**INTRODUCTION**

There is little question that the infiltrative behavior of malignant gliomas in the brain is one of the primary confounders in modern brain tumor management. Gliomas are characterized by their extensive invasion into the brain parenchyma. The primary cause of local recurrence and therapeutic failure in the treatment of malignant gliomas is the invasion of tumor cells into the surrounding normal brain. Failure in surgical cure of malignant gliomas is mainly due to those tumor cells that have invaded the normal brain far beyond the resectable areas. These remaining cells also resist radio- and chemotherapy and eventually lead to tumor regrowth and the patient’s demise within 12 months from diagnosis. The presence of treatment-resistant neoplastic glial cells beyond the surgical resection margins is the cause of therapeutic failure in patients with gliomas. The mechanisms that underlie glioma invasion are multiple and interdependent. Essentially, cells within the main tumor mass have to alter their cell adhesion such as CD44 and RHAMM, and have to produce matrix metalloproteinase (MMP) for the degradation of ECM in order to facilitate release of tumor cells into the surrounding normal brain. Surrounding environment of these neoplastic glioma cells, invaded within the normal brain, might be different comparing to the tumor core such as motility ability, cell division or BBB status. Before the identification of the mechanisms used by glioma cells to invade the brain, characterization of the invading glioma cells by molecular analysis for well known invasion associate genes could potentially indicate therapeutic strategies to reduce further spreading and/or to target the invading cells more specifically.

To elucidate the molecular profile of invading cells in glioma, we coupled the capacity of intraoperative microdissection (IOM) and laser capture microdissection (LCM) to harvest glioma cells residing in the tumor core and at the invading edge with quantitative realtime PCR for several well-known invasion associ-
Table 1. Patients’ profile according to tumor marginal demarcation

| No | A/S | Biopsy | Location | Tumor Margin |
|----|-----|--------|----------|--------------|
| 1  | 39/F | Biopsy  | F        | Relatively demarcated |
| 2  | 24/M | Glioblastoma | F        | Relatively demarcated |
| 3  | 33/M | Glioblastoma | F        | Relatively demarcated |
| 4  | 70/M | Glioblastoma | Multicentric | Relatively demarcated |
| 5  | 51/M | Glioblastoma | F        | Relatively demarcated |
| 6  | 32/M | Astrocytoma GrII | Insular | Poorly demarcated |
| 7  | 40/F | Glioblastoma | Thalamus  | Poorly demarcated |
| 8  | 52/M | Glioblastoma | Temporal | Poorly demarcated |
| 9  | 52/F | Glioblastoma | F        | Poorly demarcated |
| 10 | 51/M | Glioblastoma (with oligo) | T | Poorly demarcated |
| 11 | 52/M | Glioblastoma | F        | Poorly demarcated |
| 12 | 50/M | Glioblastoma | T        | Poorly demarcated |
| 13 | 63/F | Glioblastoma | FT       | Poorly demarcated |
| 14 | 56/F | Glioblastoma | FT       | Poorly demarcated |
| 15 | 62/F | Glioblastoma | FT       | Poorly demarcated |
| 16 | 51/M | Anaplastic oligodendro GrIII | TO | Poorly demarcated |

A/S : age/sex, F : frontal lobe, T : temporal lobe, FT : frontotemporal, TO : temporo-occipital

Table 2. Patients’ profile according to tumor recurrence pattern

| NO | Biopsy | Resection | Recurrence |
|----|--------|-----------|------------|
| 1  | GBM NO GTR | Marginal recurrence with CSF seeding |
| 2  | GBM YES STR | CSF seeding, extensive |
| 3  | GBM NO GTR | Local & distant to contralateral hemisphere |
| 4  | GBM NO GTR | Local, aggressive |
| 5  | GBM NO GTR | Local & delayed distant to same hemisphere |
| 6  | GBM NO STR | Local & aggressive |
| 7  | GBM YES PR | Local |
| 8  | GBM NO STR | Local |
| 9  | GBM YES STR | Local |
| 10 | GBM NO GTR | Local |
| 11 | GBM NO STR | Local |
| 12 | GBM NO GTR | Local |
| 13 | AO NO GTR | Local |
| 14 | A NO PR | No recurrence |
| 15 | O NO GTR | No recurrence |
| 16 | O YES GTR | No recurrence |

GBM : glioblastoma, AO : anaplastic oligodendroglioma, A : astrocytoma, O : oligodendroglioma, GTR : gross total removal, STR : subtotal removal, PR : partial removal

MATERIALS AND METHODS

Tissue samplings
Twenty-two human glioma tissues were obtained from the glioma patients, separating into the tumor core and invasive rim tissues. Pathologic diagnosis was eighteen samples of glioblastoma, two of oligodendroglioma, one of each astrocytoma and anaplastic oligodendroglioma. Tissue samplings were performed by IOM in 16 cases of clearly defined tumor margin and confirmed proper sampling of tumor core and invasive rim by pathologic slides (Table 1, 2 and) (Fig. 1). LCM was performed in six of those sampling tissues to harvest glioma cells residing in the tumor core and at the invading edge in order to reduce errors in specific sampling (Fig. 2).

Tumor specimens were prepared in consultation with the surgeon and pathologist. All tumors were histologically classified according to the WHO classification of tumors of the central nervous system. Parts of each tumor were frozen immediately after operation and stored at -80°C.

LCM
Cryopreserved glioma specimens from six patients were cut in serial 6-8 µm sections and mounted on uncoated slides treated with diethyl pyrocarbonate. The tumor core and adjacent invasive rim were identified on a coverslipped H&E-stained section. The specimen was selected for collection of 1,000 individual cells for RNA isolation and differential display analysis; the other specimens were used for quantitative, differential RT-PCR analysis. Cryostat sections intended for LCM system (Arcturus Engineering, Mountain View, CA, USA) were transferred from -80°C storage and immediately immersed in 75% ethanol at RT for 30 s. Slides were rinsed in H₂O, stained with filtered Meyer’s hematoxylin for 30 s, rinsed in H₂O, stained with bluing reagent for 20-30 s, washed in 70 and 95% ethanol for 1 min each, stained with eosin Y for 20-30 s, dehydrated in 95% ethanol (twice for 1 min each), 100% ethanol (stored over molecular sieve; three times for 1 min each) and Xylene (three times for 10 min each). Slides were air dried under a laminar flow for 10-30 min and immediately processed for LCM. Diethyl pyrocarbonate-treated, autoclaved, distilled water was used to prepare every solution. LCM was performed with a PixCell II Microscope (Arcturus Engineering, Inc., Mountain View, CA, USA) using a 7.5 µm laser beam at 50-100

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In situ hybridization

Probe preparation

Total RNA was isolated from human glioma tissues using TRIzol (Life Technologies, Gaithersburg, MD, USA) according to the manufacturer's instructions. The quality of the RNA was confirmed by a ratio of >1.8 for the optical densities at 260 nm and 280 nm. The PCR protocol of CD44 and RHAMM were 5 min at 94°C followed by 25 cycles for 30 sec at 94°C, 1 min at 60°C, and 2 min at 72°C, with a final extension for 10 min at 72°C. The following each primers were CD44; sense primer, 5'-CGGGATCATTGCAACC CGCTATGTCCAG-3', antisense primer, 5'-GAATTCCTGCAGATGATC AGCCATTC-3', allowing the amplification of 385 bp fragments. RHAMM; Sense primer, 5'-CGGGATCCTCGAGTCTCTCATCAGAAGC-3', antisense primer, 5'-GAATTCATCTCA CTGGGTGTAAAGC-3', allowing the amplification of 366 bp fragments. The each PCR products were cloned into the pGEM™-T easy vector (Promega, Madison, WI, USA) between the BamHI and EcoRI sites.
used to measure the RNA transcription level of various genes in human brain tumor samples. Primers and TaqMan probes were selected to bind specifically to human cDNA. The TaqMan probes were 5'-labeled with the reporter fluorescent dye FAM (6-carboxy-fluorescein) and carry the quencher dye TAMRA (6-carboxy-tetramethyl-rhodamine), attached to a linker-arm modified nucleotide near the 3' end. The quantitative assay was performed using Rotorgene RealTime PCR system (Corbett Research, Australia). The PCR reaction mixture consisted of cDNA template from 50 nanogram of total RNA, 0.5 μM of each primer, 0.3 μM TaqMan probe, 1 unit AmpliTaq gold polymerase (Applied Biosystems, Branchburg, NJ, USA), 200 μM each dNTP, 2.5 mM MgCl₂, and 10X AmpliTaq buffer to a final volume of 10 µL. Samples were amplified with a precycling hold at 95°C for 10 min, followed by 55 cycles of denaturation at 95°C for 15 sec, annealing at 60°C/58°C (MMP2) for 45 sec for MMP9, CD44, RHAMM, and human Reverse transcriptase II gene as a normalized gene. To compare the different gene expression levels the threshold cycles (CT) values were compared and comparative CT method (delta delta CT) for relative gene expression analysis was used. All samples were amplified in six times and the mean was obtained for further calculations.

Molecular analysis was performed for CD44, RHAMM, MMP-2, and MMP-9. Authors investigated the relative expression of these molecules between the tumor core and invading margin. The obtained value was considered to relative overexpression with significance when the relative expression ratio between the invasive rim and the tumor core (rim : core ratio) was more than 2 or less than 0.5.

Primers and probes for real-time PCR as follows:

**MMP2**

Sense and antisense probes specific for the CD44 and RHAMM were synthesized from linearized pCD44 and pRHAMM using T7 and SP6 RNA polymerases and digoxigenin-11-UTP (Boehringer Mannheim, Germany).

**Hybridization and detection**

Fresh surgical brain tumor specimens were fixed 4% paraformaldehyde. In situ hybridization was performed as described by Ahn et al. The tissue sections were hybridized with 10-15 ng/ul digoxigenin-11-UTP-labeled riboprobe. The controls were incubated with a 1 : 500 dilution of <Dig>-AP conjugate. Hybridization signal was detected colorimetrically with nitro blue tetrazolium salt and 5-bromo-4-chloro-3-indolylphosphate according to the manufacturer’s instructions. The sections were mounted in crystal mount medium.

**Quantitative real-time PCR**

From 16 glioma patients, each specimen of the tumor core and the invasive rim by IOM processed for RNA isolation [Micro RNA Isolation Kit (Stratagene, La Jolla, CA, USA)]. From six additional glioma specimens, approximately 1,000 cells were captured from the tumor core and the invasive rim by LCM and further processed for RNA isolation [Micro RNA Isolation Kit (Stratagene, La Jolla, CA, USA)]. Isolates were processed into cDNA using reverse transcriptase according to the manufacturer’s protocol (Ambion). cDNA for RPII was used for quantitative normalization of harvested material in the matched rim-core analyses.

All of the reverse-transcriptase reactions were performed using Improm II reverse-transcriptase kit (Promega, Madison, WI, USA) with oligo-dT primer. Quantitative real-time PCR was used to measure the RNA transcription level of various genes in human brain tumor samples. Primers and TaqMan probes were selected to bind specifically to human cDNA. The TaqMan probes were 5'-labeled with the reporter fluorescent dye FAM (6-carboxy-fluorescein) and carry the quencher dye TAMRA (6-carboxy-tetramethyl-rhodamine), attached to a linker-arm modified nucleotide near the 3' end. The quantitative assay was performed using Rotorgene RealTime PCR system (Corbett Research, Australia). The PCR reaction mixture consisted of cDNA template from 50 nanogram of total RNA, 0.5 μM of each primer, 0.3 μM TaqMan probe, 1 unit AmpliTaq gold polymerase (Applied Biosystems, Branchburg, NJ, USA), 200 μM each dNTP, 2.5 mM MgCl₂, and 10X AmpliTaq buffer to a final volume of 10 µL. Samples were amplified with a precycling hold at 95°C for 10 min, followed by 55 cycles of denaturation at 95°C for 15 sec, annealing at 60°C/58°C (MMP2) for 45 sec for MMP9, CD44, RHAMM, and human Reverse transcriptase II gene as a normalized gene. To compare the different gene expression levels the threshold cycles (CT) values were compared and comparative CT method (delta delta CT) for relative gene expression analysis was used. All samples were amplified in six times and the mean was obtained for further calculations.

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Primers and probes for real-time PCR as follows:

**MMP2**
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levels of expression of MMP-2, MMP-9, CD44 and RHAMM in cells at the tumor core and invasive rim. If the ratio of the message template number (cDNA) in rim : core was >2 in QRT-PCR analysis, the value was defined as higher expression significantly. The marginal tissues showed higher expression of MMP-2, MMP-9 and RHAMM than the corresponding core tissue (Table 3). On the other hand, CD44 in the core tissues of the gliomas was higher than the marginal tissues (Table 3). The mean of absolute values showed MMP-2 and CD44 were higher in the side of tumor core than in the marginal side, and the other MMP-9 and RHAMM were much more expressed in the marginal side than the core side (Table 4). The mean values from zone 1 to 3 (tumor side to normal side through the margin) demonstrated that the mean MMP-2 was 3.69 (range±SD 5.01), 3.52 (range±SD 3.69), and 2.50 (range±SD 3.38), and the mean CD44 was 4.94 (range±SD 6.45), 3.87 (range±SD 6.28), and 1.21 (range±SD 1.42). The mean MMP-9 was 5.29 (range±SD 7.03), 7.80 (range±SD 14.88), and 4.23 (range±SD 8.09), and the mean RHAMM was 3.79 (range±SD 8.18), 8.04 (range±SD 17.50), and 4.73 (range±SD 8.47).

RESULTS

Immunohistochemistry

The immunohistochemical staining showed a diverse distribution of MMP-2 and MMP-9 proteins in the various regions of pathologic specimens according to individual human glioma tissues. In general, immunoreactivity of MMP-2 was predominant in the cytoplasm of the infiltrating tumor cells at the invasive edge of the glioma, and intense immunoreactivity for MMP-9 was detected in the tumor core cells around hyperplastic endothelial cells (Fig. 6).

In situ hybridization

To verify the extent of expression of CD44 and RHAMM at tumor margin, in situ hybridization of glioma was performed. The CD44 was strongly expressed at core and margin of tumors and invaded cells. On the other hand, RHAMM was only expressed at some of invaded cells and core of the tumor (Fig. 7).

Overexpression of MMP-2, MMP-9, CD44 and RHAMM by the marginal tumor cells

Sixteen glioma specimens, obtained from IOM were analyzed for relative
Table 4. The mean of overall absolute values of molecular characteristics in tumor side to normal side through margin, obtained from Intraoperative Microdissection (IOM)

| n=16 | MMP2 (% | MMP9 (%) | CD44 (%) | RHAMM (%) |
|------|---------|----------|----------|-----------|
| N    | 2.50 ± 0.38 | 4.23 ± 0.89 | 1.21 ± 0.42 | 4.73 ± 0.47 |
| M    | 3.52 ± 0.69 | 7.80 ± 1.48 | 3.87 ± 0.68 | 8.04 ± 1.75 |
| T    | 3.69 ± 0.50 | 5.29 ± 0.70 | 4.94 ± 0.45 | 3.79 ± 0.18 |

T: tumor core, M: tumor margin, N: normal side

Table 5. The mean of overall absolute values of molecular characteristics in tumor side to normal side through margin, obtained from Laser Capture Microdissection (LCM)

| n=6  | MMP2 (%) | MMP9 (%) | CD44 (%) | RHAMM (%) |
|------|----------|----------|----------|-----------|
| N    | 0.69 ± 0.24 | 2.04 ± 1.91 | 2.34 ± 2.66 | 2.41 ± 1.84 |
| M    | 2.56 ± 3.05 | 1.75 ± 1.45 | 3.33 ± 3.64 | 2.31 ± 2.64 |
| T    | 2.72 ± 3.21 | 1.50 ± 1.73 | 0.54 ± 0.22 | 0.48 ± 0.33 |

T: tumor core, M: tumor margin, N: normal side

Table 6. The expression of the patients' molecular characteristics of tumor core (T) and tumor margin (M) according to tumor-margin demarcation

| MMP2 (%) | MMP9 (%) | CD44 (%) | RHAMM (%) |
|----------|----------|----------|-----------|
| PDM      | T<M 7 (63.6) | 6 (54.5) | 4 (36.4) | 6 (54.5) |
| n=11     | T>M 3 (27.3) | 4 (36.4) | 5 (45.5) | 4 (36.4) |
| RDM      | T<M 1 (9.1) | 1 (9.1) | 2 (18.1) | 1 (9.1) |
| n=5      | T>M 0 (0.0) | 2 (40.0) | 2 (40.0) | 3 (60.0) |
|          | T=M 3 (60.0) | 2 (40.0) | 2 (40.0) | 0 (0.0) |

PDM: poorly demarcated margin, RDM: relatively demarcated margin

Table 7. The expression of molecular characteristics of tumor core (T) and tumor margin (M) according to midline cross

| MMP2 (%) | MMP9 (%) | CD44 (%) | RHAMM (%) |
|----------|----------|----------|-----------|
| Mildline cross (+) | T<M 2 (50) | 2 (50) | 4 (100) | 2 (50) |
| n=4      | T>M 1 (25) | 2 (50) | 0 (0)  | 1 (25) |
|          | T=M 1 (25) | 0 (0)  | 0 (0)  | 1 (25) |
| Mildline cross (-) | T<M 5 (41.7) | 6 (50) | 2 (16.6) | 7 (58.4) |
| n=12     | T>M 5 (41.7) | 4 (33.4) | 7 (58.4) | 3 (25) |
|          | T=M 2 (16.6) | 2 (16.6) | 3 (25)  | 2 (16.6) |

Table 8. The expression of the patients' molecular characteristics of tumor core (T) and tumor margin (M) according to tumor recurrence

| MMP2 (%) | MMP9 (%) | CD44 (%) | RHAMM (%) |
|----------|----------|----------|-----------|
| Aggressive recurrence | T<M 3 (50) | 3 (50) | 2 (33.3) | 5 (83.3) |
| n=6      | T>M 2 (33.3) | 2 (33.3) | 2 (33.3) | 0 (0) |
|          | T=M 1 (16.7) | 1 (16.7) | 2 (33.3) | 1 (16.7) |
| Local or no recurrence | T<M 4 (40) | 5 (50) | 4 (40) | 4 (40) |
| n=10     | T>M 4 (40) | 4 (40) | 5 (50) | 4 (40) |
|          | T=M 2 (20) | 1 (10) | 1 (10) | 2 (20) |

The mean values from zone 1 to 3 (tumor side to normal side through the margin) demonstrated that the mean MMP-2 was 2.72 (range±SD 3.21), 2.56 (range±SD 3.05), and 0.69 (range±SD 0.24), and the mean MMP-9 was 1.50 (range±SD 1.73), 1.75 (range±SD 1.45), and 2.04 (range±SD 1.91). The mean CD44 was 0.54 (range±SD 0.22), 3.33 (range±SD 3.64), and 2.34 (range±SD 2.66), and the mean RHAMM was 0.48 (range±SD 0.33), 2.31 (range±SD 2.64), and 2.41 (range±SD 1.84).

Overall results of the molecular analysis from IOM method showed that relative overexpression of MMP-2, MMP-9 and RHAMM were noted at the invasive edge of human glioma specimens comparing to the tumor core, 43.8%, 50% and 56.3% respectively, but CD44 was highly expressed in the tumor core comparing to the margin (Table 3). High marginal expression of MMP-2 and MMP-9 were noted in poorly ill-defined margin on the pathological finding (Table 6). High marginal expression of CD44 and MMP-2 were demonstrated in the midline cross group on the radiological review, and of RHAMM and MMP-2 were showed in the aggressive recurrence group (Table 7 and 8). High expression of MMP-2 seems to be involved in the various invasion-related phenomena. The results of the molecular profile from LCM were similar to IOM results but a few numbers of data was needed to collect more specimens.

**DISCUSSION**

Astrocytomas are the most common primary human brain tumors. The majority of astrocytomas are histopathologically malignant lesions associated with a poor prognosis. Patients with the most malignant form of astrocytoma, the glioblastoma multiforme (GBM), have a median survival of only 12 months despite surgery, cranial irradiation, and intensive chemotherapy. Although malignant astrocytomas rarely metastasize systemically, death results from inexorable local tumor growth and brain invasion. The ability of gliomas in general and GBMs in particular to invade the surrounding tissue diffusely and eventually regrow the tumor bulk is unique among...
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In the adult brain, the ECM is filled with an amorphous matrix containing glycosaminoglycans and proteoglycans. One of the widespread glycosaminoglycans is hyaluronic acid (HA)\(^4,5\). Interestingly, HA is especially enriched in white matter tracts which are one of the most frequent routes of glioma invasion\(^1,2,7-10,12,13\). HA was defined to be important in cell migration during development\(^11,13,14,18,27,39\), wound repair\(^44,51,52\) and tumor invasion\(^13,34,37,46\). Both CD44 and RHAMM have been predicted to play critical roles in these processes. However more recently, it has been hypothesized that CD44 confers adhesive properties rather than motility functions\(^40\) and that RHAMM is involved in signaling cell locomotion\(^21,40\). RHAMM interacts with hyaluronan to promote cell locomotion\(^41\). Interaction with CD44 also stimulates cell migration\(^47\). RHAMM has emerged as a critical regulator of cell motility. The expression of RHAMM is very low in quiescent cells but becomes markedly upregulated during cell migration, following cytokine stimulation and in transformed cells. In this study, expression of CD44 was strongly observed at neuron of normal human brain by in situ hybridization. Also, CD44 was highly expressed at the tumor margin and tumor core in gliomas. On the other hand, since the expression of RHAMM was higher in invaded cells than in tumor core, it was thought that RHAMM play a more important role in glioma invasion than CD44. Overall, both CD44 and RHAMM were overexpressed at invaded cells around tumors, indicating that they may have an important role in local invasion of glioma.

The MMPs are family of extracellular matrix modifying enzymes with zinc-dependancy. MMP family have the ability to degrade macromolecules of the extracellular matrix and are responsible for tumor invasion and infiltration, limiting the complete neurosurgical resection of brain tumors. Several studies have demonstrated a correlation between tumor spread and expression of MMP-2 and MMP-9\(^3,13,14,18,27,39\), indicating that MMP-2 and MMP-9 may have prognostic potential in different epithelial cancer type and even a diagnostic potential in malignant cancers. This study showed that the expression and activity of MMP-2 and MMP-9 have been implicated in invasion of gliomas. Evidence for the role of MMP-2 and MMP-9 in gliomas invasion comes from overexpression of MMPs in malignant glioma tissues. This overexpression may be specific for tumor cells and/or interaction between tumor cells and stroma\(^11\). Recently, stromal cells are under the interest, because the tumor invasion by degrading the matrix, induce a new matrix epithelial cells\(^22\). MMP-2 was expressed in many cell types of gliomas, including the microvasculature and the tumor cells. In the immunohistochemistry, our result showed that MMP-2 was strongly expressed in glioblastoma tumor margin. It was expressed strongly at the margin of the tumor rather than tumor core. On the other hand, MMP-9 was mainly expressed in the endothelial cells of neovasculature of tumor site. Thus, it is highly plausible that the following proteolytic cascade may enhance extracellular matrix degradation in malignant gliomas: increased expression of MMP-2 and MMP-9 is correlation with tumor cell invasion of human malignant gliomas in vivo.

In this study, authors suggested that the measurement of both MMP-2 and MMP-9 in tumor margin may provide further useful correlation with tumor invasion. The results demonstrated that high expression of MMP-2 and MMP-9 was an important factor in the local invasion of gliomas. Genes that are critical for tumorigenesis are not only involved in cell-cycle regulation but also in migration/invasion and programmed cell death\(^1,2,7,8,22,28,32,33,49\). The discovery of a specific genetic profile responsible for the invasive behavior of glioma cells in vivo is therefore likely to lead to new therapeutic targets.

Improved understanding of the mechanisms used by glioma cells to invade the surrounding brain tissue is limited by the inability to reproduce this cerebral environment in vitro. In this study, authors try to characterize the profile of genes activated by glioma cells caught in the act of invading the brain tissue. To identify genetic profile of glioma marginal tissue in vivo, comparing to the tumor core, author have used the capacity of IOM or LCM to harvest specific tissue sites from surgical specimen coupled with quantitative realtime PCR of RNAs isolated from the marginal and tumor core cells. Glioma specimens, obtained from IOM were analyzed for relative levels of expression of MMP-2, MMP-9, CD44 and RHAMM in cells at the tumor core and invasive rim. The marginal tissues showed higher expression of MMP-2, MMP-9 and RHAMM than the corresponding core tissue. On the other hand, CD44 in the core tissues of the gliomas was higher than the marginal tissues. The mean of absolute values (tumor side to normal side through the margin) showed MMP-2 and CD44 were higher in the side of tumor core than in the marginal side, and the other MMP-9 and RHAMM were much more expressed in the marginal side than the core side. LCM has been developed to provide a reliable method to procure pure populations of cells from specific microscopic regions of tissue sections under direct visualization\(^11,29,30\).

Additional six glioma specimens, obtained from LCM were also analyzed for the mean of absolute values, in which only MMP-2 was higher in the side of tumor core than in the marginal side, and the other MMP-9, CD44 and RHAMM were much more expressed in the marginal side than the core. There are some discrepancies in the results obtained from IOM and LCM. Presumably, main reasons are sampling error according to tissue-harvest methods and individual tumor characteristics. But, the invasive glioma cells harvested by LCM could be precise to compare the gene expression profile of cells in the core and at the invading edge of glioma specimens by quantitative realtime PCR.

It was difficult to select tumor cells, due to requiring the differentiation from normal/reactive astrocytes even though LCM technique used in sampling. In order to successfully use the LCM at the invasive edge of a glioma specimen, the authors...
captured cells of tumor edge firstly, then bidirectionally harvest tumor core and normal astrocyte.

Overall results of the molecular analysis from IOM method showed that relative overexpression of MMP-2, MMP-9 and RHAMM were noted at the invasive edge of human glioma specimens comparing to the tumor core, 43.8%, 50% and 56.3% respectively, but CD44 was highly expressed in the tumor core comparing to the margin. High marginal expression of MMP-2 and MMP-9 were noted in poorly ill-defined margin on the pathological finding. High marginal expression of CD44 and MMP-2 were demonstrated in the midline cross group on the radiological review, and of RHAMM and MMP-2 were showed in the aggressive recurrence group. High expression of MMP-2 seems to be involved in the various invasion-related phenomena. These data suggest that invasion associated genes could be participated in the invasion process of gliomas with diversity in the various clinical situation. The results of the molecular profile from LCM were similar to IOM results but a few numbers of data was needed to collect more specimens.

The molecular characterization of the marginal invading glioma cells in this study opens new perspective for research in the field of glioma invasion. We anticipate that more accurate characterization of invading tumor cells with more precise harvest method of invading cells will be possible in the near future. Molecular analysis of pure cell populations in their native tissue environment will be an important component of the next generation of medical genetics. This method for molecular characterization in the leading edge may lead to a better understanding of the mechanisms responsible for the unique invasive behavior of glioma cells in vivo.

CONCLUSION

Up-regulation of MMP-2, MMP-9, CD44 and RHAMM was noted in invasive edge of gliomas according to the various clinical situations. By using these quantitative methods of molecular analysis, the authors could characterize tumor margin of individual glioma and make a specific target for anti-invasive therapy in the future.

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