Table 2. Scores and Correlation between Positivity for Staining and Each Area of Tumor (Grade II–IV, $n = 50$)

	Ang2			MMP-2			MT1-MMP			LN5γ2		
	Center	Border	Invasion	Center	Border	Invasion	Center	Border	Invasion	Center	Border	Invasion
3+	0	14	11	0	2	3	0	0	0	0	5	4
2+	14	32	33	5	29	22	0	16	16	11	31	27
1+	31	4	5	19	16	22	17	29	27	22	11	16
±	5	0	1	15	2	2	16	4	3	9	2	3
−	0	0	0	11	1	1	17	1	4	8	1	0
P value of correlation between positivity and area of tumor*												
Border vs center		<0.0001			<0.0001			<0.0001			<0.0001	
Invasion vs center		<0.0001			0.0001			<0.0001			0.0002	
Border vs invasion		N.S.			N.S.			N.S.			N.S.	

*Analyzed by χ^2 test for trend based on the distribution of the scores of each area.
N.S., not significant.

between the expression profiles of Ang2, MMP-2, MT1-MMP, and LN 5 γ 2 and invasiveness of these GBM was also found ($P < 0.0001$ to $P = 0.0159$ for border versus center or invasion versus center). Similarly, there is no difference in the immunoactivities of these four molecules between the border and invasive areas ($P = 0.3939$ to $P = 0.9996$, data not shown).

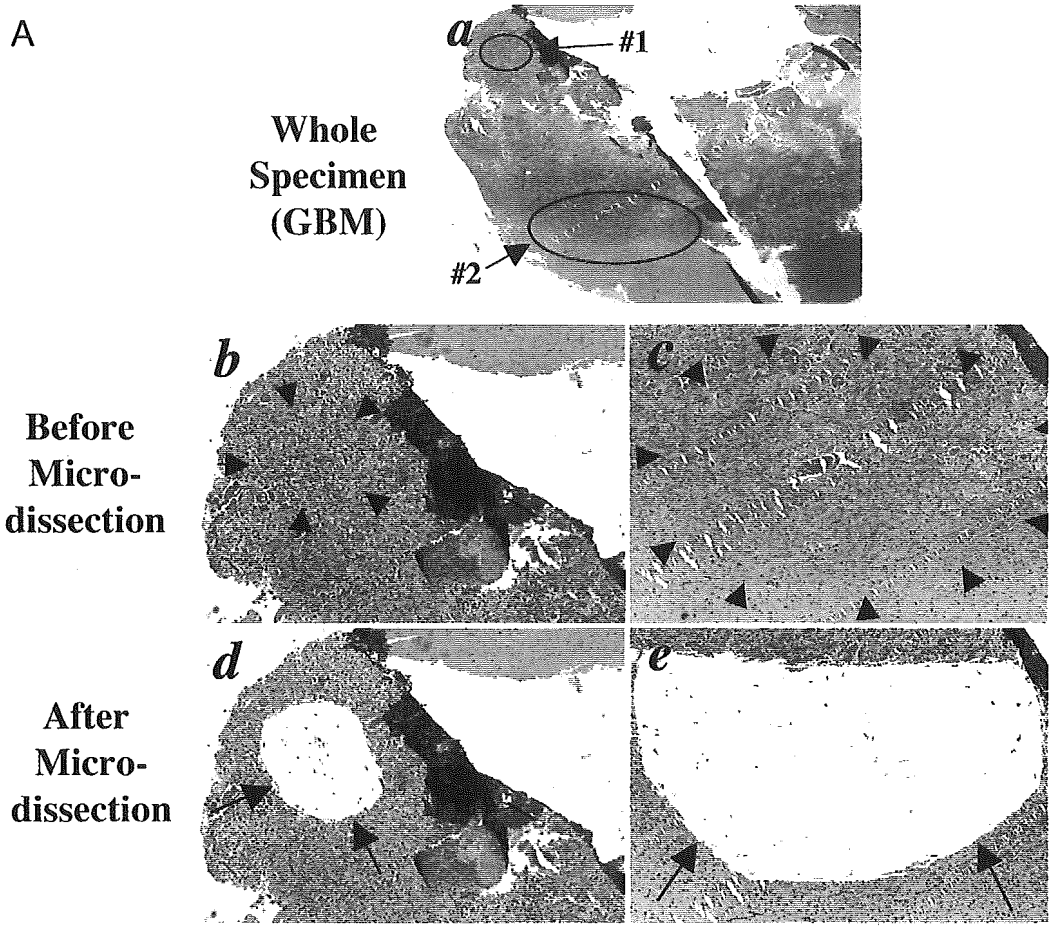
Lastly, we assessed the protein expression levels in the 39 cases of grade II to IV gliomas that only have the center area, but no tumor border and invasive area. Among these 39 samples, 25 to 33 cases (64.1 to 84.6%) have low levels of protein expression of the four molecules that were examined (scores ≤1+, data not shown). Because we do not have portions of these glioma biopsies containing tumor border and invasion areas, we cannot evaluate the correlation of the expression levels of Ang2, MMP-2, MT1-MMP, and LN 5 γ 2 with the invasive edge in these 39 human glioma specimens.

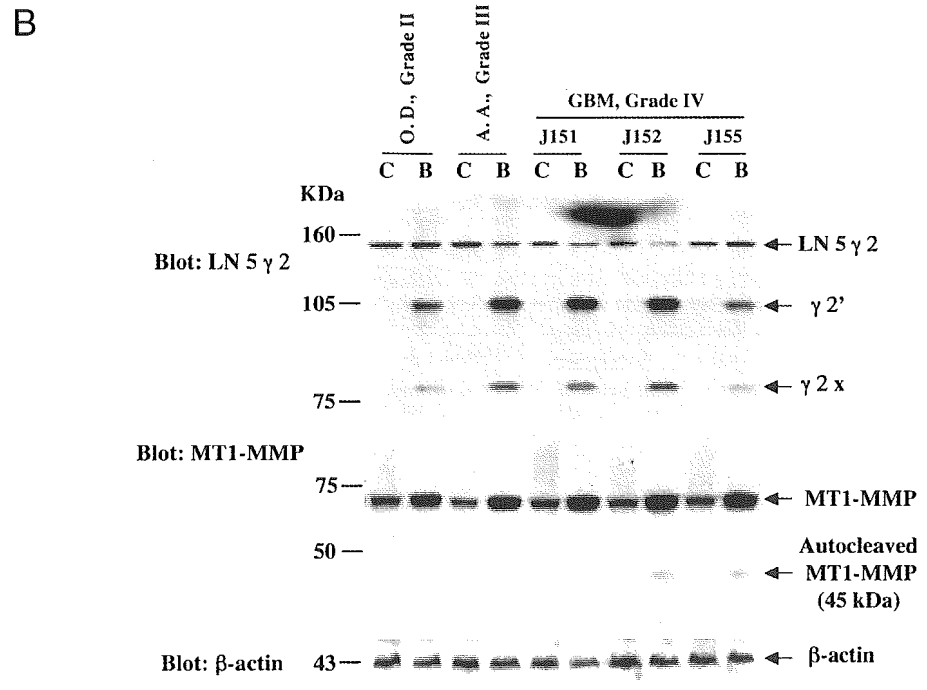
MT1-MMP and LN 5 γ 2 Are Overexpressed and Activated in the Invasive Region of Primary Human Glioma Specimens

To support our observations from the IHC analyses, we identified five primary human glioma specimens from our collection that have shown increased co-expression of Ang2, MMP-2, MT1-MMP, and LN 5 γ 2 in the invasive regions (Table 1) with sufficient tumor material: J181, WHO grade II OD; J165, a grade III AA; J152, a grade IV GBM; J152, a grade IV GBM; and J155, a grade IV GBM. We microdissected the tissues of the central region and the invasive edge of these primary glioma specimens (Figure 3A).[20] Then, we performed Western blot analyses on the total proteins extracted from the microdissected glioma tissue samples using anti-MT1-MMP and anti-LN 5 γ 2 antibodies according to the protocols that were previously reported.[21,22] As shown in Figure 3B, a twofold to threefold increase in protein expression of intact MT1-MMP (70 kd) was found in the samples from the tumor invasive edge of all of the five primary glioma specimens when compared with the central regions in the same sample. Furthermore, significant proteolytic activation of LN 5 γ 2 (155 kd) was evident by the appearance of γ2′ (105 kd) and γ2x (90 kd) fragments[8] in the samples of the tumor invasive edge of all five specimens. Autocleaved MT1-MMP protein fragments (45 kd) were also detected, albeit to a lesser degree, indicating that activation of LN 5 γ 2 and MT1-MMP occurred in these regions of the gliomas. These findings corroborate with the IHC data shown in Figure 2 and Table 1 that MT1-MMP and LN 5 γ 2 are co-up-regulated and co-activated with Ang2 and MMP-2 in the invasive edge of primary human glioma grade II to IV specimens. Taken together, our results (Tables 1 and 2 and Figures 1 to 3) demonstrate that up-regulation of Ang2, MMP-2, MT1-MMP, and LN 5 γ 2 are preferentially associated with the invasive edge of WHO grade I to IV gliomas.

MT1-MMP and LN 5 γ 2 Are Overexpressed at the Invasive Fronts and Disseminated U87MG Ang2-Expressing Gliomas in the Brain

We recently reported that overexpression of Ang2 by human U87MG glioma cells caused aggressive glioma invasion compared to isogenic control tumors. We found that Ang2 and MMP-2 were co-overexpressed in the actively invading regions of U87MG Ang2-expressing tumors.[17] To determine whether the expressions of MT1-MMP and LN 5 γ 2 were also up-regulated in the invasive regions of the Ang2-expressing U87MG gliomas, we performed IHC on various U87MG gliomas. As shown in Figure 4, the expression of MT1-MMP (Figure 4, f) and LN 5 γ 2 (Figure 4, h) was co-overexpressed with Ang2 (Figure 4, d) in the Ang2-expressing gliomas, whereas neither of these two proteins was detected in the isogenic control tumors (LacZ-expressing tumors; Figure 4, e and g). Because both MT1-MMP (Figure 4, f) and LN 5 γ 2 (Figure 4, h) are extracellular matrix proteins, the protein stains detected in the invasive Ang2 tumors were relatively diffusive. The highest expressions of MT1-MMP and LN 5 γ 2 (a fourfold to fivefold increase compared to that in LacZ tumors) were found at the invasive fronts as well as in the disseminated tumor clusters of the U87MG/Ang2 gliomas (arrows in Figure 4, f and h). Importantly, high expression of MT1-MMP (Figure 4, f) and LN 5 γ 2


A
Whole Specimen (GBM)
a
#1
#2
Before Micro-dissection
b
c
After Micro-dissection
d
e


B
O. D., Grade II
A. A., Grade III
GBM, Grade IV
J151
J152
J155
C B C B C B C B C B
KDa
160
Blot: LN 5 γ 2
105
75
LN 5 γ 2
γ 2'
γ 2 x
Blot: MT1-MMP
75
MT1-MMP
50
Autocleaved MT1-MMP (45 kDa)
Blot: β-actin
43
β-actin

(Figure 4, h) are not only co-localized in the invading malignant cells or disseminated tumor clusters, but also co-expressed in the same cell population where Ang2 (Figure 4, d) and MMP-2 are overexpressed.[17] Co-overexpression of these four molecules in the regions where Ang2-induced gliomas invasion occurs suggests that Ang2 induces human glioma invasion through the activation of the downstream effectors MMP-2, MT1-MMP, and LN 5 γ 2.

Ang2 Induces the Stimulation of MT1-MMP and LN 5 γ 2 in Vitro

Increased expression and activation of both MT1-MMP and LN 5 γ 2 are strongly associated with tumor invasion in various types of human cancers.[7,25] To determine whether Ang2 can directly stimulate the expression and activation of MT1-MMP and LN 5 γ 2 in glioma cells, we assessed the expression and proteolysis of these two molecules in various conditions. As shown in Figure 5, overexpression of Ang2 by U87MG cells or exposure to recombinant Ang2 by U87MG, U251MG, and T98G cells (data not shown) results in a 2.2- to 3.4-fold increase in expression and activation of LN 5 γ 2 (Figure 5, top) and MT1-MMP (Figure 5, the second panel) in comparison to untreated or bovine serum albumin-treated U87MG cells. Importantly, the stimulation of both MT1-MMP and LN 5 γ 2 under these conditions correlates with increased expression and activation of MMP-2 in the glioma cells (Figure 5, bottom). Because MT1-MMP is responsible for the activation of MMP-2 by interacting with tissue inhibitor-2 of MMP (TIMP-2),[5] we also determined the expression of TIMP-2 in the glioma cells under these conditions and in the invasive U87MG/Ang2 and noninvasive U87MG/LacZ gliomas. We found that there was no alteration in the expression levels of TIMP-2 in Ang2-stimulated or nonstimulated glioma cells (Figure 5, the third panel), nor in the invasive or noninvasive U87MG gliomas established in the mouse brain (data no shown). Therefore, similar to that of MMP-2,[17] the stimulation of MT1-MMP and LN 5 γ 2 is involved in human glioma invasion induced by Ang2 *in vitro* and *in vivo*.

Discussion

The involvement of Ang2, MMP-2, MT1-MMP, and LN 5 γ 2 in the invasiveness of various human cancers including gliomas has been intensively investigated using clinical tumor specimens, xenografts of human tumor cells, and cell culture models.[5,7,12,15,25,26] However, most of the studies focused on the roles of only one or two of these molecules in human tumor invasion and metastases. In this report, we use three distinct model systems to determine whether there is a significant link between the expression profiles and four important molecules, Ang2, MMP-2, MT1-MMP, and LN 5 γ 2 and human glioma invasion. First, we performed a comprehensive survey of the expression profiles of Ang2, MMP-2, MT1-MMP, and LN 5 γ 2 by analyzing a total of 96 primary human glioma specimens that included 57 samples containing a distinct invasive edge and 39 glioma biopsies containing only central regions of the tumor, and 4 normal brain biopsies. Statistical analysis of our results establishes that there is a significant association between the up-regulated expression of these molecules and the invasiveness of the various gliomas in all four WHO grades. Additionally, low levels of protein expression of these four molecules were detected in the central areas of the majority of these clinical glioma samples. Second, Western blot analyses of microdissected primary glioma specimens showed that MT1-MMP and LN 5 γ 2 proteins were not only co-up-regulated but also co-activated in the cells consisting of the invasive edge of the tumor when compared with the center regions of the same sample, thus supporting our findings of IHC analyses. Third, we further analyzed our recently established glioma invasion animal model[17] and showed that the aggressively invasive U87MG Ang2-expressing gliomas were accompanied by up-regulated MMP-2, MT1-MMP, and LN 5 γ 2 in tumor cells that are actively invading the brain. Finally, stimulation of human glioma cells by overexpressing Ang2 or exposure to exogenous Ang2 induced the expression and activation of MT1-MMP, MMP-2, and LN 5 γ 2 in glioma cells *in vitro*. Together, our data provide evidence suggesting that up-regulation of Ang2, MMP-2, MT1-MMP, and LN 5 γ 2 in actively invading tumor cells correlates with human glioma invasion and stimulation of MMP-2, MT1-MMP, and LN 5 γ 2 are involved in Ang2-induced glioma invasion.

MMP-2, MT1-MMP, and LN 5 γ 2 are important in tumor invasion. In addition to the preferential expression in the invading tumor fronts found in various types of primary human cancers,[4,7,25] these three molecules interact with each other in promoting tumor cell invasion. It has been shown that MMP-2, MT1-MMP, and TIMP-2 form a complex on the cell surface, where MT1-MMP binds to and activates pro-MMP-2 by proteolysis.[5] Both MMP-2 and MT1-MMP also cleave LN 5 γ 2 within the γ 2 chain (155 kd) and generate proteolytic fragments, γ 2′ (110 kd) and γ 2′ X (80 kd). The released γ 2′ X fragment is capable of promoting tumor cell invasion[8,9] and preventing tumor

Figure 3. Co-overexpression and co-activation of MT1-MMP and LN 5 γ 2 in the invasive edge of primary human glioma specimens. **A:** An example of performing microdissection on the center region (**a**, **circled area 1**) and invasive edge of the tumor (**a**, **circled area 2**). **a:** An overview of the specimen (J151, GBM, grade IV). **b** and **c:** Photographs of the central areas (**1** in **a**) and invasive edge (**2** in **a**) before microdissection. **d** and **e:** Photographs of the central areas (**1** in **a**) and invasive edge (**2** in **a**) after microdissection. **b** and **c:** Enlarged photos of tumor center (**circled area 1** in **a**). **c** and **e:** Tumor invasive edge (**circled area 2** in **a**). **B:** Western blot analyses of total protein extracted from microdissected glioma tissues of tumor center (**C**, illustrated as area **1** in **A**; **a**, **b**, and **d**) and tumor invasive edge (**B**, illustrated as area **2** in **A**; **a**, **c**, and **e**). OD grade II, specimen J181, an oligodendroastrocytoma; AA, grade III, specimen J165, an astrocytic astrocytoma; and J151, J152, and J155, GBM, grade IV, GBM. **Top**: Expression and activation of LN 5 γ 2; **middle**: expression and activation of MT1-MMP (the second panel), and **bottom**: β-actin (a loading control). Mature LN 5 γ 2 runs at 155 kd and cleaved γ2′ and γ2x run at 105 kd and 80 kd, respectively. Mature and autocleaved MT1-MMP run at 68 kd and 45 kd, respectively. β-Actin runs at 43 kd. The experiments were performed two independent times and similar results were obtained. Original magnifications: ×12.5 (**Aa**); ×40 (**Ab–Ae**).

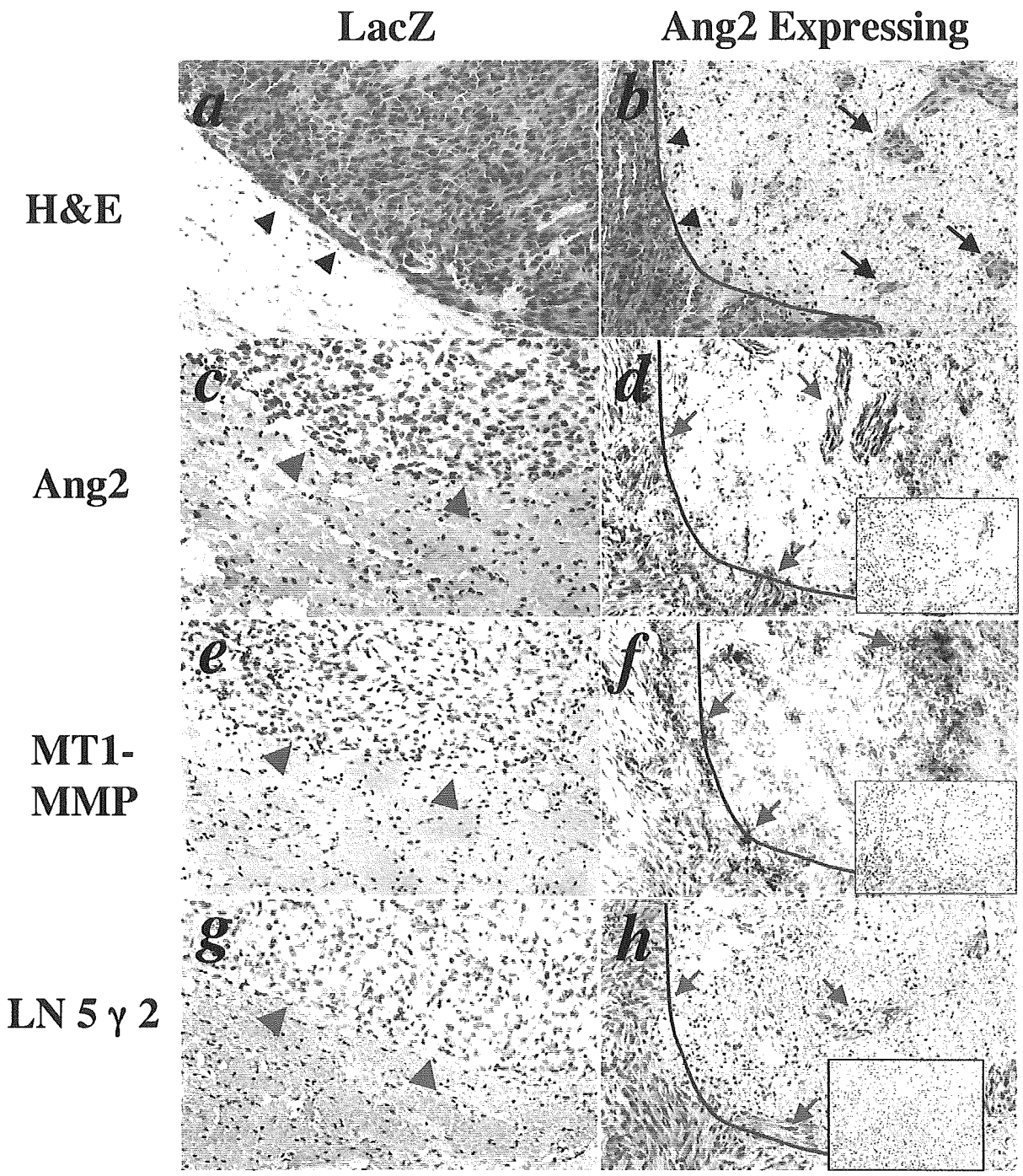


Figure 4. Overexpression of Ang2 by U87MG cells induces glioma invasion and promotes the expression of MT1-MMP and LN 5 γ 2 in the murine brain. IHC of various U87MG gliomas established in the murine brain. **a** to **d** were previously published in Hu and colleagues.[17] The only purpose that we present here again is to serve as controls showing that co-overexpression of Ang2, MT1-MMP, and LN 5 γ 2 are localized in the same invading glioma cells in the brain. **a**, **c**, **e**, and **g:** A glioma established by control U87MG/LacZ cells. **b**, **d**, **f**, and **h:** An invasive glioma formed by U87MG/Ang2 cells. **a** and **b:** H&E staining. **Arrowheads** in **a** indicate the clean edge of U87MG/LacZ tumor spheroid. **Arrowheads** in **b** indicate spikes extended from tumor mass of the U87MG/Ang2 glioma. **Arrows** in **b** show disseminated tumor clusters of U87MG/Ang2 gliomas. **c** to **h:** IHC on serial sections of U87MG/LacZ or U87MG/Ang2 gliomas using anti-Ang2 (**c**, **d**), anti-MT1-MMP (**e**, **f**), and anti-LN 5 γ 2 (**g**, **h**) antibodies. **Arrowheads** in **c**, **e**, and **g** indicate the clean edges of U87MG/LacZ tumor spheroid. **Arrows** in **d**, **f**, and **h** show the invasive tumor-spike and disseminated tumor clusters that expressed Ang2 (**d**), MT1-MMP (**f**), or LN 5 γ 2 (**h**). **Insets** in **d**, **f**, and **h** show isotype-matched IgG controls. Ten to twelve individual samples in each class were analyzed. The experiments were repeated two additional times with similar results. Original magnifications, ×200.

cell apoptosis through the interaction with the epidermal growth factor receptor.[27] LN 5 γ 2 and MT1-MMP are found to be co-localized in invasive fronts of human breast cancers.[9] Indeed, cooperative interactions of LN 5 γ 2, MMP-2, and MT1-MMP are required for tumor vascular mimicry vasculogenesis.[28,29] Our results corroborate these observations. We found that up-regulated expression of MMP-2, MT1-MMP, and LN 5 γ 2, but not

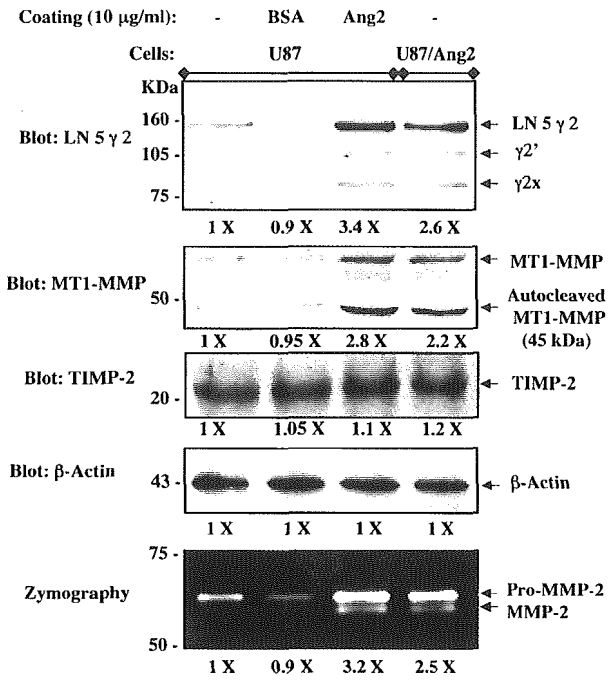


Figure 5. Stimulation of human glioma cells by overexpressing Ang2 or exogenous Ang2 promotes the expression and activation of MMP-2, MT1-MMP, and LN 5 γ 2, but not TIMP-2 *in vitro*. **Top** four panels: Western blot analyses for expression and activation of LN 5 γ 2 (the first panel from the **top**), MT1-MMP (the second panel), TIMP-2 (the third panel), and β-actin (the fourth panel, a loading control). Mature LN 5 γ 2 runs at 155 kd and cleaved γ2' and γ2x run at 105 kd and 80 kd, respectively. Mature and autocleaved MT1-MMP run at 68 kd and 45 kd, respectively. TIMP-2 runs at 22 kd. β-Actin runs at 43 kd. **Bottom**: Zymography assays for the activation of MMP-2. The pro-MMP-2 and the mature form of MMP-2 are shown at 72 kd and 64 kd, respectively.

TIMP-2, co-localized with the increased Ang2 in the invasive areas of primary human glioma specimens, in U87MG Ang2-expressing gliomas *in vivo* as well as in Ang2-stimulated glioma cells *in vitro*. Interestingly, stronger proteolytic co-activation of LN 5 γ 2 and MT1-MMP were found in the proteins extracts from microdissected primary glioma invasive tissues (Figure 3B) than the Ang2 stimulation (by overexpression or exogenous treatment with recombinant Ang2) of U87MG glioma cells *in vitro*, suggesting an optimal tumor microenvironment *in vivo* was more potent in stimulating the activities of these two extracellular matrix-modifying enzymes critical for glioma cell invasion. Thus, our data indicate that co-overexpression of these molecules is involved in glioma invasion.

The ability of glioma cells to diffusively infiltrate normal brain tissues without disrupting brain cytoarchitecture or neuronal function is a hallmark of malignant human gliomas.[1–3] Glioma invasion does not correlate with glioma grade because low-grade astrocytomas invade brain parenchyma with high frequency. However, the degree of tumor invasiveness is correlated with glioma grade.[1–3] In this study, seven of eight PAs displayed an infiltrative phenotype (Table 1) whereas grade II III, and IV glioma specimens in our collection contain distinct invasiveness with identifiable tumor border and invasive areas. Expression profiles of Ang2, MMP-2, MT1-MMP, and LN 5 γ 2 are correlated with the invasive edge of human gliomas, especially in the invasive areas that are 0.25 mm away from the tumor border. Increased levels of these proteins were evident in the invasive regions in these grade I to IV tumors (Tables 1 and 2; Figures 2A, 2B, and 2C, f, i, l, o; and data not shown) and in the tumor borders (Tables 1 and 2; Figures 2A, 2B, and 2C, e, h, k, n; and data not shown). When compared with the other three molecules, the expression profiles of LN 5 γ 2 were relatively increased in both invasive and noninvasive regions of grade IV GBM tumors (Table 1). Strong activation of LN 5 γ 2 at the invasive edge of the tumor (Figure 3B) supports our IHC data that this tumor invasion marker is involved in the progressive growth of malignant GBM tumors. These data show that up-regulation of these four molecules, particularly Ang2, MMP-2, and MT1-MMP, is correlated with the invasiveness of malignant human gliomas.

In summary, our data demonstrate that co-localization of up-regulated Ang2, MMP-2, MT1-MMP, and LN 5 γ 2 is significantly associated with human glioma invasion. Our results from the engineered human U87MG glioma (Ang2-overexpressing, Figure 4) xenografts and cell culture model (stimulation of glioma cells by overexpressing Ang2 or exposure to exogenous Ang2, Figure 5), and from previous reports[30–33] suggest that Ang2 plays a critical role in promoting human glioma invasion through the activation of MMP-2, MT1-MMP, and LN 5 γ 2 in Tie2-independent-pathways. The determination of the mechanisms by which Ang2 induces glioma invasion could provide critical information with regard to the potential of Ang2 and its effectors as new therapeutic targets in glioma treatments.

Acknowledgments

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Cancer Genetics Report

Genetic Alteration of *Poly(ADP-ribose) Polymerase-1* in Human Germ Cell Tumors

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Accumulated evidence suggests that poly(ADP-ribose) polymerase-1 (PARP-1) is involved in DNA repair, cell-death induction, differentiation and tumorigenesis. *Parp-1* deficiency also induces trophoblast differentiation from mouse embryonic stem cells during teratocarcinoma-like tumor formation. To understand the relationship of PARP-1 dysfunction and development of germ cell tumors, we conducted a genetic analysis of the *PARP-1* gene in human germ cell tumors. Sixteen surgical specimens of germ cell tumors that developed in the brain and testes were used. Two known single nucleotide polymorphisms (SNPs) (Val762Ala and Lys940Arg), which are listed in the SNP database of the NCBI (National Center for Biotechnology Information), were detected. In both cases, cSNPs encoded amino acids located within peptide stretches in the catalytic domain, which are highly conserved among various animal species. Furthermore, another novel sequence alteration, a base change of ATG to ACG, was identified in a tumor specimen, which would result in the amino acid substitution, Met129Thr. This base change was observed in one allele of both tumor and normal tissues, suggesting that it is either a rare SNP or a germline mutation of the *PARP-1* gene. Notably, the amino acid Met129 is located within the second zinc finger domain, which is essential for DNA binding and is conserved among animal species. One SNP in intron 2 and one in the upstream 5′-UTR (untranslated region) were also detected.

Key words: brain – germ cell tumor – mutations – poly(ADP-ribose) polymerase-1 – SNP

INTRODUCTION

Poly(ADP-ribose) polymerase (PARP-1) catalyzes polyADP-ribosylation of various proteins (1), including histones, PARP-1 itself, X-ray repair cross-complementing factor-1 (XRCC-1) (2), NF-κB (3) and p53 (4), using nicotinamide adenine dinucleotide (NAD) as a substrate. PARP-1 is present in nuclei and centrosomes (5) and is composed of three domains, namely: DNA-binding, automodification and NAD-binding domains. PARP-1 is activated by DNA strand-breaks and is involved in DNA repair (6–8) and also in the maintenance of genomic stability (9,10). PARP-1 takes part in cell-death induction through depletion of cellular NAD levels (11) and by activating apoptosis-inducing factor 1 (12). *Parp-1*$^{-/-}$ mice show a higher susceptibility to carcinogenesis induced by alkylating agents (13,14).

PARP-1 also participates in the transcriptional regulation of some genes (15–17) and in cellular differentiation (18–20). *Parp-1*$^{-/-}$ mouse embryonic stem (ES) cells show preferential induction of the trophoblast lineage (17), leading to trophoblast giant cells (TGCs) after subcutaneous injection into nude mice (20). The biochemical properties of TGCs resemble syncytiotrophoblastic giant cells (STGCs) of human germ cell tumors. It is thus suggested that *PARP-1* deficiency may possibly trigger differentiation of STGCs within germ cell tumors during tumor formation. The appearance of STGCs in some trophoblastic or choriocarcinomatous human germ cell tumors is reported to be associated with high metastatic potential and poor prognosis

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(21). It is also interesting to note that teratocarcinoma cells undergo differentiation *in vitro*, at least in part, in the presence of the PARP inhibitor 3-aminobenzamide (19). Therefore, Parp-1 could be involved in the development of teratocarcinomas.

The human *PARP-1* gene was previously mapped to chromosome 1q41–q42 (22) and is 43 kb in length and contains 23 exons (23). There have been few reports concerning the structural and functional analysis of the human *PARP-1* gene in tumors (24,25). Prasad et al. (26) showed overexpression of the *PARP-1* gene in human Ewing's sarcoma, and Menegazzi et al. (27) observed increased expression of the *PARP-1* gene in high-grade lymphoma. Bieche et al. (28) observed that lower expression of the *PARP-1* gene is associated with reduction in genomic stability in human breast cancer. We recently reported the reduced expression and structural alteration of the *PARP-1* gene in some human tumor cell lines (29).

In the present study, we analyzed the *PARP-1* gene in human germ cell tumors in order to clarify the relevance of *PARP-1* deficiency in the development of human germ cell tumors.

MATERIALS AND METHODS

Germ cell tumors were obtained from patients who were treated at the Saitama Medical School Hospital, Saitama, Japan, the Kanazawa University Hospital and National Cancer Center Hospital, Tokyo, Japan. Specimens of germ cell tumors of the brain were taken from 14 patients and corresponding non-cancerous tissue from 13 patients (Table 1).

Table 1. List of germ cell tumors

Tissue	Sample no.	Tumor type	Normal tissue
Testis	T1	Mixed type germ cell tumor	na
	T2	nd	na
Brain	B2	Germinoma, pineal	BN2
	B4	Germinoma with STGC, neurohypophyseal	BN4
	B5	Germinoma	BN5
	B6	Germinoma, pineal	BN6
	B7	Germinoma + immature teratoma, pineal	BN7
	B8	Yolk sac tumor + immature teratoma, pineal	BN8
	B9	Germinoma	BN9
	B10	Germinoma	BN10
	B11	Immature teratoma + yolk sac tumor + embryonal carcinoma	BN11
	B12	Immature teratoma, pineal	BN12
	B13	Immature teratoma	BN13
	B14	nd	na
	B15	nd	BN15
	B16	nd	BN16

STGC, syncytiotrophoblastic giant cells; nd, not determined; na, not available.

Oligonucleotide PCR primer sets for the 23 exons of the *PARP-1* gene were designed from intron sequences of the respective exons based on the complete human *PARP-1* sequence (Table 2). Each exon of *PARP-1* was amplified by PCR using 50–300 ng of genomic DNA with primer sets at a 1 μM concentration and with 100 mU of Ex Taq polymerase (Takara BIO Inc., Tokyo, Japan) or LA Taq polymerase (Takara BIO Inc.). The PCR amplification was performed using a thermal cycler (Perkin Elmer) for 35 cycles with 94°C for 1 min, annealing performed at the respective temperatures indicated in Table 2, for 1 min, and 72°C for 1 min, after an initial hot start at 94°C for 5 min. PCR products were purified using DNA Clean & Concentrator-^{TM5} (Zymo

Table 2. PCR primer sets

Exon	Sense primer	Antisense primer	Annealing temp (°C)*
1	aggcggcagcgtgtttctag	gttaactgtgtccgggaagg	63.0
	ccaggcatcagcaatctatcag (sense-2)	gagcggcacttggggcctat (anti-2)	66.8
2	aggctgtcctcctttcacag	gcctcgtctcagagagcaaa	62.2
3	ccaaggcataaggtgtcc	cacggtccagttctcatg	62.0
4	aggctgcccgtatcatgttc	caaaggccctacaggttcca	62.2
5	ggtgtcttctgccatcttg	ggcagttattaaatagcatggc	59.8
6	ctcctcctccagttggattc	gttgtggagtgactgtccca	62.2
7	tcccacattagtaagtgtcc	gtttatacttcaggtccctg	58.4
8	actgtctttcatccttaaagcc	ccactacttgctggtggtgt	63.4
9	tgcccagtcctttctgtgc	gagtcgaacacagcgctgaa	61.1
10	tgagggtttggaagtacac	cggaacctctccctttgtg	59.8
11	gatgggaatgagaacaactc	gcacacatccaaatgccc	59.8
12	ggtagtgtatccaagaggc	gcctggggaatgaaatct	58.4
13	aattcttccccacgtgaatg	cccccaaaaatagaagaaaata	57.1
14	ccccacagacgcagatacc	ccttcaatcttaaattgtgtgag	58.4
15	ctacagactctggatttggg	ctcaaggttaagaaactggaa	58.4
16	tgctaaaagagaaagcctgg	gccgtccctccccatcat	
	gcttttagaggatgggggtc (sense-2)	attttccctccctgcctttta (anti-2)	64.0
17	ggagggtttgccattcactg	gctggcgcccaataattcac	
	ctctcaatatgtgcagcaggt (sense-2)	cattgctgctggtggtcctt (anti-2)	62.0
18	aagtagatgaagaggtgg	ctgggtagaaaagagcag	55.0
19	ctgcacagtaatactaaccagc	gggggattctgccagacat	62.2
20	gagaattagaagtgatggattgc	gcaccaattaggattcttttctt	59.8
21	agcagtttagatggtctggg	gtgtgtgtgcgttgttgttg	59.8
22	cactgagctgaagtcacacc	ggtccctccctgtgcatag	62.2
	gaccacatggacccaacatc (sense-2)		63.0
23	gccccatgaagaggccttag	gggaagaccaaaggaaggaa	60.0
	gatgggtagtacctgtacta (sense-2)	tgtgttgtgtctgtggggag (anti-2)	

*Used as annealing temperature for PCR.

Reseach, USA), and then directly sequenced for both strands using a DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, USA). The residual dye terminator was removed using an Autoseq G-50 kit (Amersham Pharmacia Biotech), and samples were analyzed on an ABI Prism 310 Auto Sequencer (Applied Biosystems). Sequence comparison was carried out against the sequence of the human *PARP-1* gene (accession no. NT004559) and its cDNA (accession nos M18112, M32721, M17081, J03473, BC037545 and BC014206).

RESULTS

Thirteen of 16 germ cell tumors were analyzed for all 23 exons. Sequence alterations and single nucleotide polymorphisms (SNPs) found in the *PARP-1* gene are listed in Table 3. Eight SNPs were detected and two SNPs accompanied amino acid substitution, Val762Ala and Lys940Arg, respectively, as shown in Fig. 1 and Table 3. Three brain tumors (B5, B6 and B13) and their normal counterparts contained Val/Ala heterozygous alleles at amino acid position 762. Two other brain tumors (B10 and B11) and their normal counterparts only possessed an Ala allele. Two brain tumors (B8 and B13) and their normal counterparts only contained an Arg allele at amino acid position 940. These two SNPs, Val762Ala and Lys940Arg, are already in the list of the NCBI (National Center for Biotechnology Information) database (http://www.ncbi.nls.nih.gov/) of SNPs. Interestingly, a brain tumor (B10) contained a novel sequence alteration, ATG→ACG, causing a Met129Thr amino acid substitution in one allele, in both the tumor and normal counterpart (Fig. 1). This sequence alteration has not been listed in the NCBI database of SNPs, suggesting that it is either a rare allele or a germline mutation. Four SNPs, which did not cause amino acid substitution, were also found at Asp81, Ala284, Lys352 and Phe638 (Table 3). A novel sequence alteration of G to C was also found in the upstream 5′-UTR (untranslated region) 17 bases upstream of the translation initiation site, immediately downstream of a putative ETS-1-binding site (base −26 to −22) (30) in one testicular tumor (T1). Although the normal counterpart tissue for T1 was not available, we observed the non-heterozygous C allele also in normal colon tissue (data not shown), which suggests that this sequence alteration is an SNP. In addition, an SNP in intron 2, which is located six bases upstream of exon 3, was found.

DISCUSSION

In this study, we report a novel sequence alteration that causes amino acid substitution, namely Met129Thr, in the *PARP-1* gene. This sequence alteration has not been reported as an SNP and could be a germline mutation. Met129 is located at the second zinc finger motif and a Met129Thr substitution may possibly affect the DNA binding activity of PARP-1.

Two SNPs that accompanied amino acid substitutions, Val762Ala and Lys940Arg, were found. Both amino acid residues are located in the catalytic domain of PARP-1 and are highly conserved among species. The incidence of the non-heterozygous GCG allele, which causes a Val762Ala amino acid substitution, was higher (normal, 15.3%; tumor, 12.5%) than the reported value of 5.9% in the SNP 500 Cancer Database of the NIH Cancer Genomic Anatomy Project (http://snp500cancer.nci.nih:gov). The incidence of the non-heterozygous AGG allele, which causes amino acid substitution Lys940Arg, was also higher than expected (normal, 15.4%; tumor, 12.5%) and the heterozygous allele was not detected in the 16 samples analyzed. This may well be caused by the small sample size. However, there is an alternative possibility that non-heterozygous alleles may promote development of germ cell tumors. It should be further investigated whether the Ala/Ala allele at amino acid residue 762 and the Arg/Arg allele at amino acid residue 940 could contribute to the development of germ cell tumors.

Recently, Lockett et al. (31) showed that the Ala/Ala genotype of the Val762Ala SNP in the *PARP-1* gene is associated with an increased risk for prostate cancer in Caucasian subjects. They reported decreased PARP-1 activity of Ala/Ala compared to the Val/Val genotype. Detailed analysis of genetic effects of Val762Ala, Lys940Arg and Met129Thr on PARP-1 function may be helpful in understanding the significance of these SNPs in germ cell tumor development.

A promoter allelotype of *PARP-1* is reported to relate to susceptibility to rheumatoid arthritis (32). In this study, we observed a novel sequence alteration of G to C at the 5′-UTR (see Table 3) in tumor sample T1. Although the normal counterpart tissue was not available for T1, this sequence alteration was likely to be an SNP because we found this non-heterozygous C allele in the normal colon tissue from an unrelated sample as well. This sequence alteration in the 5′-UTR, located 17 bases upstream from the first ATG, does not overlap with Kozak's consensus sequence (33), therefore the effect on translation is not currently understood well. The major transcription initiation site is present at 146 bases upstream of this sequence alteration. The sequence alteration by this SNP is located nine bases upstream of a putative binding site for transcription factor Ets-1 (30) and, in addition, this region is GC-rich. Therefore, if the alternative transcription starts from the nearby sequence, the transcription efficiency may be affected by the secondary structural changes. Further studies of the effect on the transcription and translation of the *PARP-1* gene may facilitate our understanding of the role of the sequence alteration in the 5′-UTR.

We observed both a heterozygous and non-heterozygous SNP in intron 2 at the same frequency (3/16) in germ cell tumors. This SNP is located within close proximity to the splicing acceptor site for exon 3. Nakata et al. (34) reported that a relatively conserved stretch of $(C/T)_6NCAGG(C/T)$ at a splicing acceptor site is generally observed in the introns. The SNP at intron 2 in Table 3 located within this pyrimidine-rich

Table 3. SNPs and sequence alteration found in the *PARP-1* gene

	Exon	Nucleotide*	Normal tissue (n = 13)	Germ cell tumor (n = 16)	Tumor sample	Information on NCBI database of SNPs	
						ID of SNP	Average estimated heterozygosity+
Amino acid substitution							
Met129Thr	3	ATG	12	15	T1, T2, B2, B4, B5, B6, B7, B8, B9, B11, B12, B13, B14, B15, B16		
		ATG/ACG	1	1	B10	nl	nl
		ACG	0	0			
Val762Ala	17	GTG	8	11	T1, T2, B2, B4, B7, B8, B9, B12, B14, B15, B16		
		GTG/GCG	3	3	B5, B6, B13	rs1136410	0.500
		GCG	2	2	B10, B11		
Lys940Arg	21	AAG	11	14	T1, T2, B2, B4,B6, B7, B8, B10, B11, B12, B14, B15, B16		
		AAG/AGG	0	0		rs321945	0.429
		AGG	2	2	B8, B13		
SNPs without amino acid substitution							
Asp81	2	GAC	nd**	12	T2, B2, B4, B5, B6, B7, B8, B10, B12, B14, B15, B16		
		GAC/GAT	nd	0		rs1805404	0.338
		GAT	1	4	T1, B9, B11, B13		
Ala284	7	GCT	nd	8	T2, B2, B4, B11, B13, B14, B15, B16		
		GCT/GCC	nd	2	B6, B7	rs1805414	0.500
		GCC	1	6	T1, B5, B8, B9, B10, B12		
Lys352	8	AAA	13	15	T1, B2, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14, B15, B16		
		AAA/AAG	0	1	T2	rs3219061	0.332
		AAG	0	0			
Phe638	13	TTC	12	15	T1, T2, B2, B4, B5, B6, B7, B9, B10, B11, B12, B13, B14, B15, B16		
		TTC/TTT	0	0		nl	nl
		TTT	1	1	B8		
SNPs in non-coding region							
5′-UTR (−17 bp)		G	13	15	T2, B2, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14, B15, B16		
		G/C	0	0		rs907187	nl
		C	0	1	T1		
Intron 2 (15 610 bp)		C	nd	10	T2, B4, B5, B6, B8, B11, B12, B13, B14, B16		
		C/A	1	3	B2, B7, B10	rs1805405	0.440
		A	nd	3	T1, B9, B15		
3′-UTR exon 23 (46 584 bp)		G	11	14	T1, T2, B2, B4, B5, B6, B7, B9, B10, B11, B12, B14, B15, B16		
		G/A	1	1	B13	nl	nl
		A	1	1	B8		

*Underline indicates the altered nucleotide.
+Calculated based on all variation data and their sample sizes, according to the method established by Sponge et al. (http://www.ncbi.nlm.nih.gov/SNP/Hetfreq.html).
SNP, single nucleotide polymorphism; UTR, untranslated region; nl, not listed; nd, not determined.

stretch [TTTGATT(C/A)TCC*AG*G, where italic *AG* corresponds to the intronic sequence at the intron–exon boundary] and alteration of C to A may possibly affect the splicing efficiency at exon 3.

Germ cell tumors are known to be highly sensitive to chemotherapeutic agents, including cisplatin, and radiation therapies. Since PARP-1 is involved in DNA repair, dysfunction of *PARP-1* may affect the outcome of cancer therapy besides

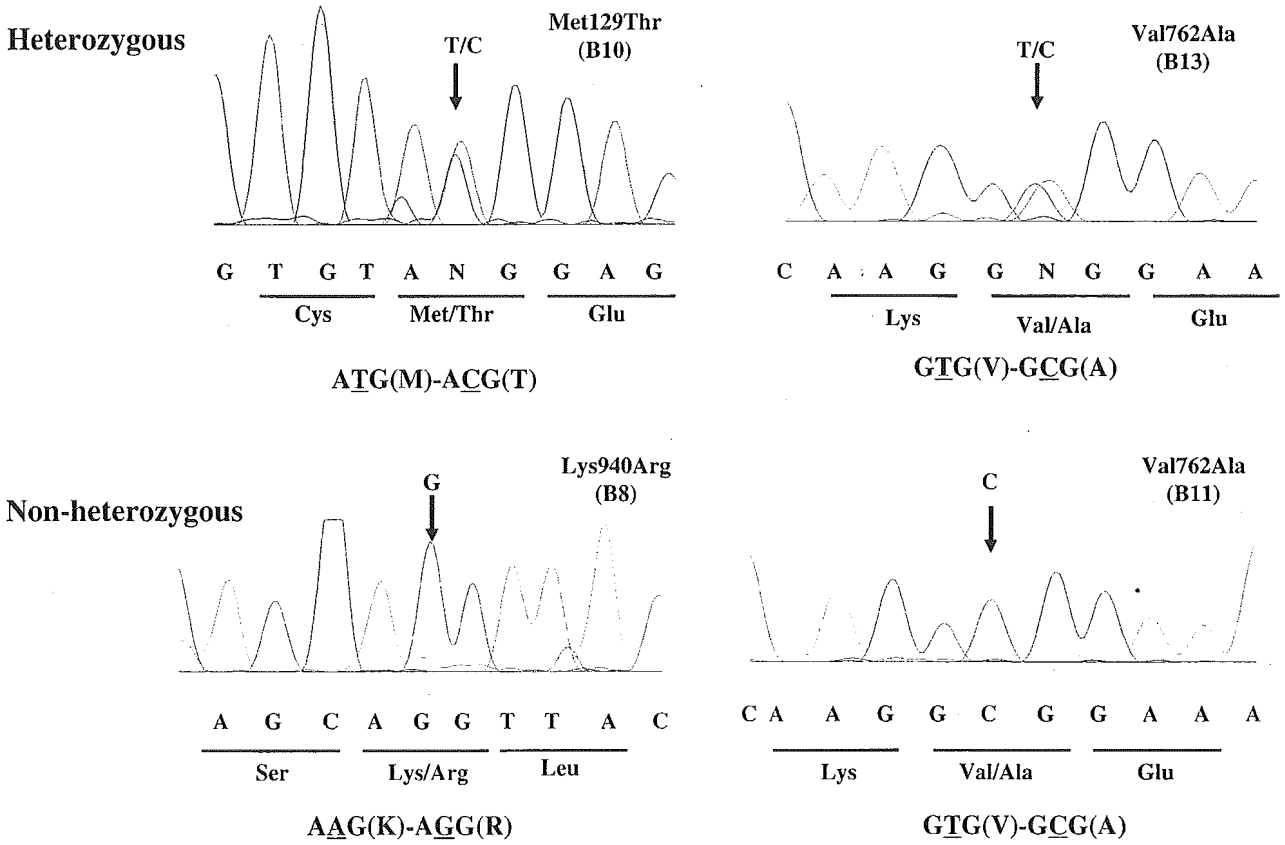

Figure 1. Sequence alteration and SNPs of the *PARP-1* gene associated with amino acid substitution. The upper and lower panels show heterozygous and non-heterozygous alleles, respectively.

its impact on cancer susceptibility, indicating that functional and genetic analysis of the *PARP-1* gene is important not only for the elucidation of the mechanism of cancer development but also from a therapeutic point of view.

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専門医に求められる最新の知識

脳腫瘍

頭蓋内胚細胞腫瘍の最新知見

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Ⅰ．はじめに

頭蓋内胚細胞腫瘍は，髄芽腫と並んで治癒が期待できる悪性脳腫瘍である．1995年に組織された厚生労働省がん研究助成金による研究班の努力によってgerminomaの5年生存率は98％，immature teratomaなどの予後中間群で94％，choriocarcinoma，yolk sac tumor，embryonal carcinomaなどの予後不良群においても64％という成績が達成された（松谷雅生，unpublished data）．

その治療の基本方針については別稿に譲り[10]，本稿ではいくつかの最近のトピックについて解説する．

Ⅱ．神経内視鏡導入の意義

頭蓋内胚細胞腫瘍治療の第一歩は，手術による診断の確定と腫瘍の摘出である．腫瘍マーカーの測定は組織学的診断を下すうえで重要な情報となるが，例えばα-fetoproteinやhuman chorionic gonadotropin-β（HCG-β）が陰性でもgerminomaとは限らず，immature teratomaやembryonal carcinomaあるいはそれらを含むmixed tumorである可能性がある．また，placental alkaline phosphatese[13]やc-Kit[7]が有意に高値である腫瘍はgerminoma成分を含んでいると考えられるが，これらの腫瘍マーカーはnon-germinomatousな成分の混入に関しては情報をもたらさない．

ただ，前述のように予後中間群の治療成績が著しく向上しgerminomaに匹敵する生存期間が得られるようになったことから，例えばgerminomaにimmature teratomaが混じていても依然としてその5年生存率は90％以上が期待される．Germinomaと予後中間群との厚生労働省研究班による治療方針の相違は，後者における腫瘍局所へのboost照射と維持化学療法である．仮に腫瘍マーカーの値からgerminoma成分を含んでいると考えられる症例をすべて予後中間群に準じて治療するとすれば，組織学的に確認したpure germinomaとの間の生存期間の差を検出することはほとんど不可能であろう．しかし，不要な局所照射と維持療法の追加は患者の高次機能や内分泌学的機能に不要な影響を与える可能性が否定されていない．また，仮に腫瘍マーカーの値からgerminoma成分を含んでいると考えられる症例をpure germinomaに対する厚生労働省班研究治療プロトコールで治療したとしても，おそらく組織学的に確認した場合としていない場合との生存期間の差は数％で，これを検出するには多数例のよくコントロールされた臨床研究が必要であろう．しかし，pure germinomaと予後中間群との間には後者の治療強度を上げても5年生存率で4％の差が存在す

ることから，予後中間群を誤ってpure germinomaと考えて治療する危険を冒すことは許されないのではないだろうか．したがって，現時点ではあくまで組織型を確認する姿勢が必要であろう．

さて，進歩著しい神経内視鏡の導入によって開頭手術に比べて低侵襲で組織型の確認ができるようになった．松果体腫瘍に対して内視鏡的に生検を行った場合の組織学的診断率は48/54（89％）と報告されている．神経学的症状の一過性の出現を15％に認めているが，手技による死亡症例や後遺症を残した症例はなかったとのことである[16]．ただし，報告は欧米からのものがほとんどで，したがって対象疾患として胚細胞腫瘍以外のpineoblastomaなどを多く含んでいる．組織学的に混合腫瘍が多い頭蓋内胚細胞腫瘍における診断率についてはわが国において何らかの方法で多数例のデータを集積することが期待される．

神経内視鏡による手術では同時に第3脳室底開窓術を行うことができるという点も大きな利点であるが，内視鏡による観察によって頭蓋内胚細胞腫瘍における新しい知見が追加されたことも特筆すべきである．すなわち，術前のMRIにおいて病変が松果体あるいは神経下垂体部に限局していると思われた症例でも実際には脳室壁に多発病変が認められることが少なくない[6, 15]．

図1に示したように第3脳室壁を観察すると，特にchiasmatic recessなどに色調が赤味を帯びた限局性病変が存在し，これを生検すると腫瘍組織であることがしばしば経験されている．腫瘍が明らかに脳室壁の表面に付着しているように存在し播種病変を思わせる場合と，脳室上衣下に腫瘍が存在して多発病変である可能性を示唆するものとの両方を経験する．

いずれにせよ，MRI画像上は単発腫瘍であっても実際には脳室内に多発している症例があることは，局所照射後の再発の多くが照射野外脳室内に発生する事実とあわせて，胚細胞腫瘍治療においては少なくとも全脳室照射が必要であることを強く示唆している．

Ⅲ．治療による障害

はじめに述べたように頭蓋内胚細胞腫瘍の治療成績は著しく進歩し，多くの症例で治癒が望めるようになった．しかし，放射線照射や化学療法は特に小児においては正常組織に対する重大な影響を及ぼす可能性がある．したがって，次のステップとして治療成績を維持しつつ治療による障害を軽減する方向が模索されなければならない．

本腫瘍の好発部位の一つは神経下垂体近傍であるが，厚生労働省研究班の提唱した治療法前後の内分泌機能について検討した結果が発表されている．

対象はgerminoma症例9例で，24Gyの全脳室照射と3クールのcarboplatinとetoposideによる化学療法を受けている．治療前後に刺激試験を行った結果，正常機能を維持していると判定された症例はホルモン別にACTH治療前2例/治療後2例（以下同じ順序），GH 2/2，LH 1/6，FSH 2/6，PRL 4/8，TSH 2/4であった．特にLH，FSH，PRL，TSHにおいて治療によって腫瘍が消失するとともに内分泌機能が改善している症例が認められている[14]．

24Gyの全脳室照射によって治療したgerminomaにおいて，治療前から存在した内分泌学的異常は改善しなかったが，治療によって新たに出現あるいは悪化した障害はなかったという報告もある[1]．

全脳照射による高次機能障害については，

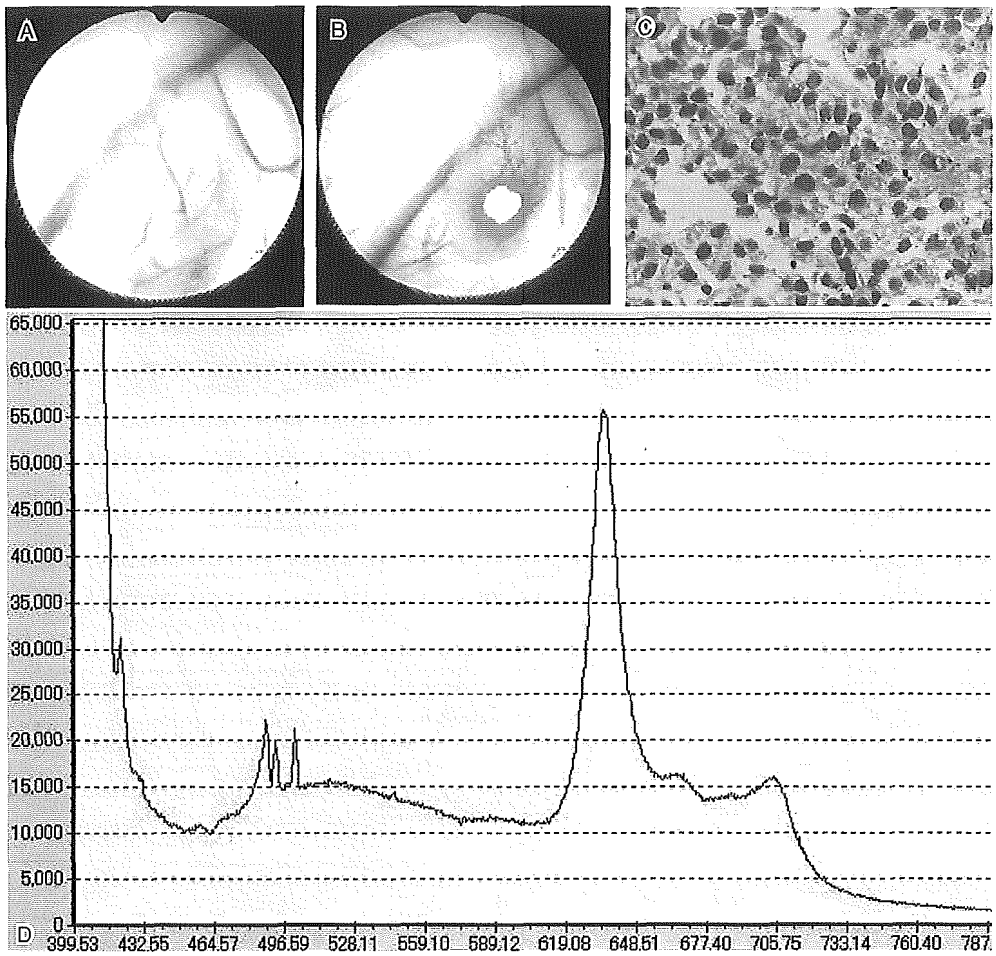

図1　あらかじめ5-aminolevulinic acidを内服させて手術を行った松果体胚細胞腫瘍患者の神経内視鏡所見

第3脳室壁において術前MRIでは腫瘍が検出されなかった部位にやや赤味を帯びた病変が観察された（A）．青色光を照射し（B），同時にスペクトラムを計測すると赤い光が検出され（D，中央やや右のピーク），腫瘍性病変であることが確認された．生検した組織はgerminomaであった（C）．

18～24Gyの線量でもmildで遅発性ではあるが障害が出る可能性があると報告されている．障害は年少児ほど顕著であり，線量が少ない場合は注意力散漫と記銘力障害が主体で，高線量では認知障害を生じるとされている[12]．高度の認知障害であれば発見は比較的容易であるが，注意力散漫や記銘力障害を呈している場合はそれを見落とさないことが必要である．

ドイツにおける前向き多施設共同研究MAKEI 83/86/89では，全脳全脊髄30Gyに加えて局所15Gyを照射した結果，52/56（92.8％）が学校在学中あるいは卒業し，35/56（62.5％）がdegreeを得たので，高次機能の面でも全脳照射には問題は少ないと報告されている（ここでいうdegreeが大学卒業を意味するとすると相当に高い数字である）．しかし，具体的な高次機能の検査は行われていない[2]．

一方，全脳室照射例の報告では24Gyの照射前後において知能検査が行われ，成績の悪化をみていない[1]．

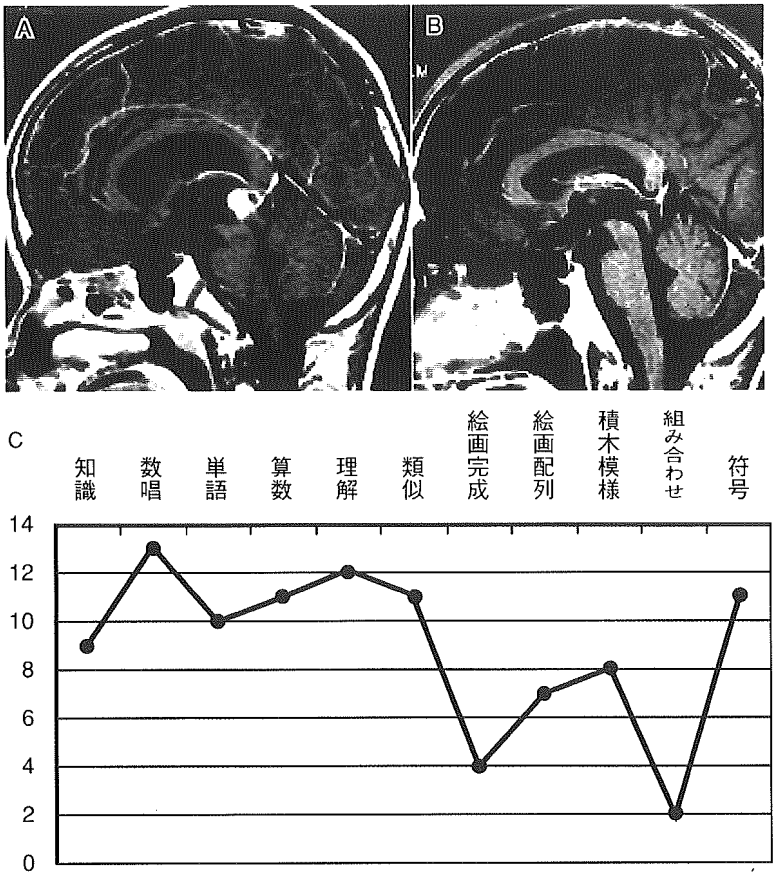

図2　松果体胚細胞腫瘍患者のWAIS-R知能検査

全脳室照射24Gyとcarboplatin＋etoposideによる化学療法3コースによって腫瘍は消失した（B）．治療開始3年後に行った知能検査では言語性IQ＝107であったが，動作性IQ＝68と有意に後者が低値であった．なかでも絵画完成と組み合わせのスコアが際立って低かった（C）．下位項目のこのパターンは動作性IQが低値であった松果体胚細胞腫瘍患者に共通していた．

松果体胚細胞腫瘍患者においては知能検査を行うと治療前から動作性IQの低下が観察されることが多い[9]．自験13例においては6例（46％）で言語性IQに比べて25ポイント以上の動作性IQの低下が観察された．典型例を示す（図2）．動作性IQが低下している症例の下位項目を分析すると，共通して組み合わせ（図3）や絵画完成などが不得手であった．これは視覚—運動の早さ（visual-motor speed）や視覚的全体把握（visual closure）の障害と解釈される．眼球運動障害によるものではないかとの指摘もあるが，治療後Parinaud徴候を認めなくなった症例においても観察された現象である（図2）．ただし，動作性IQの低下は腫瘍径が大きい症例に顕著な傾向があった（図4）．

したがって，微細な眼球運動障害が残存している可能性が否定できないので，眼球運動や視覚認識についてはさらに踏み込んだ生理学的な検討が課題である．

一見障害を残していないように見える患者においてもこのような異常がみられたという事実は患者の学業や職業の選択に影響を与え

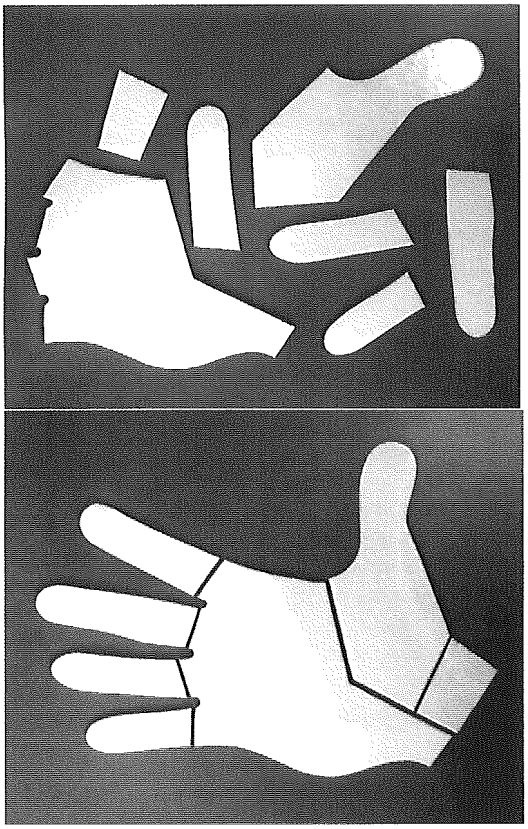

図3　WAIS-Rにおける組み合わせ問題

上段のようなピースが与えられ，下段の図をつくることができれば正解．

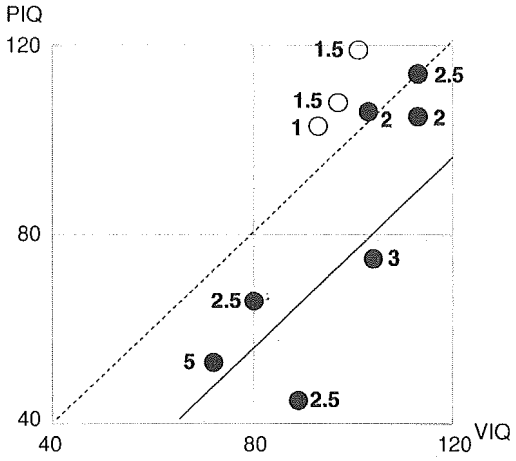

図4　松果体腫瘍患者の腫瘍径と言語性・動作性IQ

縦軸は動作性IQ，横軸は言語性IQ．黒丸は胚細胞腫瘍患者，白丸は松果体嚢胞患者．実線は言語性IQに比べて動作性IQが25ポイント低いライン．症例に添えて示した数字は腫瘍径（cm）．動作性IQが有意に低かった症例の腫瘍径は2.5～3cm以上であった．

る可能性を示唆するので，臨床医としては記憶すべきデータであると考える．

Ⅳ．基底核胚細胞腫瘍とHCG-β超高感度測定法の意義

基底核胚細胞腫瘍は，その特異な画像所見と臨床経過の特徴を銘記すべき腫瘍である．MRIにみられる初期像については多数の報告がある[4, 8, 11]．T1強調画像では一部わずかに増強される不整な淡いmixed signal intensityを示し，T2強調画像では境界のやや不鮮明なhigh signal intensity病変が主体で，基底核から大脳脚に至る萎縮像を認めることが特徴である（図5）．特徴的な症状は片麻痺である．

定位手術によって生検を行った後，放射線照射と化学療法による治療を行うことになるが，多くの場合症状の改善は軽度で，片麻痺が残存し，またMRI所見においても腫瘍像が消失することはまれで，図5に示したようにirregularな信号強度を示す病変が残存しているかのような所見を呈することが多い．

したがって，実際に治療を行っていると治療効果の判定に困ることになる．

自験4例の基底核胚細胞腫瘍患者において血液と髄液のHCG-βを測定したところ，血中HCG-βが高値を示した症例は1例のみであった．ところが，最近宮崎大学の片上らが開発した超高感度測定法を用いると，髄液中HCG-βは254～12,800pg/mL（中央値480pg/mL）と全例で高値を示した．この測定法は従来の測定法による値の1,000倍の感度を有し，測定感度は

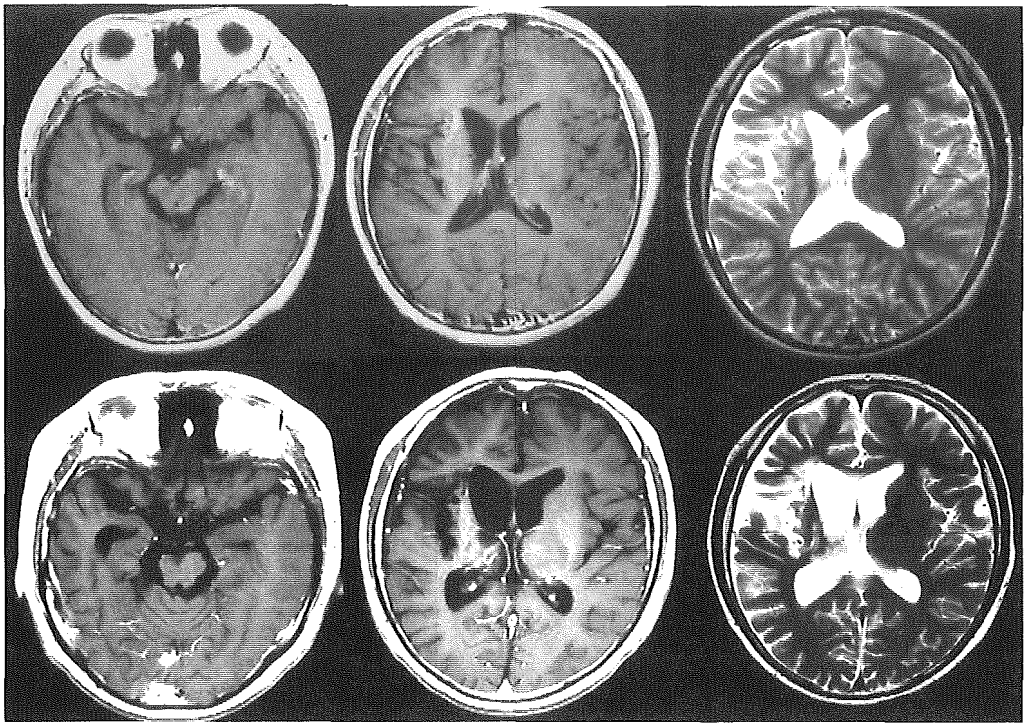

図5　基底核胚細胞腫瘍症例のMRI

上段は治療前，下段は全脳30Gyおよび局所20Gy，さらにcarboplatin＋etoposideによる化学療法を8コース行った後．上下段とも左と中央は造影T1強調画像，右はT2強調画像．治療後に脳委縮を認めるが，右基底核の腫瘍像は酷似している．

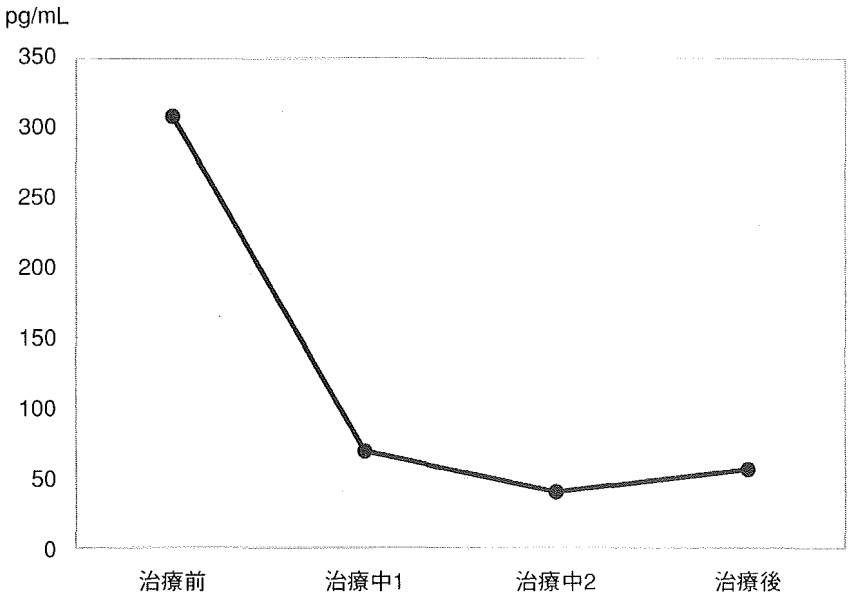

図6　図5の症例の髄液中HCG-βの推移

超高感度測定法による測定で，縦軸のHCG-β値の単位はpg/mLである．治療中1は全脳30Gyと局所20Gyの放射線照射および3コースの化学療法終了の時点，治療中2は6コースの化学療法終了時点，治療後は8コースの化学療法が終了した図5の下段の画像の時点である．

ng/mL単位ではなく0.1pg/mLである．正常値は血清と髄液ともに30pg/mL以下で，かつ髄液／血清比＜2.0と規定されている[3]．この測定法を用いてさらに治療経過を追跡すると，治療前に高値であったHCG-βが治療とともに低下していく様子が明らかであった（図6）．

また，画像上の腫瘍再発に先行して髄液中HCG-βの上昇を確認した症例も経験している．

Ⅵ．終わりに

予後不良群や，再発してプラチナ製剤に抵抗性になってしまったgerminoma，予後中間群の腫瘍に対する新しい化学療法の開発が必要である．また，特に予後不良群においては画像上の腫瘍を消失させることが治療成績の改善に繋がるという考え方から，放射線照射と化学療法後の残存腫瘍の積極的な摘出も提唱されている[5]．

現在厚生労働省研究班は休止中であるが，新たな全国的な臨床試験が計画されている．また，謎に包まれた本腫瘍の発生機序を解明するために網羅的な遺伝子解析も進行中である．頭蓋内胚細胞腫瘍はさらなる治療の発展が期待される分野であり，若い脳神経外科医の積極的な貢献が期待される．

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総説

中枢神経胚細胞腫瘍治療の基本方針

西川　亮

Some Principles for the Treatment of Central Nervous System Germ Cell Tumors

by

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Although the optimum treatment of intracranial germ cell tumors remains controversial, there are some basic underlying principles. 1) It is important to confirm the histological type of the tumors by biopsy or extirpation. 2) The appropriate extent of surgery for germinoma still remains to be discussed. The reliability of endoscopic biopsy should be compared with the results of the definite pathology after open biopsy or extirpation of the lesion. 3) In germinoma, it is not necessary to irradiate the whole spine. A reduced-volume radiotherapy such as whole-ventricular irradiation, plus chemotherapy should replace craniospinal radiotherapy to avoid the late sequelae of large-volume radiotherapy, such as impairment of endocrinological function or neurocognitive development. Focal irradiation would lead to an increased risk of recurrence. 4) Chemotherapy alone without irradiation is not sufficient to cure germinoma patients. 5) Neoadjuvant chemo-radiotherapy followed by complete excision of the residual tumors should be considered in patients with non-germinomatous germ cell tumors.

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Key words: germinoma, germ cell tumors, chemotherapy, radiotherapy

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はじめに

中枢神経胚細胞腫瘍には多彩な組織型が含まれているが，これを3群に分ける分類が提唱されている（Table 1）[11]．この分類は，多数の症例の生存期間解析を基にした日本独自の成果である．欧米では germinoma/non-germinomatous germ cell tumor，あるいは secreting/non-secreting germ cell tumor という2群への分類が一般的であるが，これらの分類ではきめ細かな治療法の選択ができない[12]．中枢神経胚細胞腫瘍の治療は，1980年以前は放射線照射が主体であったが，1980年代に化学療法が導入され治療成績が向上した．1990年代前半の治療成績としては，5年生存率が germinoma 95%，予後中間群約70%，予後不良群約20%あるいはそれ以下と報告されている[11]．したがって1990年代後半からの課題は，すでに放射線照射単独によってもおよそ90%の症例が治癒可能となっている germinoma においては，治癒率を維持しつつ，治療による障害，特に放射線照射による障害を軽減すること，また予後中間群と不良群においては生存期間のさらなる延長を図ることと総括された．この課題の達成を目的として，1995年から厚生労働省がん研究助成金による研究班が組織された．2005年の時点における成果は，5年生存率において germinoma 98%，予後中間群94%，予後不良群64%である（松谷雅生，unpublished

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