19 In meningiomas, MCP-1 expression may be regulated by PDGF-like molecules produced by the meningioma. Thus, a study of the correlation between MCP-1 expression and PDGF expression in meningiomas is warranted.

Monocyte chemoattractant protein-1 is not only a chemoattractant but also a novel cytokine with a variety of biological functions: it regulates adhesion molecule expression5 and releases superoxide and lysosomal protease.20 Furthermore, MCP-1 is capable of directly inducing histamine release by basophils.16

Cerebral edema results from the disruption of the blood-brain barrier with increased vascular permeability and excessive interstitial fluid accumulation. Bruce, et al.,2 reported that brain tumors produce and release a specific substance that evokes cerebral edema by increasing vascular permeability; some meningiomas are accompanied by perifocal edema. Shimonaga, et al.,16 suggested that macrophage infiltration may play an important role in the pathogenesis of peritumoral edema in meningiomas. We theorized that the perifocal edema is attributable to the release of vascular permeability factors induced by macrophage accumulation in meningioma tissues; however, this study showed that the degree of MCP-1 mRNA expression has no relationship to the extent of cerebral edema. Many other factors including tumor size, tumor location, and the extent of vascularity must be examined to understand the pathogenesis of perifocal edema in brain tumors.

At present, we cannot discern the significance of MCP-1 expression in meningiomas. Recently, MCP-1 was reported to possess the ability to suppress tumor formation by attracting monocytes in vivo, and the possibility of use as an adjuvant to surgery or cytotoxic chemotherapy was discussed.13

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References


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