Expression of CD163 prevents apoptosis through the production of granulocyte colony-stimulating factor in meningioma

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Background. CD163 is a 130-kDa transmembrane protein expressed in human monocytes and macrophages, and the aberrant expression of CD163 in breast and colorectal cancer associated with patients’ poor prognosis was reported. Here, we analyzed the expression of CD163 in meningioma, a common intracranial tumor, and its molecular mechanism in association with meningioma progression.

Methods. First, we performed immunohistochemical analysis using 50 human meningioma specimens. Next, we established CD163-overexpressing human meningioma cell lines and investigated its roles in tumor progression in vitro and in vivo.

Results. Immunohistochemically, 26 of 50 human meningioma specimens (52.0%) were positive for CD163 in tumor cells, including benign grade I (48.5%) and grade II (71.4%) cases. Furthermore, CD163 expression was correlated with histological atypical parameters that directly predict the prognosis of meningioma. CD163-overexpressing meningioma cells showed significant suppression of apoptosis and accelerated tumor growth in nude mice. In addition, unexpected splenomegaly affiliated with the xenograft predicted tumor-derived granulocyte colony-stimulating factor (G-CSF) production, which was confirmed by reverse-transcription polymerase chain reaction and enzyme-linked immunosorbent assay.

Conclusions. To our knowledge, this is the first report that demonstrates CD163 expression in meningioma not only by immunohistochemistry but also by reverse-transcription polymerase chain reaction, using primary culture cells, and provides the novel molecular function of CD163 to prevent apoptosis through the production of G-CSF in meningioma.

Keywords: apoptosis, CD163, G-CSF, malignancy, meningioma.
grade II or III, exhibiting an unfavorable clinical course, although it has been recently reported that atypical meningioma occupied up to 20% of meningiomas with use of the latest WHO classification, in which meningioma with increased mitotic activity or \( \geq 3 \) of the following histological features, including hypercellularity, macronucleoli, small cell formation, patternless architecture, and necrosis, is defined as atypical meningioma (grade II). Grade II and III meningiomas represent a risk of recurrence of 30%–40% and 50%–80%, respectively, after surgical resection, and the 5-year survival rates among patients with grade II and III meningiomas were 67.5% and 60%, respectively. In addition, even benign meningioma of grade I sometimes shows recurrence and/or malignant progression. The therapeutic modality to high-grade meningioma includes surgical resection, preoperative embolization, adjuvant radiotherapy, and multidrug chemotherapy. There have been also some trials and retrospective reports on targeted molecular therapies, including gefitinib, erlotinib, imatinib, and bevacizumab. However, the treatment efficacy, especially of chemotherapy, was limited, and there is currently no standardized treatment for recurrent high-grade meningiomas after surgery and radiation therapy. Thus, the proper diagnosis of high-grade meningioma based on reliable molecular markers is requested to the neuropathologists, and the management of high-grade meningioma and recurrent benign meningioma is a clinically significant theme for neurosurgeons.

Before starting this study, we were aware that CD163 is expressed in a subset of high-grade meningioma during the process of differential diagnosis to histiocytic tumors. Here, we analyzed the correlation of CD163 expression in meningioma and pathological atypical features. Moreover, we also examined the molecular function of CD163 in vitro and in vivo with use of meningioma cell lines, including primary culture cells derived from surgically resected meningioma specimens, and elucidated the novel mechanism of CD163 to promote tumor growth by preventing apoptosis.

### Materials and Methods

#### Ethical Requirements

The study using human samples was performed with the approval of the Internal Review Board on Ethical Issues of Hokkaido University Hospital and Graduate School of Medicine, Sapporo, Japan.

#### Patients

We used 50 cases of primary and recurrent meningiomas diagnosed from January 2010 through March 2011 in our faculty for histological examination and immunohistochemical analysis. The characteristics of the patients are summarized in the right column of Table 1. Twenty-one were men, and 29 were women. Median age at surgery was 59.9 years (range, 23–79 years). The 50 meningiomas consisted of 33 grade I tumors (66.0%), 14 grade II tumors (28.0%), and 3 grade III tumors (6.0%).

#### Evaluation of Histologic Parameters

According to the previous report, we counted mitoses per 10 high-power fields (\( \times 400 \)) and evaluated 5 prognostic histological parameters, including hypercellularity, macronucleoli, small cell formation, patternless architecture, and necrosis, as 0 (no) or 1 (yes). The sum of each parameter was designated as an atypical score. According to the latest WHO classification, the

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CD163(–)</th>
<th>CD163(+)</th>
<th>Total (% of positive case)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M:F</td>
<td>14:10</td>
<td>7:19</td>
<td>21:29</td>
</tr>
<tr>
<td>age (range)</td>
<td>57.6 (43–67)</td>
<td>61.8 (23–79)</td>
<td>59.9 (23–79)</td>
</tr>
<tr>
<td>WHO Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>17</td>
<td>16</td>
<td>33 (48.5%)</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>10</td>
<td>14 (71.4%)</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>0</td>
<td>3 (0%)</td>
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<tr>
<td>Atypical score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>4</td>
<td>14 (28.6%)</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>4</td>
<td>9 (44.4%)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>9</td>
<td>11 (81.8%)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>7</td>
<td>9</td>
<td>16 (56.2%)</td>
</tr>
<tr>
<td>Mitosis (/10HPF)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>21</td>
<td>25</td>
<td>46 (54.3%)</td>
</tr>
<tr>
<td>≥4</td>
<td>3</td>
<td>1</td>
<td>4 (25.0%)</td>
</tr>
<tr>
<td>mean MIB-1 index</td>
<td>7.4% (0–29.8)</td>
<td>7.0% (0.8–17.9)</td>
<td>7.2% (0–29.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>26</td>
<td>50 (52.0%)</td>
</tr>
</tbody>
</table>

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case with ≥ 4 mitoses per 10 high-power fields or with atypical score ≥ 3 corresponds to atypical meningioma (grade II). In this study, 14 cases (28.0%) were atypical score 0, 9 cases (18.0%) were score 1, 11 cases (22.0%) were score 2, and 16 cases (32.0%) were 3–5, corresponding to high-grade meningioma.

**Immunohistochemical Analysis**

Formalin-fixed paraffin-embedded tissues were sectioned and stained using anti-CD163 (Leica Microsystems, Clone 10D6, NCL-CD163, 1:100 dilution), anti-Ki67 (Dako, Tokyo, Japan, Clone MIB-1, M7240, 1:100 dilution), and anti-EMA (Dako, Clone E29, M0613, 1:200 dilution). The positivity of CD163 staining in meningioma cytoplasm was evaluated at 0%–100%, and the cases with >30% positive tumor cells were evaluated as positive cases. We confirmed equable staining of all sections (Supplementary Fig. S4A). Tumor stroma, including macrophages, lymphocytes, and endothelial cells, were isolated from CD163-positive meningioma cells by suitable immunostaining (Supplementary Fig. S4B). The MIB-1 index was calculated in the field of maximal activity as a percentage of positive cells. Mean MIB-1 index of 50 meningiomas was 7.2% (range, 0%–29.8%).

**Cell Lines**

A human malignant meningioma cell line, HKBMM, obtained from the Riken Cell Bank (Tsukuba, Japan), was cultivated in DMEM/Nutrient Mixture F-12 Ham (Sigma-Aldrich, St. Louis, MO), 15% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL). Primary culture cells of meningioma were established from surgical specimens. The surgically resected specimens were minced in the 20-mL culture medium containing 200 μL collagenase and hyaluronidase and incubated for 30 min. After the filtration through a cell strainer, the cell suspension was centrifuged at 1500 rpm for 3 min. The sediments were resuspended in 15% FBS-containing DMEM/Nutrient Mixture F-12 Ham and incubated at 37°C in a humidified atmosphere containing 5% CO₂ in air.

**Establishment of CD163-Overexpressing Cell Lines**

cDNA of CD163 was obtained from Open Biosystems (catalog no. MHS1011-9199674). The preparation of lentiviral vectors expressing human CD163 was performed using the Gateway System (Invitrogen, Carlsbad, CA). For generation of infectious lentiviral particles, expression vectors were cotransfected with ViraPower packaging plasmids mix: pLP1, pLP2, and pLP/VSVG (Invitrogen) into 293 FT cells using lipofectamine 2000 (Invitrogen). Forty-eight hours after transfection, the viral supernatant was harvested. The lentiviral construct was introduced into HKBMM or primary culture cells, followed by selection with 2 μg/mL or 1 μg/mL blasticidin S HCL, respectively (Invitrogen).

**Cell Proliferation Assay**

For drawing the growth curve, cells were seeded at a density of 5 × 10⁴ cells in 60-mm dishes and cultivated in DMEM/Nutrient Mixture F-12 Ham containing 15% FBS. After 12 h, the medium was shifted to 3% FBS-containing medium. The cells were counted in triplicate after 12 h and, afterward, every 24 h up to 5 days.

For bromodeoxyuridine (BrdU) assay, 5 × 10³ cells per well were seeded into 96-well plates in DMEM/Nutrient Mixture F-12 Ham containing 15% FBS. Forty-eight hours later, 10 μmol/L BrdU (Cell Proliferation ELISA, Roche, Roche, Penzberg, Germany) was added to the wells and, after 2 h proliferation, was analyzed according to the manufacturer’s instructions.

**Determination of Apoptosis**

The cytoplasmic histone-associated DNA fragments were detected using Cell Death Detection ELISA Plus (Roche Applied Science, Mannheim, Germany) according to the manufacturer’s instructions. In brief, 1 × 10⁴ cells were seeded in 96-well plates and incubated for 24 h at 37°C. For induction of apoptosis, camptothecin (CPT) or a UV exposure was used. Cells were treated with 2 μg/mL CPT for 4 h before assays. For UV exposure, cells were exposed to UV for 2 min (HKBMM) or 15 min (WY08) with use of UVP Transilluminator (Funakoshi, Tokyo, Japan), followed by a 12-h incubation. After the induction of apoptosis, the supernatant of the cells was collected for assay. Twenty microliters of each supernatant was transferred into the streptavidin-coated plate, and 80 μL of immunoreagent containing 4 μL of anti-histone-biotin and 4 μL of anti-DNA-POD were added to each well, followed by incubation at room temperature for 2 h. Fifteen minutes after addition of 100 μL of 2, 2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) solution, the absorbance was detected immediately in a microplate reader.

**In Vivo Tumorigenicity Assays**

Tumor formation was examined using subcutaneous injection of 7 × 10⁶ cells in a final volume of 200 μL into the flank region of 4–6-week-old nude mice. Growth of the resulting tumors was monitored by measuring tumor size and calculating the tumor volume.

**Western Blot Analysis**

Western blots were performed as described elsewhere. Equal amounts of protein (20 μg/lane) were loaded on 6%–12% polyacrylamide gels and transferred electrophoretically onto polyvinylidene difluoride membrane (Immobilon-P; Millipore, Billerica, MA). The blots were blocked for 1 h at room temperature with 2%
nonfat dry milk or 1% bovine serum albumin and were incubated with primary antibodies at 1:1000 overnight at 4°C. The primary antibodies were obtained from the following sources: anti-CD163 monoclonal antibody (Leica Microsystems, Tokyo, Japan, Clone 10D6, NCL-CD163) and anti-α-tubulin monoclonal antibody (Sigma-Aldrich, Clone B-5-1-2, T6074). Bound antibodies were probed with peroxidase-conjugated anti-mouse IgG at a dilution of 1:5000 for 1 h. The target proteins were detected using enhanced chemiluminescence (GE Healthcare, formerly Amersham Biosciences, Tokyo, Japan).

**RNA Isolation and Reverse-Transcription Polymerase Chain Reaction (RT-PCR)**

RNA was isolated using the TRIzol-Reagent (Ambion, Carlsbad, CA), according to the manufacturer’s instructions. Reverse transcription was performed using Superscript II RT (Invitrogen). Resulting cDNA was used as a template and amplified by PCR with use of GoTaq Master Mix (Promega, Tokyo, Japan) or used as a template and amplified by PCR with use of KOD-Plus DNA polymerase (Toyobo, Osaka, Japan). Reverse transcription was performed using M-MLV Reverse Transcriptase (Promega, Tokyo, Japan), according to the manufacturer’s instructions. Reverse transcription was performed using Superscript II RT (Invitrogen). Resulting cDNA was used as a template and amplified by PCR with use of GoTaq Master Mix (Promega, Tokyo, Japan) or used as a template and amplified by PCR with use of KOD-Plus DNA polymerase (Toyobo, Osaka, Japan).

**Immunohistochemical Analysis**

To investigate the CD163 expression in meningioma, we first performed immunohistochemical analysis using 50 human meningioma samples. The spindle cells with whorl formation and epithelial membrane antigen were defined as meningeal tumor cells by hematoxylin and eosin sections, which was consistent with typical meningioma histology. CD163 was positive in the cytoplasm of meningeal cells in which nuclei were larger than the macrophages, in addition to macrophages in tumor stroma. For statistical analysis, a Mann-Whitney test and a Student’s t test were performed.

**Expression of CD163 in Meningioma Cell Line HKBMM and Primary Culture Cells**

We next examined the expression of CD163 in HKBMM, a malignant meningioma cell line, and also in primary cultured meningioma cells established from surgically resected meningioma specimens (Supplementary Fig. S1). RT-PCR analysis showed no CD163 expression in HKBMM (Fig. 2A), whereas mRNA of CD163 was found in 2 of 4 primary culture cells, WY01 and WY09, which were derived from grade II and grade I meningioma specimens, respectively (Fig. 2B). Consistent with this result, paraffin-embedded tissues from the same patients’
samples showed immunopositivity for CD163 (Fig. 2C), whereas WY02, which did not exhibit CD163 expression in RT-PCR, was also negative for CD163 in immunohistochemistry (Fig. 2C). These data confirmed the prevalence of CD163 expression in meningioma by immunohistochemistry.

**Table 2. Summary of CD163 immunoreactivity**

<table>
<thead>
<tr>
<th>Variable</th>
<th>CD163 (−)</th>
<th>CD163 (+)</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Grade I Atypical score</td>
<td>0</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Grade II</td>
<td>4</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Grade III</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>24 (48.0%)</td>
<td>26 (52.0%)</td>
<td>50</td>
</tr>
</tbody>
</table>
CD163 Overexpression Promotes Cell Growth through Suppression of Apoptosis In Vitro

To elucidate the molecular role of CD163 on meningioma cells, we established CD163-overexpressing HKBMM (HKBMM-CD163) and also green fluorescent protein (GFP)–expressing HKBMM (HKBMM-GFP) for control with use of the lentiviral system (Fig. 3A). First, we examined the growth rate of these cells and found that CD163 overexpression significantly enhanced the growth rate of HKBMM in vitro under 3% FBS containing medium (Fig. 3B), although there was no significant growth enhancement under 15% FBS containing medium (data not shown). Because previous reports indicated that CD163 expression was associated with colon cancer aggressiveness, we also established the CD163-overexpressing WiDr, a colon cancer cell line, and obtained a similar result of cell growth (Supplementary Figs. S2A, S2B). However, we failed to find significant promotion in the proliferation rate measured by BrdU incorporation of HKBMM-CD163, compared with the control cells (Fig. 3C); then, we examined the amount of apoptosis in HKBMM-CD163 by Cell Death Detection ELISA. Twelve hours after the induction of apoptosis by 2-min UV exposure, HKBMM-CD163 showed an 18.4% reduction in apoptosis, compared with the control (Fig. 3D). In addition, HKBMM-CD163 demonstrated a 28.2% decrease in apoptosis, compared with the control, after incubation with 2 μg/mL CPT (Fig. 3E).

Furthermore, we established a CD163-overexpressing primary meningioma cell, WY08-CD163 (Fig. 3A), and obtained the similar result that WY08-CD163 demonstrated a 31.1% reduction in apoptosis, compared with the control, WY08-GFP (Fig. 3F). These results suggest that CD163 overexpression promotes cell growth through suppression of apoptosis in meningioma cells.
data indicate that CD163 expression promotes the growth rate of meningioma cells through the suppression of apoptosis.

CD163 Overexpression Promotes Tumor Growth and Induces Splenomegaly In Vivo

To confirm the tumor-promoting function of CD163 in vivo, HKBMM-CD163 and HKBMM-GFP were inoculated subcutaneously into nude mice. The tumor volume and mean weight of the excised xenografts of HKBMM-CD163 were significantly higher than those of the control cells (Fig. 4A and B). Moreover, the histological examination of the xenografts of HKBMM-CD163 demonstrated a relatively smaller central necrosis area, compared with those of control cells (Fig. 4C), whereas there was no significant difference in MIB-1 labeling index between HKBMM-CD163 and the control (Fig. 4C). These results of in vivo analyses supported the idea based on in vitro experiments that CD163 expression promotes the growth rate of meningioma through suppression of apoptosis.

Cytokine Expression in CD163-Overexpressing HKBMM

During the in vivo analysis, we noticed unexpected splenomegaly in mice inoculated with HKBMM-CD163.
Fig. 4. Enforced expression of CD163 in HKBMM promotes tumor growth and xenograft-affiliated splenomegaly. (A) In vivo growth of subcutaneous tumors after injection of HKBMM-CD163 or HKBMM-GFP. The tumor volume was measured on days 7, 14, 21, 28, 35, and 42. The graph indicates mean tumor volume ± SEM of 5 mice per group (*P < .05, **P < .01). (B) The tumor weight of resected xenograft from HKBMM-GFP and HKBMM-CD163 on day 49. (*P < .05). (C) Representative sections from resected xenograft formed by HKBMM-GFP and HKBMM-CD163 on day 49. Top: The xenograft of HKBMM-GFP demonstrated a large area of necrosis in the central region. The dotted line indicates the area of necrosis. Bottom: The xenograft of HKBMM-CD163 showed a much larger tumor, but a relatively smaller necrosis area (dotted line). Original magnification, left, ×20; right 3 columns, ×400. Scale bar, left, 1 mm; right 3 columns, 20 μm. (D) The resected xenografts and spleens on day 49 are shown. The mean weight of spleen was 437.0 mg in HKBMM-GFP and 755.4 mg in HKBMM-CD163, respectively (Scale bar, 1 cm). (E) Tumor weight plotted against spleen weight, showing significant positive correlation between tumor weight and spleen weight. (Pearson’s correlation coefficient, $R^2 = 0.7192$, $P < .005$) Black circles represent the mice injected with HKBMM-GFP, and white boxes represent the mice injected with HKBMM-CD163.
CD163 induced the production of G-CSF. Moreover, they suggest that the G-CSF/G-CSFR autocrine loop plays a pivotal role in tumor progression through the inhibition of apoptosis.

Discussion

CD163, a monocyte/macrophage lineage marker, is a type I transmembrane protein containing 9 scavenger receptor cysteine-rich (SRCR) domains and belongs to the SRCR superfamily. 3 Previously, the expression of CD163 was thought to be restricted to the monocyte/macrophage lineage; however, Shabo et al. recently explored the aberrant expression of CD163 in breast cancers and rectal cancers and its association with poor prognosis. 12,13 Here, we demonstrated that meningioma is another CD163-expressing tumor, and expression of CD163 in meningioma was correlated with histological atypical parameters that directly predict the prognosis of meningioma. 26 Moreover, in vitro and in vivo experiments revealed that enforced expression of CD163 in meningioma cells resulted in significant suppression of apoptosis and accelerated tumor growth in nude mice. This is the first report, to our knowledge, to prove the novel biological role of CD163 to promote tumor cells in vitro and in vivo.

The molecular function of CD163 has not been fully understood to date, especially for tumor promoting ability. In this study, we clearly demonstrated that expression of CD163 induced G-CSF production in meningioma cells, and it was previously reported that cross-linking of CD163 with monoclonal antibody induced secretion of GM-CSF and IL-6 in macrophages. 27 Although G-CSF was originally identified as a hematopoietic growth factor, 28 an alternative role of G-CSF associated with tumor progression was also analyzed in various types of cancer, such as skin, head and neck, bladder, and brain cancer. 29–32 In addition, many case reports indicated that G-CSF–producing tumors represent a relatively poor clinical course, 33–37 suggesting that aberrant production of G-CSF from tumor cells assists tumor progression. In fact, several in vitro and in vivo studies revealed that G-CSF promoted tumor proliferation, migration, invasion, angiogenesis, and neutrophil recruitment and suppressed apoptosis 29–32; thus, our result that G-CSF derived from CD163-overexpressing tumor cells promotes meningioma progression through the inhibition of apoptosis should be acceptable even if inquired for these previous reports, although the molecular mechanisms responsible for the production of G-CSF after CD163 expression, such as transcriptional regulation, remain to be elucidated.

It is known that CD163 is not only present as a transmembrane protein, but also detected as a soluble protein in serum after being shed from the plasma membrane by metalloproteinases. 38,39 Moreover, serum CD163 levels were reported to be increased in various pathological conditions and work as a marker monitoring macrophage activity in some diseases, including sepsis, liver disease, and...
autoimmune disorders. The serum CD163 level in patients with meningioma is of great interest as a diagnostic or prognostic marker, although future investigations to measure the serum CD163 levels in patients with meningioma should be performed to establish the clinical value of CD163 expression in meningioma.

The mechanisms that allow tumor cells to express CD163 have not been fully understood. It was hypothesized that the aberrant gene expression in tumor cells could be a result of fusion of the tumor cells with bone marrow–derived cells. Previous reports have also shown that cancer cell fusion with macrophages was associated with a more proliferative and metastatic phenotype. Recently, it was demonstrated that coculturing of human macrophages and bladder cancer cells induced cancer cells to express CD163; thus, a cross-talk between tumor cells and inflammatory cells, including macrophages, might be involved in meningioma progression. To elucidate the involvement of inflammatory cells in CD163 expression in meningioma, we evaluated the level of infiltration of inflammatory cells and the extent of bleeding in tumors and analyzed the correlation with CD163 expression (Supplementary Table 1). Of interest, the meningioma cells that harbor a much higher level of inflammatory cells exhibited a higher frequency of CD163 expression, and the histological subtype and the extent of bleeding were not associated with CD163 expression. These results gave us the idea that CD163 expression in meningioma cells might be induced by surrounding inflammatory cells via cytokines.

Establishing a standard treatment protocol for high-grade meningioma and recurrent benign meningioma is a clinically significant theme for neurosurgeons. In particular, a molecular targeting therapy based on the various molecular alternations of meningioma tumorigenesis and progression is requested. The expression of several growth factor receptors, including platelet-derived growth factor receptor, epidermal growth factor receptor, and basic fibroblast growth factor receptor, have been identified in meningioma thus far; however, the clinical trials with molecular targeting drugs against such growth factor receptors demonstrated minimal efficacy. In this report, we explored that CD163 expression was correlated with histological atypical parameters that directly predict the prognosis of meningioma, and G-CSF derived from CD163 expression promoted meningioma progression through the inhibition of apoptosis. Furthermore, one previous report indicated that G-CSF immunoreactivity in meningiomas was correlated with tumor proliferation and vascularization. These data suggest that CD163 and G-CSF could be not only diagnostic markers for malignancy but also attractive target molecules for meningioma treatment.

A limitation of this study is that we exhibited the correlation between CD163 expression and histological atypical features only in grade I and II meningiomas and that all of the 3 grade III meningiomas were negative for CD163. A possible reason for why we could not find any significant CD163 expression in grade III meningiomas was the limited number of grade III cases. In addition, we noticed the heterogeneity of CD163 expression, especially in grade III tumor; in fact, as shown in Supplementary Fig. S3, 1 grade III tumor exhibited a high intensity level of CD163 but only in a limited area (<30%), resulting in the final evaluation of CD163 negative. Further analysis using a large number of grade III meningiomas might elucidate the biological role of CD163 in grade III meningioma. Furthermore, we should also consider the correlation between CD163 expression and the malignant behavior without histological atypia, because it was reported that some meningiomas recurred without histological change. Clinopathological mass analysis of CD163 expression in recurrent grade I meningioma or grade II meningioma with brain invasion but not with morphological atypia will give us the answer. In summary, we demonstrated the expression of CD163 in meningioma and its correlation with histological atypical parameters. Moreover, in vitro and in vivo experiments demonstrated the novel molecular function of CD163 to prevent apoptosis through the production of G-CSF.

Supplementary Material
Supplementary material is available online at Neuro-Oncology (http://neuro-oncology.oxfordjournals.org/).

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Conflict of interest statement. None declared.

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References


