原著

全国統計からみた最近のわが国のグリオーマの治療成績

渋井壮一郎　野村和弘

Recent Results of Glioma Therapy in Japan based on the Data of Brain Tumor Registry of Japan

by

Soichiro Shibui, M.D., and Kazuhiro Nomura, M.D.

from

Neurosurgery Division, National Cancer Center Hospital

Report of Brain Tumor Registry of Japan (1969-1993) 10th Edition was issued as a supplement of Neurologia medico-chirurgica, Vol. 40 in January, 2000. More than 80,000 cases of brain tumors were registered by 281 neurosurgical institutes all over Japan. According to the report the frequency of registered cases of gliomas has been decreasing while that of the benign tumors such as meningiomas and pituitary adenomas has been increasing. The frequency of meningiomas was 26% of all the primary brain tumors and that of gliomas was 24%. The five-year survival rates of malignant astrocytomas and glioblastomas were only 23% and 6%, respectively, even for the cases after 1991. Histological diagnosis, mode of surgery, preoperative performance status and age have been considered to be the factors influencing the survival rates of gliomas.

The report demonstrated that the more tumors were removed, the longer survival rates were expected for any kind of gliomas. The elder patients over 70 years of age showed poor survival rates. Radiotherapy was beneficial to the longer survival of malignant gliomas but did not always contribute to that of low grade gliomas. These data have not resulted from the controlled studies but they represent the recent standard of neurosurgery in Japan.

Precise registration is essential in order to establish the reliability of the registry.

(Received October 3, 2001; accepted November 13, 2001)

Key words : brain tumor registry, glioma, survival rate, prognostic factor, treatment

Jpn J Neurosurg (Tokyo) 11 : 355-361, 2002

はじめに

1974年，わが国の脳腫瘍の頻度，治療成績などを調査する目的で脳腫瘍全国統計委員会が組織され，1977年には脳腫瘍全国集計調査報告第1巻が発行された．その後，発行を重ね，2000年1月には第10巻がNeurologia medico-chirurgicaのsupplementとして英文にて刊行された．第10巻には，国内の280余りの脳神経外科施設より年間5,000例前後の症例が登録され，1969～1993年までの8万例を超える症例について解析が行われている[3]．人口10万人に対し11～12人という原発性脳腫瘍の発生頻度からの単純計算によれば，わが国で発生する原発性脳腫瘍の40%程度が登録されていることになる[2]．

原発性脳腫瘍の登録症例数

1984～1993年までの登録症例の腫瘍別頻度としては，全体ではgliomaが28.3%で最も頻度が高いが，15～69歳の成人例に限ればmeningiomaが26.4%で最も多く，続いてglioma 25.1%，pituitary adenoma 20.2%となっている．また，15歳未満の小児例ではgliomaが58.5%を

国立がんセンター脳神経外科／〒104-0045 中央区築地5-1-1〔連絡先：渋井壮一郎〕

Address reprint requests to: Soichiro Shibui, M.D., Neurosurgery Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

Table 1 Frequency of primary brain tumors by age (1984-1993)

Tumor	All	Age		
		15-69	<15	70≦
Glioma	28.3%	25.1%	58.5%	28.0%
Meningioma	26.3	26.4	2.2	44.4
Neurinoma	10.8	12.3	1.5	6.9
Pituitary adenoma	17.4	20.2	1.4	9.0
Germinoma	2.1	1.6	9.8	0.0
Oraniopharyngioma	3.4	3.2	8.9	1.5
Dermoid, epidermoid	1.7	1.8	1.3	0.6
Hemangioma	1.8	2	0.4	1.0
Sarcoma	0.2	0.2	0.5	0.1
Malignant lymphoma	2.7	2.5	0.4	6.0
Others	5.3	4.7	14.8	2.5
Total	100% (n=38,273)	100% (n=30,803)	100% (n=3,198)	100% (n=4,272)

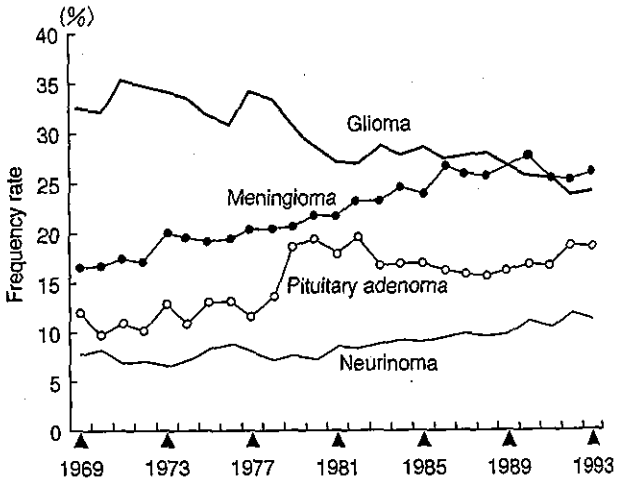


Fig. 1 Trend of frequency of primary brain tumors

Table 2 Frequency of gliomas (%)

Histological type	1969-1983	1984-1993
Glioblastoma	28.7%	31.9%
Astrocytoma	30.3	28.1
Malignant astrocytoma	15.6	17.6
Oligodendroglioma	5.9	3.8
Malignant oligodendroglioma	0.6	0.6
Ependymoma	4.8	3.0
Malignant ependymoma	1.5	1.0
Mixed *glioma*	1.0	1.8
Medulloblasotma	7.7	4.3
Others	3.9	7.9
Total	100% (n=9,925)	100% (n=10,834)

占めるが，70歳以上の高齢者では*meningioma*が44.4%で最多となっている（Table 1）．これを年代別にみると，1970年代には原発性脳腫瘍の30%以上をgliomaが占めていたが，徐々に減少傾向をたどり，1980年代には30%を切り，1992年には23.6%となった．これに対しmenigioma, pituitary adenoma, neurinomaなどの良性腫瘍の頻度は上昇し，1992年には25.1%となったmeningiomaがgliomaを上回り，原発性脳腫瘍のなかで最多となった（Fig. 1）．

また，グリオーマの組織別頻度を1969～1983年の症例と1984～1993年の症例で比較してみると，glioblastomaやmalignant（anaplastic）astrocytomaなどのhigh grade gliomaが増加傾向にあり，逆にastrocytomaやoligodendrogliomaなどのlow grade gliomaが減少傾向にある（Table 2）．これは脳腫瘍患者の高齢化とも関係が深く，高齢者ほどhigh grade gliomaが多いという事実を反映しているものとも考えられる[5]．

グリオーマの予後規定因子

1 病理診断

脳腫瘍全国統計では，Cultler-Ederer法により生存率の算出を行っている[9]．従来の相対生存率（累積生存率を生命表より算出した期待生存率で割ったもの）のみでなく，vol. 10では累積生存率が加えられて，各施設での成績との比較がしやすくなっている．

代表的な脳腫瘍の累積生存率をTable 3に示す．脳腫瘍は多数の病理学的診断により分類され，その診断により予後がほぼ規定されてしまう点，他の臓器がんと異なっている．そのなかでastrocytic tumorとして総括されるastrocytoma（grade 2), malignant astrocytoma (grade 3), glioblastoma（grade 4）の3者も病理診断がそのままgradeの違いとなっており，大きく予後を規定していることがわかる．そのほかにgliomaの予後に影響を与える因子として，従来より，年齢，治療前のperformance status，手術摘出度などがあげられている[1)4)6)]．

2 年 齢

Fig. 2およびTable 4に年齢別の*glioma*の5年生存率を示す．high grade, low gradeを問わず，乳児期の生存率は悪く，小児期で上昇するが，高齢になるにつれ徐々に下降し，特に70歳以上での低下が著しい．glioma全体についてはχ^2検定を行うと，55歳未満とそれ以上の間に有意差がみられ，以降65歳未満とそれ以上，75歳未満とそれ以上，85歳未満とそれ以上のそれぞれに有意

Table 3 Five-year cumulative survival rate of brain tumors (cases between 1981 and 1993)

	n	1y	2y	3y	4y	5y
Astrocytoma	788	88.0%	78.2%	72.9%	69.6%	66.2%
Malignant astrocytoma	534	70.4	44.8	33.7	27.0	22.7
Glioblastoma	982	54.2	20.0	11.6	8.6	6.3
Oligodendroglioma	76	91.9	90.5	89.1	84.0	81.7
Ependymoma	77	90.0	79.1	72.1	67.9	60.3
Medulloblastoma	103	89.5	75.5	64.4	57.8	57.8
Neurinoma	1,292	99.0	98.3	97.8	97.4	97.3
Meningioma	2,837	98.1	96.9	95.6	94.6	93.3
Germinoma	237	96.5	94.0	92.9	90.5	89.7
Pituitary adenoma	2,086	98.4	97.4	97.1	96.0	95.5
Craniopharyngioma	388	95.1	94.2	92.5	91.3	90.4
All glioma	2,790	73.1	52.0	44.5	40.4	37.3
Malignant glioma	1,703	62.7	33.4	24.3	19.9	17.0
Metastatic tumors	2,280	42.1	24.6	17.5	14.8	12.9

n : number of cases y : year(s)

Table 4 Cumulative survival rate of all glioma by age (1981-1993)

Age	n	1y	2y	3y	4y	5y
0-4	687	80.7%	67.1%	60.5%	55.9%	54.5%
5-9	555	85.1	72.1	67.6	65.3	61.7
10-14	458	85.8	74.0	68.7	63.9	61.9
15-24	427	90.8	76.5	70.8	65.1	60.4
25-34	521	90.1	77.3	69.0	63.7	60.2
35-44	660	89.8	73.8	63.5	57.2	52.2
45-54	830	89.1	73.9	65.4	60.1	53.2
55-64	834	83.1	65.0	56.4	49.8	45.1
65-74	814	78.5	56.3	48.3	42.4	38.4
75-84	925	71.6	45.3	35.1	31.4	28.4
85-94	922	64.8	35.4	26.1	20.7	17.5

n : number of cases y : year(s)

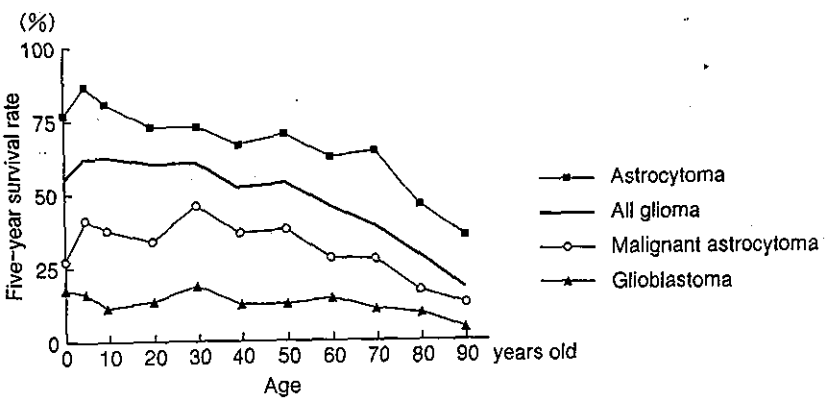


Fig. 2 Five-year culmulative survival rates (CSR) of gliomas by age

差があり，高齢者では10歳進むごとに有意差をもって生存率の下降がみられる．

また，glioblastomaでは75歳以上と65歳未満の間に有意差があり，malignant astrocytomaおよびastrocytomaでは75歳以上とそれ未満との間に有意差がみられた．

以上より，若年発症に比べ，高齢者のグリオーマは予後は不良であるといえる．

3 臨床悪性度

通常，臨床症状の指標としてKarnofsky scaleが用いら

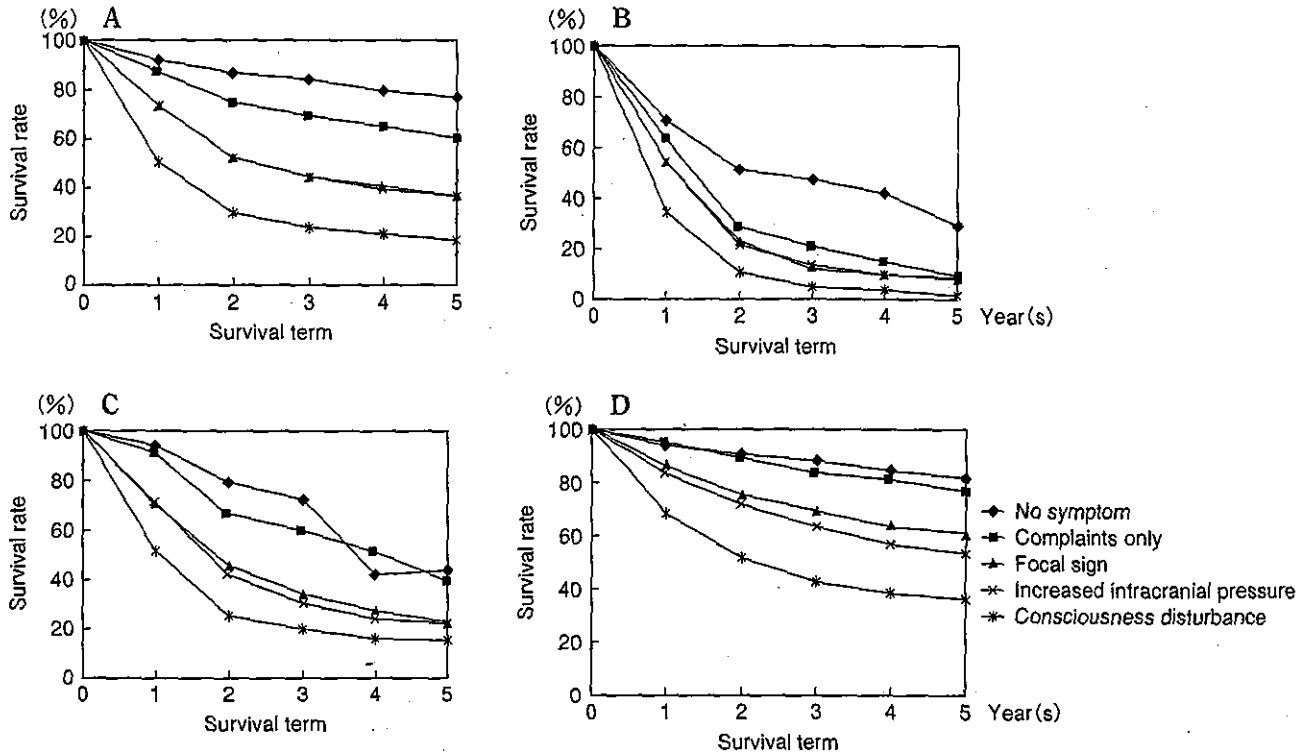


Fig. 3 Cumulative survival rate by clinical grading
A: All glioma
B: Glioblastoma
C: Malignant astrocyotma
D: Astrocytoma

れることが多いが，脳腫瘍全国統計では臨床悪性度を無症状，自覚症状のみ，巣症状，頭蓋内圧亢進，意識障害，昏睡，呼吸中枢障害の7段階に分けている．呼吸障害例および昏睡例はいずれも1%以下の症例であるため，これらを除いたgliomaの生存曲線をFig. 3に示す．glioma全体では巣症状と頭蓋内圧亢進とがほとんど一致した生存率を示すほかは，上記臨床症状の進行とともに有意差をもって生存率の低下がみられる（Fig. 3A）．

glioblastomaではほぼ同様に巣症状と頭蓋内圧亢進との間のみ有意差が認められなかったが，malignant astrocytoma，astrocytomaと分化型になるにつれ，無症状と自覚症状との差が狭まり有意差が認められなくなっている（Fig. 3B～D）．悪性gliomaほど早期発見，早期治療開始の重要性を示すものと考えられる．

4 手術摘出率（残存腫瘍量）

手術的に腫瘍を除去することにより，確実に腫瘍細胞は減少し，それにより予後が改善されることが期待できる．悪性gliomaの場合，何をもって全摘とすべきかは論議の分れるところであるが，通常はCT，MRI上で造影される箇所を摘出できたもの，造影されない場合はMRIのT1強調画像で低信号域を摘出できたものを全摘とよぶことが多い．

病理学的には造影箇所より数cm先まで腫瘍の浸潤があるといわれ，組織レベルでの全摘は不可能であるにせよ，可及的な切除により頭蓋内圧亢進症状や神経症状は軽減され，performance status（PS）の改善が期待できる．さらに残存腫瘍が少ないことは，術後の放射線治療や化学療法がより効果的に行われることになり，このことも予後の改善につながっている．

glioblastoma, malignant astrocytoma, astrocytomaのおのおのについて，手術摘出率と生存率の関係をFig. 4A～Cに示す．glioblastomaでは，全体的に予後不良であるために各群の差が小さいが，50%以下の摘出と75%摘出の間に有意差があり，さらに75%と95%，95%と100%の間にも有意差が認められ，75%以上の摘出であれば，摘出率が増すに従い生存率の上昇がみられる．それに対しmalignant astrocytomaおよびastrocytomaでは，50%摘出と75%摘出の間には有意差がなく，95%以上の摘出ではじめて有意差が出現する．さらに

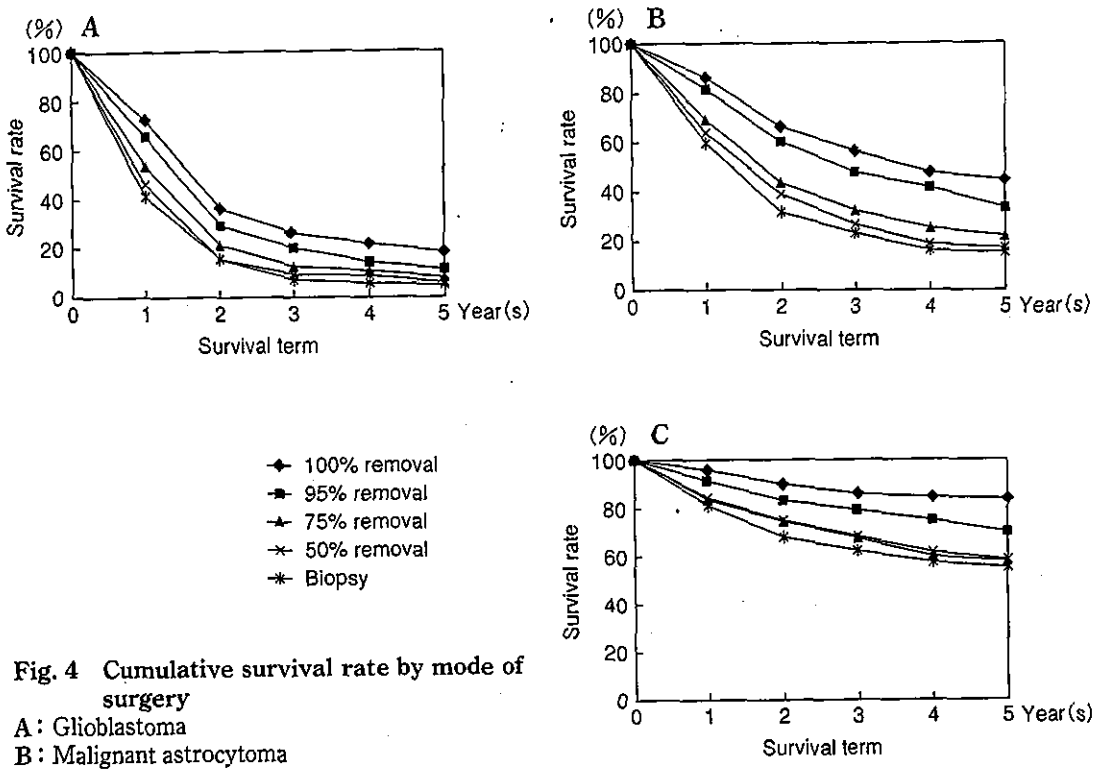


Fig. 4 Cumulative survival rate by mode of surgery
A: Glioblastoma
B: Malignant astrocytoma
C: Astrocytoma

malignant astrocytomaでは，95％摘出と100％摘出の間に有意差が認められないが，astrocytomaでは有意差があり，low gradeであればあるだけ，手術が予後に及ぼす影響が強まり，確実な手術的摘出の重要性を示すものと考えられる．

5 放射線治療

悪性gliomaに対する放射線治療が有効であることは広く認められている[10]．脳腫瘍全国統計においてもglioblastomaでは，生検，部分摘出（50～75％摘出），亜全摘のいずれも手術のみの群に対し，それぞれに放射線治療を加えたものと比較すると照射群の生存率が有意に高くなっている（Fig. 5A）．しかしながら，この疾患については通常放射線照射が行われており，されていない症例では術後のPSの悪さで照射ができなかったものも多く含まれている可能性があり，必ずしもこれだけの有意差は存在しないことも考えられる．

これに対しmalignant astrocytomaでは，唯一biopsy群で放射線照射による有意な予後の改善がみられるものの，部分摘出や亜全摘では必ずしも放射線照射群の生存率が高いとはいえない（Fig. 5B）．low grade astrocytomaでは，生検および部分摘出群において放射線による予後の改善が若干認められるが，有意な差ではなく，亜全摘群ではむしろ照射群の生存率が悪くなっている（Fig. 5C）．しかしながら同じ亜全摘群でもMRIのT2強調画像で広範な高信号領域を示すような浸潤性の高い症例には積極的に放射線を行い，より限局性で確実に摘出された可能性のある例には放射線照射が行われていない可能性もあるため，この結果からlow grade astrocytomaには手術的な摘出で十分であると判断することはできない．ただし照射なしで長期生存のあり得る疾患であることも事実であり，特に長期生存例での放射線による後遺症の問題もあるため，照射についてはさらに検討する必要があると考えられる．

6 化学療法

化学療法の効果についてはまだ明らかでなく，唯一glioblastoma例でACNUを使用した群で有意な生存率の上昇が示されている．照射との併用により奏効率が高まることはいわれているが[8]，維持療法の効果はまだ不明で，現在進行中の厚生労働省がん研究助成金野村班の研究成果を待ちたい．

各種gliomaの生存率の推移

悪性gliomaに対して各腫の治療法が工夫され，その

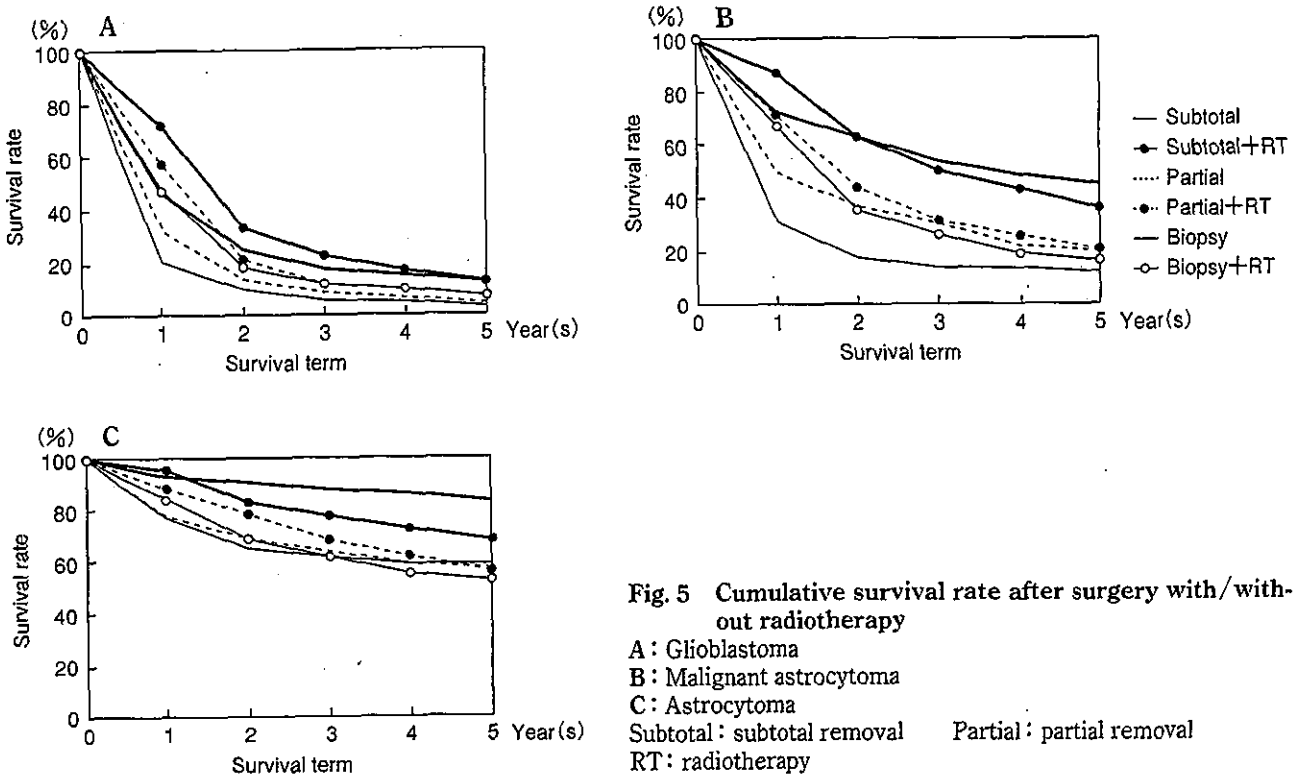


Fig. 5 Cumulative survival rate after surgery with/without radiotherapy
A: Glioblastoma
B: Malignant astrocytoma
C: Astrocytoma
Subtotal: subtotal removal Partial: partial removal
RT: radiotherapy

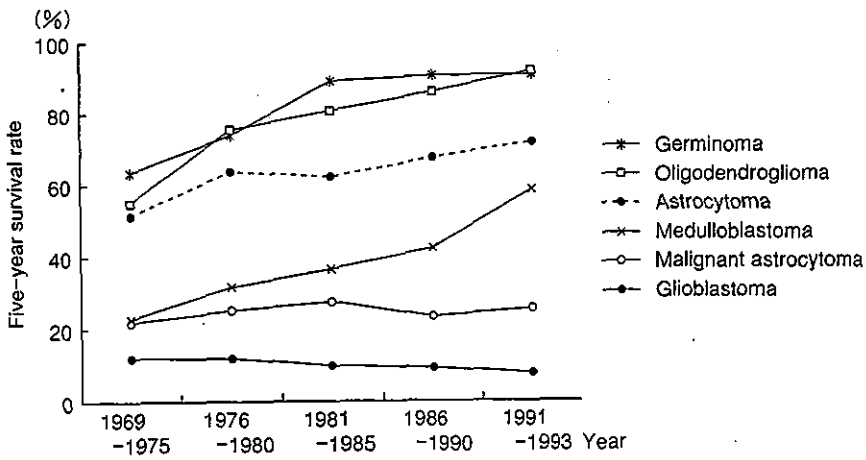


Fig. 6 Trend of 5-year cumulative survival rates of various gliomas

生存率は徐々に上昇しつつある．Fig. 6に年代ごとの各種悪性脳腫瘍の5年生存率の推移を示したが，特に生存率の上昇が目立つのはgerminomaおよびmedulloblastomaである．

germinomaについては，播種性病変が存在しても放射線治療が有効であり，大半の症例を治癒にもっていくことが可能である．また，プラチナ系化学療法剤が奏効することから，最近ではさらに治療後のPSも考慮し，化学療法を優先して放射線照射量を減らす試みもなされている[7]．

medulloblastomaについては手術的な摘出と術後の全脳・全脊髄に対する放射線治療を行い得た多くの症例で，再発を防ぐことができるようになり，20年間に5年生存率は20%から60%にまで上昇している．

oligodendrogliomaやastrocytomaなどについても年代とともに生存率は上昇している．前述のようにこれらのlow grade gliomaについては手術的な摘出度が生存率に大きく影響しているため，近年の診断技術，さらに術中

モニタリングやナビゲーションの進歩により，早期に発見された腫瘍が確実に摘出されることが多くなったことが大きく関与しているものと考えられる．

現在なお治療成績のよくない悪性gliomaについても，可及的な摘出，効果的な放射線治療，感受性のある化学療法剤の使用により生存率の向上が期待できるが，現段階ではいずれも十分とはいえず，さらに効果的な新しい治療法の開発が望まれる．

おわりに

脳腫瘍全国統計は，国内の多くの脳神経外科施設の協力により，8万例を超える症例が蓄積され，世界でも有数の脳腫瘍統計となっている．ここに示されたデータは国内の脳神経外科の水準を示すものであり，新しい治療の開発に当たっては，この成績を凌駕することが1つの条件ともなり得る．

このような統計の解析に当たっては，一つひとつのデータが正確であることが前提条件であることはいうまでもない．今後も各施設から正確で詳細な報告が集積されていくことを期待したい．

本稿の一部は，第21回日本脳神経外科コングレス（2001，山形）プレナリーセッションにて発表した．

文　献

1) Burger PC, Green SB: Patient age, histologic features, and the length of survival in patients with glioblastoma multiforme. *Cancer* 59: 1617-1625, 1987.
2) Central Brain Tumor Registry of the United States: CBTRUS (2000). Statistical report: Primary brain tumors in the United States, 1992-1997.
3) Committee of Brain Tumor Registry of Japan: Report of brain tumor registry of Japan (1969-1993) 10th ed. *Neurol Med-Chir* 40 (Suppl), 2000.
4) Curran WJ Jr, Scott CB, Horton J, Nelson JS, Weinstein AS, Fischbach AJ, Chang CH, Rotman M, Asbell SO, Krisch RE Nelson DF: Recursive partitioning analysis of prognostic factors in three radiation therapy oncology group malignant glioma trials. *J Natl Cancer Inst* 85: 704-710, 1993.
5) Legler JM, Gloeckler Ries LA, Smith MA, Warren JL, Heineman EF, Kaplan RS, Linet MS: Brain and other central nervous system cancers: Recent trends in incidence and mortality. *J Natl Cancer Inst* 91: 1382-1389, 1999.
6) Levin VA, Wara WM, Davis RL, Vestnys P, Resser KJ, Yatsko K, Nutik S, Gutin PH, Wilson CB: Phase III comparison of BCNU and the combination of procarbazine, CCNU, and vincristine administered after radiotherapy with hydroxyurea for malignant gliomas. *J Neurosurg* 63: 218-223, 1985.
7) Matsutani M, Ushio Y, Abe H, Yamashita J, Shibui S, Fujimaki T, Takakura K, Nomura K, Tanaka R, Fukui M, Yoshimoto T, Hayakawa T, Nagashima T, Kurisu K, Kayama T, the Japanses Pediatric Brain Tumor Study Group: Combined chemotherapy and radiation therapy for central nervous system germ cell tumors: Preliminary results of a phase II study of the Japanese Pediatric Brain Tumor Study Group. *Neurosurg Focus* 5: 1-5, 1998.
8) Takakura K, Abe H, Tanaka R, Kitamura K, Miwa T, Takeuchi K, Yamamoto S, Kageyama N, Handa H, Mogami H, Nishimoto A, Uozumi T, Matsutani M, Nomura K: Effects of ACNU and radiotherapy on malignant glioma. *J Neurosurg* 64: 53-57, 1986.
9) 富永祐民：治療効果判定のための実用統計学．生命表法の解説．東京，蟹書房，1983.
10) Walker MD, Strike TA, Sheline GE: An analysis of dose-effect relationship in the radiotherapy of malignant gliomas. *Int J Radiat Oncol Biol Phys* 5: 1725-1731, 1979.

要　旨

全国統計からみた最近のわが国のグリオーマの治療成績

渋井壮一郎　野村　和弘

2000年1月に発行された脳腫瘍全国集計調査報告第10巻よりグリオーマの治療成績を検討した．近年の脳腫瘍発生頻度の動向としてグリオーマが減少し，髄膜腫や神経鞘腫の頻度が高くなる傾向がある．

グリオーマの予後を規定する因子として組織診断，手術摘出率，術前のperformance status，年齢などがあげられているが，本統計においても，手術摘出率が高い症例，臨床悪性度の低い症例，70歳未満の症例で予後が有意に良好であった．悪性グリオーマに対し放射線治療は有効であるが，星細胞腫では必ずしも有効といえず，さらに検討を要するものと考えられる．

脳外誌　11：355-361，2002

Oncogene (2002) 21, 3961–3968

www.nature.com/onc

Distinction in gene expression profiles of oligodendrogliomas with and without allelic loss of 1p

Akitake Mukasa[1,2], Keisuke Ueki*[,2], Shinichi Matsumoto[1], Shuichi Tsutsumi[1], Ryo Nishikawa[3], Takamitsu Fujimaki[4], Akio Asai[2,5], Takaaki Kirino[2,5] and Hiroyuki Aburatani*[,1]

[1]*Genome Science Division, Research Center for Advanced Science and Technology, University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan;* [2]*Department of Neurosurgery, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan;* [3]*Department of Neurosurgery, Saitama Medical School, 38-2 Morohongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan;* [4]*Department of Neurosurgery, School of Medicine, Teikyo University, 2-11-2 Kaga, Itabashi-ku, Tokyo 173-8606, Japan;* [5]*CREST (Core Research for Evolutional Science and Technology), Japan Science and Technology Corporation, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan*

Oligodendrogliomas frequently, but not always show sensitivity to chemotherapy and recent studies demonstrated that allelic loss of chromosome 1p is highly associated with this chemosensitivity. To gain insight into the molecular mechanism of such difference, we examined comprehensive gene expression profiles of 11 oligodendroglial tumors, six with and five without 1pLOH (loss of heterozygosity), and two normal brain tissues using the oligonucleotide microarray (GeneChip). Statistically significant numbers of genes were expressed differentially between the two genetic subsets. Clustering analysis separated the tumor subsets well. The tumors with 1pLOH had similar expression profiles to the normal brain for those differentially expressed genes. Many genes showing higher expression in tumors with 1pLOH were presumed to have functions in nervous tissues. Notably, the majority of the 123 genes showing significant expression reduction in tumors with 1pLOH were either on chromosome 1 (50%) or on 19 (10%), and the average expression reduction ratio was about 50% (0.54 ± 0.13) possibly reflecting the chromosomal deletion. Thus, the biological difference between the genetic subsets of oligodendroglioma was indeed reflected to gene expression profile, which provided baseline information for further studies to elucidate the mechanism of chemosensitivity in gliomas.
Oncogene (2002) **21**, 3961–3968. doi:10.1038/sj.onc.1205495

Keywords: oligodendroglioma; oligonucleotide microarray; loss of heterozygosity

Introduction

Oligodendrogliomas are a major type of gliomas which constitute approximately 5% of all primary brain tumors or 10 to 25% of all intracranial gliomas (Kleihues and Cavenee, 2000). One of the important recent findings in neuro-oncology was that those oligodendrogliomas frequently showed remarkable sensitivity to chemotherapy, especially to a regimen using procarbazine, CCNU and vincristine (PCV therapy) (Cairncross and Macdonald, 1988). However, the response rate to PCV therapy remains 60–80%, and 20–30% of tumors are resistant to chemotherapy and have worse prognosis. Therefore, within this histologically indistinguishable entity, there apparently exist subgroups showing different biological behavior. Recent molecular genetic studies on oligodendrogliomas revealed that allelic loss of chromosome 1p, which is found in 60–80% of oligodendrogliomas and often accompanied with allelic loss of 19q (Smith *et al.*, 1999), was highly associated with the treatment responsiveness and also with a better prognosis (Cairncross *et al.*, 1998; Ino *et al.*, 2001). Thus, it is now being recognized that loss of chromosome 1p is a marker separating oligodendrogliomas into subgroups showing different biological behavior. In addition to its important clinical implications, understanding of the underlying molecular mechanisms of such a difference may lead to a new treatment strategy for all gliomas. Unfortunately, the putative tumor suppressor genes at chromosomes 1p and 19q, obvious keys to investigate the molecular biologic features of the tumor cells, are yet to be identified despite vigorous investigations. Several attractive candidates on chromosome 1p include *TP73* (1p36.3) and *CDKN2C* (1p32), but neither has been shown to be altered in the majority of oligodendrogliomas (Husemann *et al.*, 1999; Mai *et al.*, 1998). Although 1p loss is also found in many other neoplasms including neuroblastomas, the search for the suppressor gene in such neoplasms has not been successful either (Ohira *et al.*, 2000; Schwab *et al.*, 1996). To gain insight into the molecular basis of the biological difference among oligodendrogliomas, we turned to recently developed oligonucleotide microarray technology. By analysing comprehensive gene expressions, several studies have now shown that the expression profiles correlated well with the histology

*Correspondence: K Ueki or H Aburatani;
E-mail: kueki-tky@umin.ac.jp or E-mail: haburata-tky@umin.ac.jp
Received 18 July 2001; revised 28 February 2002; accepted 18 March 2002

and clinical grades in human neoplasms including gliomas (Golub *et al.*, 1999; Huang *et al.*, 2000; Watson *et al.*, 2001). Therefore, we performed a comparative study of the gene expression profiles between the genetic subgroups of oligodendrogliomas based on the 1p status.

Results

Genetic alterations in oligodendroglial tumor samples

Of 40 oligodendroglial tumors we could collect, we selected six cases with 1pLOH (loss of heterozygosity) and five cases without 1pLOH from which we could obtain good quality RNA evaluable with the GeneChip system (Affymetrix, Santa Clara, CA, USA). Histological diagnoses and the results of molecular genetic analysis are summarized in Table 1. There were seven oligodendrogliomas, one oligoastrocytoma and three anaplastic oligodendrogliomas. In all six tumors with 1pLOH, all of the informative 1p markers showed LOH, indicating that deletion involved the whole arm of chromosome 1p (data not shown). Of the six cases with 1pLOH, five cases also had 19qLOH and one case was non-informative on examined 19q markers. None of the six tumors with 1pLOH had *TP53* mutation, and three of the five tumors without 1pLOH had *TP53* mutation. No case had 10qLOH.

The statistical analysis of genes differentially expressed by 1p status

To select genes that were expressed differentially by 1p status, we used prediction value (*P*-value) in neighborhood analysis, which was recently described as useful for extracting genes expressed uniformly high in one group and low in the other (Golub *et al.*, 1999). We listed a total of 209 genes that had an absolute *P*-value of more than one, of which 86 genes showed higher expression and 123 genes showed lower expression in tumors with 1pLOH. These numbers of the genes were significantly higher than expected in random grouping tested by 1000 times permutation test ($P<0.01$), indicating that these two subgroups indeed have significantly different gene expression profiles. When Mann–Whitney test with cut-off *P*-values of 0.05 and 0.01 were used, 288 and 123 genes were detected as differentially expressed by the 1p status, and those numbers were higher than the expected numbers in permutation test which were 115 and 33 in median, respectively. Of the 209 genes selected by prediction value, more than 90% (192 genes) were also included in the 288 genes selected by a *P*-value of 0.05 by Mann–Whitney test, indicating the consistency of those two methods in selecting differentially expressed genes. We used the 209 genes for further analysis.

Clustering analysis was performed to classify all 13 samples using Pearson correlation with these extracted 209 genes (Figure 1). The tumor subsets were separated well and the normal brain samples were clustered into the same group with the tumors with 1pLOH. Among the five tumors without 1pLOH, expression profiles were not markedly different between the tumors with and without *TP53* mutation in this clustering analysis.

Genes showing higher expression in tumors with 1pLOH

Of the 86 genes selected by *P*-value, 24 genes whose mean average difference had more than threefold difference between the two groups were listed in Table 2. The average differences of those genes in normal brain RNA were close to those in tumors with 1pLOH as expected by the clustering analysis. Based on the UniGene on National Center for Biotechnology Information (NCBI), 14 of the 24 genes were predominantly expressed in brain or neural tissues (*KIAA0985*, *RGS7*, human clone 23695, *INA*, *KIAA0750*, *MYT1L*, human clone 23560, *PTPRN*, *SLC1A2*, *HAPIP*, *SNCB*, *SNAP25*, *L1CAM* and *OLFM1*), and were likely to have some function in the nervous system. In the normal brain samples, genes that are predominantly expressed in glial cells such as glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) were also well expressed, indicating that these samples contained many glial cells.

Table 1 Summary of oligodendroglial tumors used in GeneChip experiments

Tumor no.	*Gender*	*Age*	*Histology*	*1pLOH*	*19qLOH*	*10qLOH*	*TP53*	*CDKN2A*
1	F	47	AOG	(+)	(+)	(−)	Wt	HD
2	F	23	OG	(+)	(+)	(−)	Wt	Normal
3	M	44	OG	(+)	(+)	(−)	Wt	Normal
4	M	49	OG	(+)	(+)	(−)	Wt	HD
5	F	22	OA	(+)	NI	(−)	Wt	Normal
6	F	48	AOG	(+)	(+)	(−)	Wt	Normal
7	M	44	OG	(−)	(−)	(−)	Mutation	Normal
8	F	60	OG	(−)	(−)	(−)	Mutation	Normal
9	F	25	OG	(−)	(−)	(−)	Mutation	Normal
10	M	67	AOG	(−)	(−)	(−)	Wt	HD
11	F	27	OG	(−)	(−)	(−)	Wt	Normal

OG: oligodendroglioma, OA: oligoastrocytoma, AOG: anaplastic oligodendroglioma, LOH: loss of heterozygosity, NI: non-informative, Wt: wild type, HD: homozygous deletion

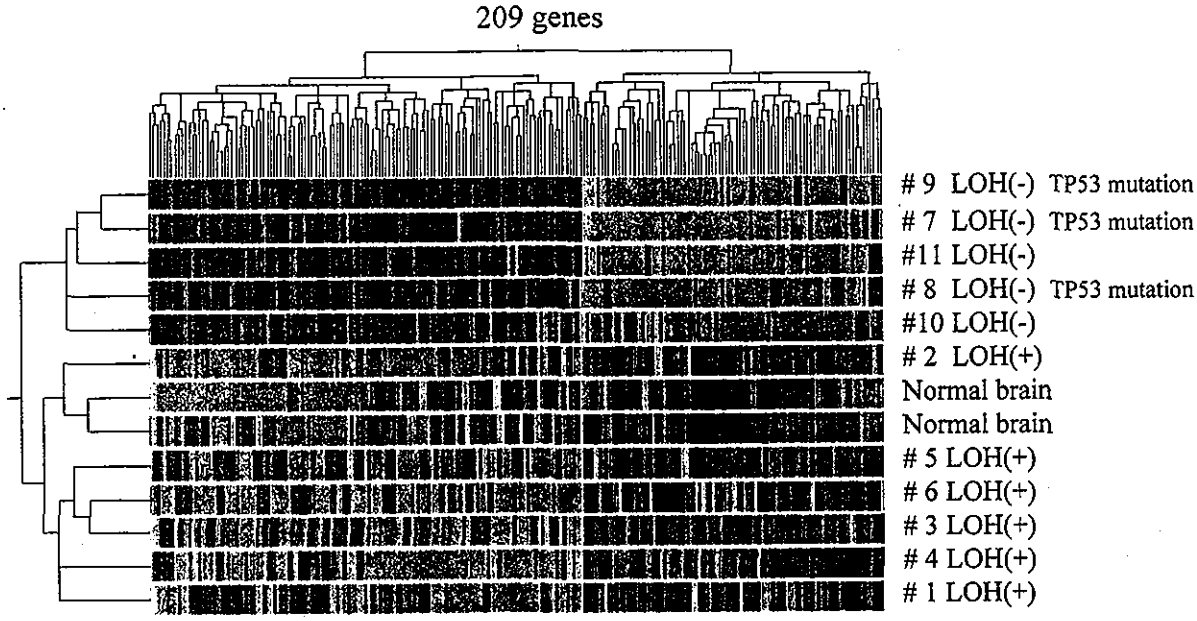


Figure 1 Hierarchical clustering of 11 oligodendroglial tumors and two normal brain samples using the 209 genes selected by *P*-value. Each column represents a gene and each row represents a sample. Red indicates increased expression, and blue indicates decreased gene expression. Expression of each gene is normalized to its median in this figure. The dendrogram indicates the degree of similarity between their expression profiles. Normal brain samples were clustered into the same group with the tumors with 1pLOH. LOH: loss of heterozygosity

Table 2 List of genes showing higher expression in tumors with 1pLOH

P-value	*Accession number*	*Gene*	*Symbol*	*Expression levels* 1pLOH(+)	1pLOH(−)	*Normal brain*	*Chromosome*
3.02	L12350	Thrombospondin 2	THBS2	167±36	46±9	117±26	6
2.1	AB023202	KIAA0985 protein	KIAA0985	213±77	65±13	519±137	12
2.1	U32439	Regulator of G-protein signaling 7	RGS7	133±32	43±15	320±2	1q
1.86	U79289	Human clone 23695		72±35	22±2	188±11	1q
1.83	S78296	Internexin neuronal intermediate filament protein α	INA	622±182	177±54	825±97	10
1.57	AB020639	Estrogen-related receptor γ	ESRRG	66±21	18±12	68±4	1q
1.51	AB018293	KIAA0750 gene product	KIAA0750	67±59	6±3	630±54	11
1.42	AB029029	*Myelin transcription factor 1-like	MYT1L	125±70	9±8	303±35	2
1.4	U79242	Human clone 23560		97±33	17±13	127±21	11
1.4	L18983	*Protein tyrosine phosphatase, receptor type N	PTPRN	99±81	2±0	628±76	2
1.31	L39833	Potassium voltage-gated channel, shaker-related subfamily	KCNAB1	81±36	23±10	205±29	3
1.25	U01824	Solute carrier family 1, member 2	SLC1A2	52±33	8±7	15±7	11
1.2	M25756	*Secretogranin II (Chromogranin C)	SCG2	269±180	62±35	354±16	2
1.18	U94190	Huntingtin-associated protein interacting protein	HAPIP	42±55	2±0	319±95	3
1.18	U78575	Phosphatidylinositol-4-phosphate 5-kinase, type Iα	PIP5K1A	110±41	34±20	92±6	1q
1.08	AA021140	cDNA clone IMAGE 363856		50±46	3±1	85±15	2
1.08	X96381	Ets variant gene 5	ETV5	280±151	77±58	224±71	3
1.07	AF053136	*Synuclein β	SNCB	145±147	6±5	741±174	5
1.07	D21267	Synaptosomal-associated protein, 25 kD	SNAP25	553±301	169±117	2167±135	20
1.04	K03000	*Aldehyde dehydrogenase 1 family, member A1	ALDH1A1	118±57	16±11	196±86	9
1.02	U52112	*L1 cell adhesion molecule	L1CAM	479±273	56±63	498±58	X
1.02	U71364	Serine proteinase inhibitor, clade B, member 9	SERPINB9	58±31	18±21	59±12	6
1.01	U72936	Alpha thalassemia/mental retardation syndrome X-linked	ATRX	166±82	55±65	131±49	X
1.01	D82343	Olfactomedin 1	OLFM1	762±599	236±56	2109±128	9

P-value: prediction value, which reflects the difference between two groups (the details are described in Materials and methods). Expression level of each gene was demonstrated as a mean value and a s.d. of average differences in each subgroup. Of the 86 genes selected by P-value, 24 genes whose mean average difference had more than threefold difference between the tumors with and without 1pLOH are listed. The genes examined by semi-quantitative RT–PCR are indicated by *

Genes showing lower expression in tumors with 1pLOH and their chromosomal location

Of the 123 genes selected by *P*-value, 61 genes (50%) were mapped to chromosome 1 (58 to 1p, 1 to 1q, and 2 to 1p or 1q) and 12 genes (10%) were mapped to chromosome 19 (11 to 19q, and 1 to 19p), while 50 genes (41%) were mapped to other chromosomes. When we focused on top 30 genes that had an absolute

P-value of more than 1.5, 83% (25 genes) were mapped to chromosome 1 or 19. Relative expressions of the 73 genes, 61 on chromosome 1 and 12 on chromosome 19, in tumors with 1pLOH compared to tumors without 1pLOH were 0.54±0.13 in average. Of the 123 genes, 16 genes whose mean average difference had more than threefold difference between the two groups were listed in Table 3 (whole list of the selected genes would be available on request).

The validation using semi-quantitative RT–PCR

Of the 24 higher expressed and 16 lower expressed genes in 1pLOH tumors, semi-quantitative RT–PCR was performed on nine known genes whose differences were more than fourfold and also more than 40 in mean average difference between the two groups (indicated by * in Tables 2 and 3). The results of RT–PCR corresponded well to the GeneChip data (Figure 2). The additional tumor samples showed similar expression pattern to the same 1p status cases examined by GeneChip, although there were two cases (case 18 and 19) which showed exceptional expression pattern. Those two cases neither had allelic loss on 1p/19q nor had *TP53* mutation. The case 19 was rather similar to the 1pLOH tumors. Case 18 showed lower expression in some of the genes that had higher expression in other tumors without 1pLOH.

Expression of genes on chromosome 1p

The relative expressions of the genes on chromosome 1p ($n=158$) in tumors with 1pLOH against tumors without 1pLOH were arranged on the chromosome map to see their relationship with chromosomal loci (Figure 3). Genes showing lower expression in tumors with 1pLOH were distributed over the whole chromosome 1p arm. There also were many genes whose expressions were not decreased in 1pLOH tumors, which were also found in various chromosomal loci.

Discussion

Using the oligonucleotide microarray technology, we could identify genes that were differentially expressed between the subgroups of oligodendroglioma by the 1p status. Results of semi-quantitative RT–PCR performed on some of the identified genes were concordant with the chip analysis data, confirming the fidelity of the system in general. Additional oligodendrogliomas studied by RT–PCR showed similar expression pattern to the GeneChip cases according to their 1p status. Of the five tumors without 1pLOH and without *TP53* mutation, however, one additional case was rather similar to the tumors with 1pLOH and another additional case also showed some inconsistency. Such variations of gene expression pattern suggest heterogeneity in tumors without 1pLOH and that more than two subgroups may exist in oligodendroglial tumors, as reported recently (Ino *et al.*, 2001). The numbers of cases analysed in our study were still limited, and a larger-scale study would enable detailed classification of oligodendroglial tumors based on gene expression profiles. Nonetheless, our data clearly showed that oligodendrogliomas of different genetic subsets indeed had distinct gene expression pattern, and could identify many differentially expressed genes.

Five of 10 cases without 1pLOH had *TP53* mutation, and the expression patterns of the genes examined by RT–PCR were not significantly different between tumors with and without *TP53* mutation. There was no apparent difference in the expression pattern of the 209 genes among five tumors without

Table 3 List of genes showing lower expression in tumors with 1pLOH

P-value	*Accession number*	*Gene*	*Symbol*	*Expression levels* 1pLOH(+)	1pLOH(−)	*Normal brain*	*Chromosome*
−1.96	J04177	*Collagen type IX α 1	COL11A1	13±16	230±111	22±7	1p21
−1.94	M97388	Down-regulator of transcription 1	DR1	23±10	82±18	31±5	1p22
−1.87	AB029000	KIAA1077 protein	KIAA1077	2±0	50±39	2±0	8
−1.50	D49493	Growth differentiation factor 10	GDF10	2±0	37±49	2±0	10
−1.49	AB011173	KIAA0601 protein	KIAA0601	47±38	225±79	25±23	1p
−1.48	X74262	*Retinoblastoma-binding protein 4 (RbAp48)	RBBP4	19±17	109±51	49±1	1p
−1.31	AL109671	cDNA clone EUROIMAGE 29222		19±25	103±60	80±6	15q
−1.25	AI806222	Arachidonate 5-lipoxygenase-activating protein	ALOX5AP	7±12	42±34	15±13	13
−1.17	AB028967	*Potassium voltage-gated channel, Shal-related subfamily	KCND2	35±20	165±68	33±9	7q
−1.12	M59830	Heat shock 70 kD protein 1B	HSPA1B	25±22	90±35	119±60	6p
−1.12	AF056490	Phosphodiesterase 8A	PDE8A	103±38	318±133	217±99	15
−1.11	J04111	v-jun avian sarcoma virus 17 oncogene homolog	JUN	62±50	226±116	80±8	1p32-p31
−1.09	S78203	Solute carrier family 15, member 2	SLC15A2	2±0	31±42	2±0	3
−1.08	AF104922	Growth differentiation factor 8	GDF8	6±4	43±37	2±0	2q
−1.06	D11151	Endothelin receptor type A	EDNRA	27±22	94±45	11±2	4
−1.05	U80055	Cystein dioxygenase type I	CDO1	28±24	97±37	2±0	5q

P-value: prediction value, which reflects the difference between two groups (the details are described in Materials and methods). Expression level of each gene was demonstrated as a mean value and a s.d. of average differences in each subgroup. Of the 123 genes selected by P-value, 16 genes whose mean average difference had more than threefold difference between the tumors with and without 1pLOH are listed. The genes examined by semi-quantitative RT–PCR are indicated by *

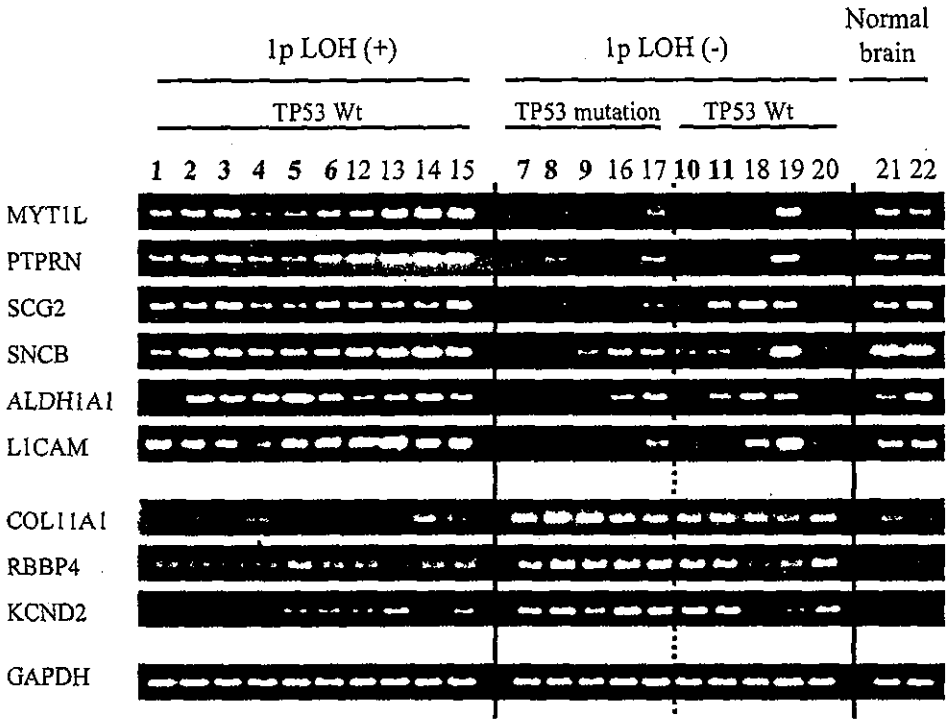


Figure 2 Results of semi-quantitative RT–PCR. Eleven tumors (#1–11) used in the GeneChip experiments (printed in boldface), nine additional tumors (#12–20), and two normal brain tissues (#21, 22) were analysed. The upper six genes and the middle three genes showed higher and lower expression in 1pLOH tumors in the GeneChip experiment, respectively. GAPDH was used as a control

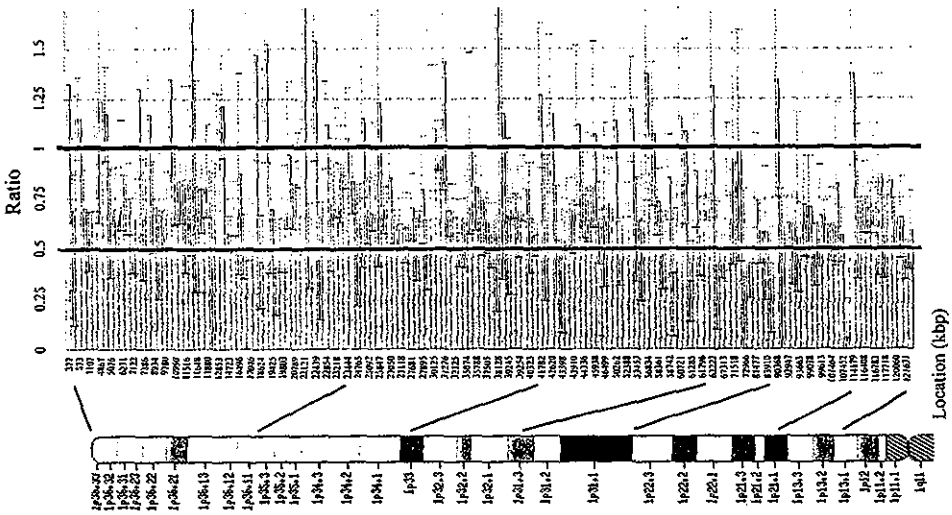


Figure 3 The relative expressions of the genes on chromosome 1p. Horizontal-axis represents the location of the examined 158 genes demonstrated from telomere (left) to centromere (right). Vertical-axis represents relative expressions with error bar, dividing the mean expressions (average difference) of tumors with 1pLOH by those of tumors without 1pLOH. The value beyond 1.7 is not shown in this figure

1pLOH examined by GeneChip, regardless of the *TP53* mutation status (data not shown). Therefore, the listed genes were most likely extracted by the status of 1pLOH than that of *TP53*.

Of the genes showing higher expression in tumors with 1pLOH (see Table 2), we noticed several genes that were expressed predominantly in normal brain or neural tissue, indicating that those genes are functional in the normal nervous system. Contamination of normal tissue cells was not likely, because allelic losses observed on the microsatellite analysis were almost complete in all cases, indicating that the examined

tissues consisted mostly of tumor cells. For example, *MYT1L* encodes a zinc finger protein which plays a role in the development of neurons in the central nervous system (Kim *et al.*, 1997), and *PTPRN*, which had especially similar expression pattern to *MYT1L* in the RT–PCR analysis, is implicated in neuroendocrine secretory processes. *SNCB* plays a role in neuronal plasticity, *SLC1A2* is a glial high affinity glutamate transporter, and *HAPIP* is also abundantly expressed in the neural tissues. *L1CAM* is an axonal glycoprotein involved in neuronal migration and differentiation (Kenwrick *et al.*, 2000). In combination with the results of clustering, these data may suggest that tumors without 1pLOH are more distant from normal brain, possibly reflecting their differentiation status.

Analysis on the differentially expressed genes provided potentially interesting information on their chromosomal locations. Of the top 123 genes whose expressions were most significantly decreased in tumors with 1pLOH, nearly 60% were located either on chromosome 1 or chromosome 19, with the ratio of expression levels to tumors without 1pLOH around 50%. It was reported that nearly all oligodendrogliomas with 1p and 19q LOH lose the entire arm of 1p and 19q (Bigner *et al.*, 1999; Nigro *et al.*, 2001; Smith *et al.*, 1999), which was also confirmed by our microsatellite analysis on 1p. Therefore, reduced expression of genes in a wide range of 1p is likely to be a consequence of losing one copy of each gene. On the other hand, some genes on chromosome 1p had higher expressions in tumors with 1pLOH, suggesting that the expression regulations of those genes were not simply dependent on the copy number. In a few genes such as *COL11A1* and *RBBP4*, the relative expressions were remarkably low probably because of their overexpression in tumors without 1pLOH, rather than their expression reduction in 1p losing tumors (see Table 3). Despite the rather comprehensive expression analysis, we still could not pinpoint a particular gene that would affect the chemosensitivity of oligodendrogliomas. None of the genes previously suggested to be related with chemosensitivity, such as O^6-methylguanine-DNA methyltransferase (MGMT), multidrug resistance 1 (MDR1), multidrug resistance-associated protein (MRP), glutathione S-transpherase pi, metallothionein and topoisomerase IIα (Nutt *et al.*, 2000; Tanaka *et al.*, 2000), were detected as differentially expressed genes in our study. On the other hand, we showed that significant numbers of genes were differentially expressed between oligodendroglioma subsets, including expression reduction of numerous genes in the chromosome 1p. An interesting question is whether about 50% reduction of such numerous genes in the same chromosomal region could have any biological effect on tumorigenesis or chemosensitivity. Recent studies showed that loss of one copy of a gene and subsequent reduction of its expression level is possibly related to tumorigenesis, a phenomenon called haplo-insufficiency (Fero *et al.*, 1998; Gutmann *et al.*, 1999). Whether similar phenomenon underlies the biological features of oligodendroglioma remains to be investigated.

From a technological point of view, it could be an important observation that the oligonucleotide microarray may be quantitative enough to detect expression reduction caused by the allelic loss in numerous genes. cDNA microarray and serial analysis of gene expression (SAGE) have been tried with some success to detect increase or decrease of expressions of certain genes which were altered by gene amplifications or deletions (Caron *et al.*, 2001; Pollack *et al.*, 1999). Our data indicated that oligonucleotide microarray would be a good system to identify such genes.

In summary, we showed that genetic subsets in oligodendrogliomas by 1p status were reflected in gene expression profile. Some of the interesting genes differentially expressed included genes implicated in the function of nervous tissues, genes on chromosome 1p and 19q. Molecular mechanism of chemosensitivity and chemoresistance may well be represented by those differentially expressed genes, and our data would serve as good baseline data for the future studies to solve that clinically important question.

Materials and methods

Sample preparation

Tumor samples obtained at surgery were snap frozen in liquid nitrogen and stored at −80°C until use. Histological diagnosis was made on formalin-fixed paraffin-embedded tissues processed separately. To minimize the notorious variability of the histological diagnosis in oligodendroglial tumors, the histology slides were reviewed by four independent neuropathologists to make consensus diagnoses following the WHO classification (Kleihues and Cavenee, 2000). Paired blood samples were obtained after written informed consents, and were subjected to DNA extraction for the microsatellite analysis. Of 40 oligodendroglial tumors, six tumors with 1pLOH and five without 1pLOH were selected for expression profiling using GeneChip system (Affymetrix). Total RNA from normal whole brain was purchased from two different providers (Clontech, Palo Alto, CA, USA and Life Technologies, Inc., Rockville, MD, USA), which were used to see the expression profile of the normal neurons and glial cells.

Genetic analysis

LOH assay on chromosomes 1p, 19q and 10q to detect allelic losses were performed using Genetic Analyzer 310 (Applied Biosystems, Foster City, CA, USA) as previously described. The following microsatellite markers located at the commonly deleted in gliomas were used: *D1S244*, *D1S2734*, and *D1S402* for 1p (1p36), *D19S112*, *D19S596*, *D19S412* and *D19S219* for 19q (19q13), *D10S1744*, *D10S1680* and *D10S583* for 10q (10q22-23) (Ueki *et al.*, 2000). For tumors with 1pLOH, four additional 1p markers were further examined to see the range of the deletion: *D1S1166* (1p13), *D1S495* (1p22), *D1S2835* (1p32) and *D1S2657* (1p34). The SSCP assay for exons 5 to 8 of *TP53* was performed using previously published primer pairs (Fults *et al.*, 1992), again using Genetic Analyzer 310. Exons showing migration shift were PCR amplified again and were directly sequenced using BigDye Terminator Kit (Applied Biosystems) following the manufacturer's protocol. Established comparative multiplex

PCR assays were used to detect homozygous deletion of *CDKN2A* (Ueki *et al.*, 1996). For RNA extraction, the frozen tumor sample was homogenized in Isogen (Nippon Gene, Osaka, Japan) and total RNA was isolated following manufacturer's instructions.

Gene Chip experiment

Five μg of total RNA from each sample were used to synthesize biotin-labeled cRNA, which was then hybridized to the high-density oligonucleotide array (GeneChip Human U95A array; Affymetrix) following the previously published protocol with minor modifications (Ishii *et al.*, 2000). Arrays contain probe sets for approximately 12 626 human genes and ESTs, which were selected from Build 95 of the UniGene Database (derived form GenBank 113, dbEST/10-02-99). After washing, arrays were stained with streptavidin-phycoerythrin (Molecular Probes, Inc., Eugene, OR, USA) and analysed by a Hewlett-Packard Scanner to collect primary data. The GeneChip 3.3 software (Affymetrix) was used to calculate the average difference for each gene probe on the array, which was shown as an intensity value of gene expression defined by Affymetrix using their algorithm. The average difference has been shown to quantitatively reflect the abundance of a particular mRNA molecule in a population (Ishii *et al.*, 2000; Lockhart *et al.*, 1996). To allow comparison among multiple arrays, the average differences were normalized for each array by assigning the average of overall average difference values to be 100. A value of two was assigned to every average difference below two. Of the total 12 626 probe sets represented on the array, control probes and genes scored as absent (not detected) by the expression algorithm in GeneChip software (Affymetrix) or less than 100 in all 13 samples were excluded from the analysis because of low confidence of scarcely expressed genes, and 5668 probe sets were left.

Selection of differentially expressed genes

For the selection of differentially expressed genes by 1p status, we used prediction value (*P*-value) which reflects the difference between two groups, given by $(\mu 1-\mu 2)/(\sigma 1+\sigma 2)$ when ($\mu 1$, $\sigma 1$) and ($\mu 2$, $\sigma 2$) denote the means and standard deviations of the log of the expression level of gene for the sample in group 1 and group 2, respectively (Golub *et al.*, 1999). Pre-filtering was applied to select probe sets whose maximum and minimum average difference among 11 tumor samples differed by more than 100, and had more than two-fold difference. For the remaining 3875 probe sets, the prediction values were calculated. We also used Mann–Whitney test, which measures whether the distribution of gene expression level between two groups is overlapped.

Clustering analysis

The expression patterns of samples were statistically analysed using GeneSpring 4.0 software (Silicon Genetics, Redwood City, CA, USA). Average differences were converted into logarithm, and hierarchical clustering was carried out using Pearson correlation coefficient of 0.8 (Eisen *et al.*, 1998).

Semi-quantitative RT–PCR

Semi-quantitative RT–PCR was performed using 13 samples used for GeneChip analysis and additional nine oligodendroglial tumors. Of the nine additional cases, four cases had combined 1p and 19q LOH, while five cases had neither genetic alteration. Two of the five additional cases without 1pLOH had *TP53* mutation. cDNA was synthesized with oligo-dT primer from 2 μg total RNA, using SuperScript Preamplification System (Life Technologies, Inc.). The concentration of the cDNA was equalized using the *GAPDH* gene expression as a control. PCR was then performed with 2 μl of cDNA for 31–37 cycles, consisted of 30 s of denaturing at 94°C, 30 s of annealing at 63–70°C and 1 min of extension at 72°C. The primer sets used are listed in Table 4. PCR products were separated by electrophoresis on 1.5% agarose gels and were visualized with ethidium bromide staining. Numbers of PCR cycles were optimized to ensure product intensity within the linear phase of amplification. For each primer set, the amplicon was sequenced after subcloned into pGEM-T Easy vector (Promega, Madison, WI, USA) to confirm that the correct target gene was amplified.

Identification of gene location

Chromosomal loci of the genes were identified using the locus information from the web sites of GenBank, UniGene and LocusLink on NCBI, by referring to the corresponding GenBank accession number of each probe set.

Detailed chromosomal locations of 950 genes mapped on 1p were obtained from the web site of Map Viewer (Homo sapiens build 26) on NCBI, in which the gene locations are shown by distances from the telomere of the short arm. These 950 genes were matched to the probe sets on Human U95A array by referring to the LocusLink ID, UniGene ID and GenBank accession number, which identified 502 probe sets represented on U95A array. Genes with expressions (average difference) scored as absent or less than 100 in average of 11 tumors were excluded because of low confidence in evaluating genes with low expression. For the 158 genes remaining, we calculated relative expressions by dividing the mean expressions in tumors with 1pLOH by those in tumors without 1pLOH, which were then arranged on the chromosome map.

Table 4 Primer pairs used in RT–PCR

Gene	*Sense (5′→3′)*	*Antisense (3′→5′)*
MYT1L	AAACAGCGGGCCAGCAACGGTATAG	CAGCAGCAAAAAACAAGAGGCATCC
PTPRN	GTGGAGGATGGTGTCAAGCAGTGTG	GGCTGTCAGGGCAAATTCAAACTGG
SCG2	GTTCTGCCAAGGCTCCCTTATGGTG	GGATTTGCTTGGGGTGGGAGGAATG
SNCB	ATGGACGTGTTCATGAAGGGCCTG	GGACAGGGACAGAATTGTGCTGCT
ALDH1A1	CCTCTGACCCCAGGAGTCACTCAA	TTCATGGAAACCGTACTCTCCCAG
L1CAM	TACCACCCGGTCCCCACTTTATTGC	ATGTTGTGTGGTGGGTACCGAAGGC
COL11A1	GCCAAAGGAGAAACCAGGAAGTTGG	CACAAAATGGGTTGGTGGCACCAAG
RBBP4	CCAAACCAAGCCACTCAGTTGATGC	CCTTGTCCTTCTGGATCCACGCTTC
KCND2	TCGAGCCCTGGCTGTGAAAAGAATC	TTATTTGCACAGCCCACCATGGAAC
GAPDH	CATGTGGGCCATGAGGTCCACCAC	AATGCCTCCTGCACCACCAACTGC

Acknowledgments

We thank Drs Yoichi Nakazato, Takanori Hirose, Nobuaki Funada and Junko Hirato for reviewing the histology. We also thank Xijin Ge and Makoto Kano for their help in statistical analysis and Hiroko Meguro for her technical assistance. This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan (No. 11671357 and No. 13470184). This study was carried out as a part of The Technology Development for Analysis of Protein Expression and Interaction in Bioconsortia on R&D of New Industrial Science and Technology Frontiers which was performed by Industrial Science, Technology and Environmental Policy Bureau, Ministry of Economy, Trade and Industry, and entrusted by New Energy Development Organization (NEDO).

References

Bigner SH, Matthews MR, Rasheed BK, Wiltshire RN, Friedman HS, Friedman AH, Stenzel TT, Dawes DM, McLendon RE and Bigner DD. (1999). *Am. J. Pathol.*, **155**, 375–386.

Cairncross JG and Macdonald DR. (1988). *Ann. Neurol.*, **23**, 360–364.

Cairncross JG, Ueki K, Zlatescu MC, Lisle DK, Finkelstein DM, Hammond RR, Silver JS, Stark PC, Macdonald DR, Ino Y, Ramsay DA and Louis DN. (1998). *J. Natl. Cancer Inst.*, **90**, 1473–1479.

Caron H, van Schaik B, van der Mee M, Baas F, Riggins G, van Sluis P, Hermus MC, van Asperen R, Boon K, Voute PA, Heisterkamp S, van Kampen A and Versteeg R. (2001). *Science*, **291**, 1289–1292.

Eisen MB, Spellman PT, Brown PO and Botstein D. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 14863–14868.

Fero ML, Randel E, Gurley KE, Roberts JM and Kemp CJ. (1998). *Nature*, **396**, 177–180.

Fults D, Brockmeyer D, Tullous MW, Pedone CA and Cawthon RM. (1992). *Cancer Res.*, **52**, 674–679.

Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, Coller H, Loh ML, Downing JR, Caligiuri MA, Bloomfield CD and Lander ES. (1999). *Science*, **286**, 531–537.

Gutmann DH, Loehr A, Zhang Y, Kim J, Henkemeyer M and Cashen A. (1999). *Oncogene*, **18**, 4450–4459.

Huang H, Colella S, Kurrer M, Yonekawa Y, Kleihues P and Ohgaki H. (2000). *Cancer Res.*, **60**, 6868–6874.

Husemann K, Wolter M, Buschges R, Bostrom J, Sabel M and Reifenberger G. (1999). *J. Neuropathol. Exp. Neurol.*, **58**, 1041–1050.

Ino Y, Betensky RA, Zlatescu MC, Sasaki H, Macdonald DR, Stemmer-Rachamimov AO, Ramsay DA, Cairncross JG and Louis DN. (2001). *Clin. Cancer Res.*, **7**, 839–845.

Ishii M, Hashimoto S, Tsutsumi S, Wada Y, Matsushima K, Kodama T and Aburatani H. (2000). *Genomics*, **68**, 136–143.

Kenwrick S, Watkins A and de Angelis E. (2000). *Hum. Mol. Genet.*, **9**, 879–886.

Kim JG, Armstrong RC, v Agoston D, Robinsky A, Wiese C, Nagle J and Hudson LD. (1997). *J. Neurosci. Res.*, **50**, 272–290.

Kleihues P and Cavenee WK. (ed). (2000). *Pathology and Genetics of Tumours of the Nervous System*. IARC Press: Lyon.

Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H and Brown EL. (1996). *Nat. Biotechnol.*, **14**, 1675–1680.

Mai M, Huang H, Reed C, Qian C, Smith JS, Alderete B, Jenkins R, Smith DI and Liu W. (1998). *Genomics*, **51**, 359–363.

Nigro JM, Takahashi MA, Ginzinger DG, Law M, Passe S, Jenkins RB and Aldape K. (2001). *Am. J. Pathol.*, **158**, 1253–1262.

Nutt CL, Noble M, Chambers AF and Cairncross JG. (2000). *Cancer Res.*, **60**, 4812–4818.

Ohira M, Kageyama H, Mihara M, Furuta S, Machida T, Shishikura T, Takayasu H, Islam A, Nakamura Y, Takahashi M, Tomioka N, Sakiyama S, Kaneko Y, Toyoda A, Hattori M, Sakaki Y, Ohki M, Horii A, Soeda E, Inazawa J, Seki N, Kuma H, Nozawa I and Nakagawara A. (2000). *Oncogene*, **19**, 4302–4307.

Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D and Brown PO. (1999). *Nat. Genet.*, **23**, 41–46.

Schwab M, Praml C and Amler LC. (1996). *Genes Chromosomes Cancer*, **16**, 211–229.

Smith JS, Alderete B, Minn Y, Borell TJ, Perry A, Mohapatra G, Hosek SM, Kimmel D, O'Fallon J, Yates A, Feuerstein BG, Burger PC, Scheithauer BW and Jenkins RB. (1999). *Oncogene*, **18**, 4144–4152.

Tanaka S, Kamitani H, Amin MR, Watanabe T, Oka H, Fujii K, Nagashima T and Hori T. (2000). *J. Neurooncol.*, **46**, 157–171.

Ueki K, Ono Y, Henson JW, Efird JT, von Deimling A and Louis DN. (1996). *Cancer Res.*, **56**, 150–153.

Ueki K, Nishikawa R, Nakazato Y, Hirose T, Hirato J, Funada N, Fujimaki T, Hojo S, Kubo O, Ide T, Usui M, Ochiai C, Ito S, Takahashi H, Mukasa A, Asai A and Kirino T. (2002). *Clin. Cancer Res.*, **8**, 196–201.

Watson MA, Perry A, Budhjara V, Hicks C, Shannon WD and Rich KM. (2001). *Cancer Res.*, **61**, 1825–1829.

手術手技・周術期管理

中心前回に存在する神経膠腫の手術

隈部俊宏　中里信和*　岩崎真樹
永松謙一　清水宏明*　吉本高志

Surgical Removal of the Glioma at the Precentral Gyrus

by

Toshihiro Kumabe, M.D., Nobukazu Nakasato, M.D.*, Masaki Iwasaki, M.D., Ken-ichi Nagamatsu, M.D., Hiroaki Shimizu, M.D.*, and Takashi Yoshimoto, M.D.

from

Department of Neurosurgery, Tohoku University Graduate School of Medicine, and
* Department of Neurosurgery, Kohnan Hospital

A 15-year-old female presented with a 10-month history of generalized epileptic activity. Neurological and neuropsychological examination revealed no abnormalities.

Magnetic resonance imaging (MRI) demonstrated a slightly enhanced round expansive tumor, which appeared identical on both T1- and T2-weighted imaging, in the left precentral gyrus medial to the precentral knob. Magnetoencephalography, functional MRI, surface anatomy scan with venography, and fiber mapping using diffusion-weighted MRI disclosed that the tumor was located just before the leg motor cortex and had displaced the corticospinal tract posterolaterally.

Surgery was performed with the patient under general anesthesia. Stimulation mapping techniques for localization of the motor cortex and the descending motor pathway was applied under guidance from a neuronavigation system. Direct cortical stimulation of the anterior half of the precentral gyrus overlying the tumor did not evoke leg motor movements. Thus, the cortex was resected up to the location of the leg motor cortex confirmed by the direct cortical stimulation technique. The tumor was completely resected with preservation of the descending motor pathway. The histological diagnosis was *ganglioglioma*.

Postoperatively, the patient only had transient weakness of the right leg, and was discharged home 11 days after the operation without neurological deficit.

The whole of the precentral gyrus does not correspond to primary motor area (area 4). If the patient has no neurological deficit and the tumor has an expansive nature, as evaluated by MR imaging, gliomas at the anterior side of the precentral gyrus can be resected without permanent motor deficit.

(Received September 8, 2001 ; accepted October 18, 2001)

Key words : brain mapping, descending motor pathway, glioma, precentral gyrus, primary motor cortex

Jpn J Neurosurg (Tokyo) 11 : 271-277, 2002

東北大学医学部脳神経外科／〒980-8574 仙台市青葉区星陵町1-1〔連絡先：隈部俊宏〕
Address reprint requests to : Toshihiro Kumabe, M.D., Department of Neurosurgery, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai-shi, Miyagi 980-8574, Japan
*広南病院脳神経外科

はじめに

eloquent area近傍の神経膠腫の手術は，術前に解剖と機能を把握することによって手術戦略をたて，それを術中検証しながらいかに確実に手技をこなすかにかかっている．近年，運動野周辺の脳機能解析は急速に進歩し，解剖学的特徴からその機能を推測することがかなりの部分で可能となってきた．Yousryら[7]が報告した，「手指運動野は，MRIの水平断において中心溝が形成する逆Ωもしくはε構造の前方の“precentral knob”に存在する」という事実は，運動野に外科的侵襲を加える脳外科医にとってきわめて重要なものである．

中心前回に存在する神経膠腫は顔面の運動野より外下方では，より上位の手指の運動野から下降する錐体路に注意を払えば永続する神経脱落症状なく摘出することが可能であると報告されてきた[3~5]．それでは手指の運動野より内上方の中心前回に存在する腫瘍は，すべて永続的な麻痺症状なしに摘出することは不可能なのであろうか．今回痙攣にて初発し，術前麻痺症状のない下肢運動領域前方の中心前回自体に存在する神経節膠腫症例に対して，術前術中マッピングを行い，中心前回前半部を摘出し，麻痺症状なく腫瘍全摘が可能であった症例を経験したので報告する．

症例呈示

患　者：15歳，女性

既往歴・家族歴：特記事項を認めない．

現病歴：2000年1月中旬睡眠中に右下肢から始まる全身痙攣発作にて発症した．同様の発作が3月，8月，10月と計4回出現し，近医にてMRIを施行され左前頭葉に腫瘍を認めたため，11月17日当科紹介となった．

神経学的所見：神経学的脱落症状は認めなかった．

術前検査所見：magnetic resonance imaging（MRI）にて左前頭葉にT1強調画像にて低信号，T2強調画像にて高信号，わずかに造影効果を示す長径約3cmの腫瘍を認めた．なおT1強調画像とT2強調画像の異常信号域は完全に一致していた．水平断MRIによって確認される逆Ω構造との関係から，腫瘍は下肢の運動野を後方に強く圧排して中心前回およびその皮質下に存在すると考えられた（Fig. 1A～C）．

脳磁図（magnetoencephalography；MEG）では，正中

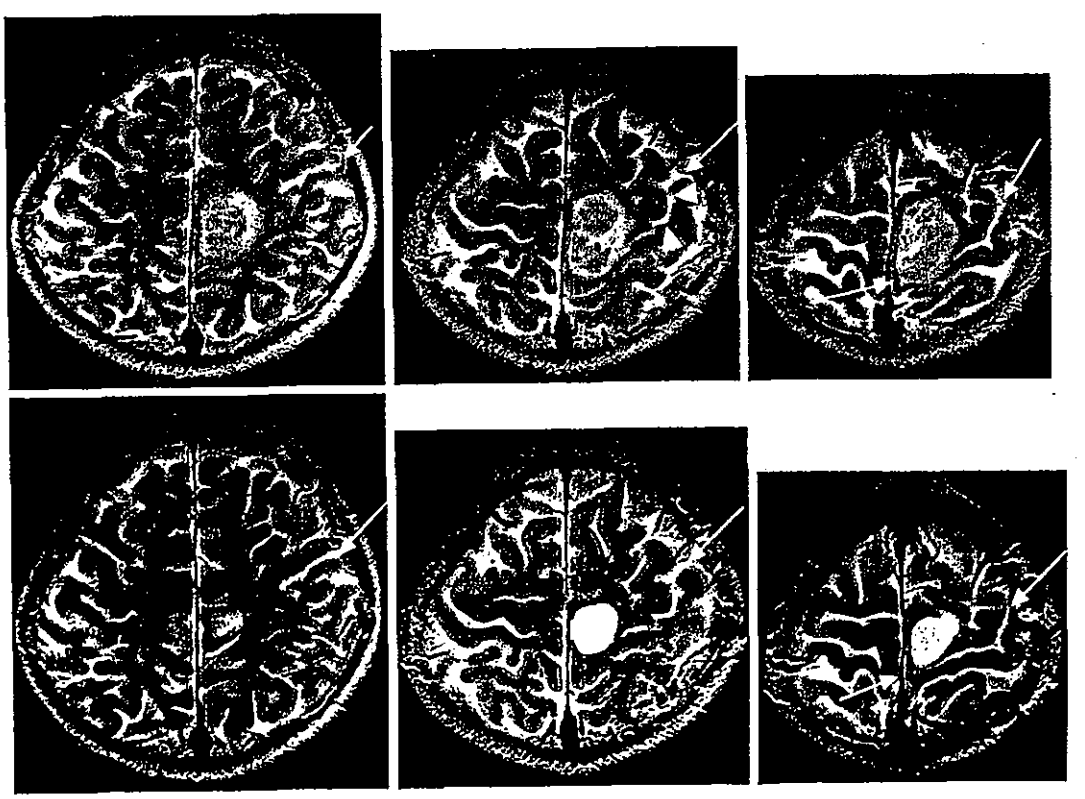


Fig. 1　Pre-（A～C）and postoperative（D～F）T2-weighted magnetic resonance images
Arrows and arrowheads indicate the central sulcus and the inverted omega-shaped sulcus, respectively.

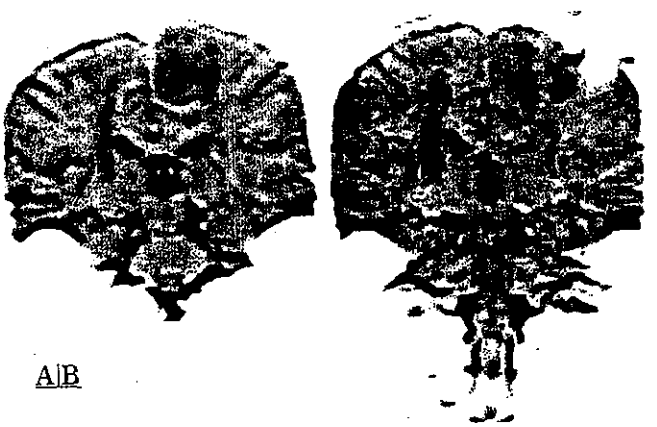
神経刺激による体性感覚誘発磁界（somatosensory evoked field; SEF）は逆Ω構造の後外側に，後脛骨神経刺激によるSEFは腫瘍にほぼ接するような形で腫瘍後方に同定された（Fig. 2）.

離握手賦活によるfunctional MRI（fMRI）にて，腫瘍外側の逆Ω構造内側に賦活領域が確認された．足関節の背屈底屈負荷によるfMRIにて腫瘍の後方に賦活領域が確認されたが，これはMRIによる解剖学的情報およびMEGの後脛骨神経刺激によるSEFから考えて，下肢運動領域を示すものではないと判断された（Fig. 2）.

MRI拡散強調画像（diffusion-weighted image; DWI）では上下方向の線維を赤，左右方向の線維を緑，前後方向の線維を青となるように合成した[2]．この結果，上下方向に走行する皮質脊髄路を含む白質線維は腫瘍によって障害されておらず，腫瘍の後方から外側に接して走行していることが確認された（Fig. 3A）.

脳表静脈を重像したsurface anatomy scan（SAS）にて，脳回脳溝の構造学的所見から，腫瘍は脳表に投影すると中心溝の下方にその中心をもち，下肢運動野を後方に強く圧迫していることが予想された（Fig. 4）.

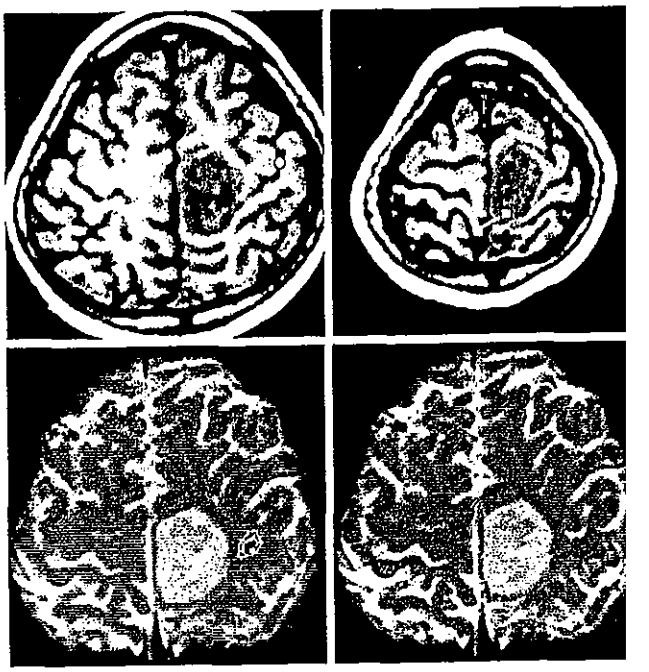
術前日に開頭部位の剃毛を行い頭皮上にマーカーを設置した後，ニューロナビゲーションシステム用にMRIを撮影した．2001年2月15日プロポフォール，フェン



A|B

Fig. 3 Pre- (A) and postoperative (B) coronal diffusion-weighted images (DWIs)

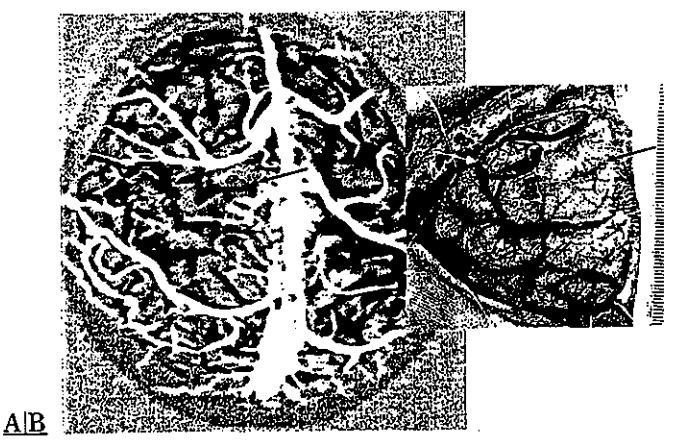
The grayscale images using superior-inferior, left-right, and anterior-posterior diffusion gradients were first reversed in gradation, and then colored red, green, and blue, respectively. These colored images were combined into a single RGB color image. Thus, a nerve fiber running superior-inferior, left-right, or anterior-posterior appears as red, green, or blue, respectively. Mixed colors indicate oblique orientation of the nerve fibers. These DWIs demonstrated the descending motor pathways running just beside the tumor before the resection and postoperative preservation.



A|B
C|D

Fig. 2 Preoperative magnetoencephalograms (A, B) and functional MR images (C, D)

The open circle and square indicate the estimated N20m dipoles of the somatosensory evoked magnetic fields caused by stimuli of the median nerve and the posterior tibial nerve, respectively. The bars indicate the orientation of the dipole. The highest activation caused by opening and closing of the right hand was located at the posterior bank of the precentral knob (C). The highest activation caused by dorsiflexion and extension of the right ankle joint was located posterior to the tumor, and was anatomically identified as the postcentral sulcus.



A|B

Fig. 4 Superficial venous image obtained by MR using a three-dimensional phase contrast technique superimposed on the surface anatomy scan (SAS) obtained by the multiple slice SAS method (A) and an intraoperative photograph (B)

The superficial venous image on the SAS is apparently identical to the initial intraoperative photograph. White and black arrows and arrowheads indicate the central sulcus, the precentral sulcus, and the middle bend, respectively.

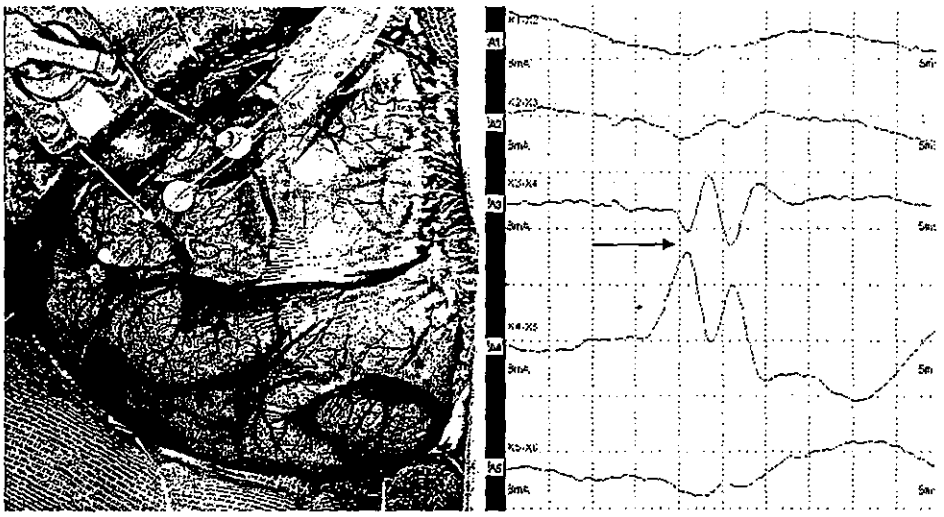


Fig. 5 Intraoperative somatosensory evoked potentials (SEPs) recorded from a strip electrode crossing the thumb sensory cortex
White and black arrows indicate the central sulcus and the phase reversal of the SEPs, respectively.

タニル，笑気を用いた全身麻酔下に腫瘍摘出術を施行した．なお術中電気刺激によるマッピング時には，筋弛緩モニターで筋弛緩剤の効果が完全に消失していることを確認したうえで行った．

手術所見：仰臥位で頭部は正中にして下顎を引き，上体を起こして，腫瘍存在部位がなるべく水平に近くになるようにして3点固定した．ニューロナビゲーションシステム（Viewscope, Leika）を設定した．筋電図モニタリングとして，上腕二頭筋，上腕三頭筋，腕橈骨筋，尺側手根屈筋，短母指外転筋，大腿四頭筋，大腿二頭筋，前脛骨筋，下腿三頭筋に表面筋電図を設定した．

ニューロナビゲーションシステムにて腫瘍の位置を確認し，これを取り囲むように左前方に開く弧状切開を行った．再度腫瘍の位置をニューロナビゲーションシステムにて確認し，開頭を行った．

腫瘍存在部位の内側においてクモ膜顆粒が発達しておりやや癒着が強かったが，正中まで硬膜を翻転することが可能であった．脳表静脈を重像したSAS画像は，脳表の状態をきわめて正確に反映していることが確認された（Fig. 4）．ニューロナビゲーションシステムにて，腫瘍の存在範囲および術前機能マッピングにおいて予想された機能領域を確認した．

この段階で筋弛緩剤が完全に失効している状態にした．まず，術前検査にて予想され，ニューロナビゲーションシステムにて確認された中心溝の拇指の感覚野をまたぐ位置に電極を設置して，体性感覚誘発電位（somatosensory evoked potential; SEP）を測定すると，明瞭な極性の反転が得られた（Fig. 5）．

続いて脳表に脳波電極を設置した．脳表電気刺激には5mm間隔の双極電極を用い，双極性の矩形波を持続時間0.3 msec，60Hzで1～2秒間連続刺激した．刺激強度は4mAから1mAずつ上げ，皮質脳波で後発射が出現しない範囲とした．4mAから手指運動野刺激により良好な筋収縮が確認された．さらに内側の上肢，下肢〔下肢を引きずり上げるような全体の動き（Fig. 7），さらにその内側で下肢を内転するような動き〕に関しては8mAにて安定して筋収縮が確認された（Fig. 6～8）ため，以後この刺激電流を使用することとした．

まず中心前溝を底部まで開放した．この後方の中心前回皮質はやや色調が青白く変色しており，直下に腫瘍があることが予測された．再度脳表電気刺激を行い，中心溝から次第に前方に向かって脳表電気刺激を行うと，ちょうど中心前回の半分から後方の皮質で下肢の運動誘発が生じ，それより前方では運動誘発が生じないことが判明した．そこでこの中心前回前半部の皮質を除去して腫瘍を露出させることとした．

皮質を約2mm除去すると明らかに周囲白質と色調の異なる境界鮮明な弾性軟の腫瘍が露出した．皮質領域では後方に下肢運動野が存在すること，白質領域では下肢運動野からの皮質脊髄路が後方から腫瘍外側に回り込んで走行すること，内側には皮質が薄く存在するものの前大脳動脈の分枝が走行することに注意して，腫瘍と正常白質の間ぎりぎりを剥離して腫瘍を摘出した（Fig. 8）．

腫瘍摘出後，再度脳表電気刺激を行い，それぞれの筋

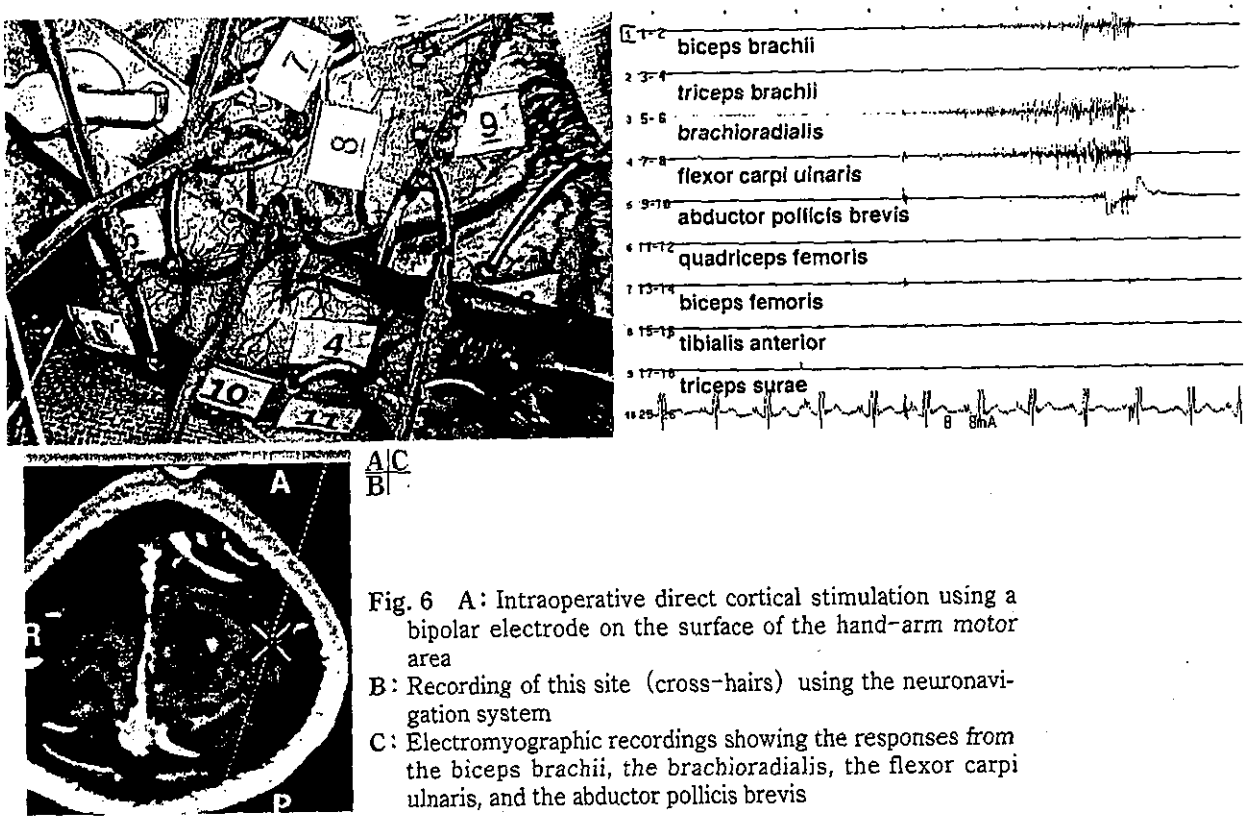


Fig. 6 A: Intraoperative direct cortical stimulation using a bipolar electrode on the surface of the hand-arm motor area
B: Recording of this site (cross-hairs) using the neuronavigation system
C: Electromyographic recordings showing the responses from the biceps brachii, the brachioradialis, the flexor carpi ulnaris, and the abductor pollicis brevis

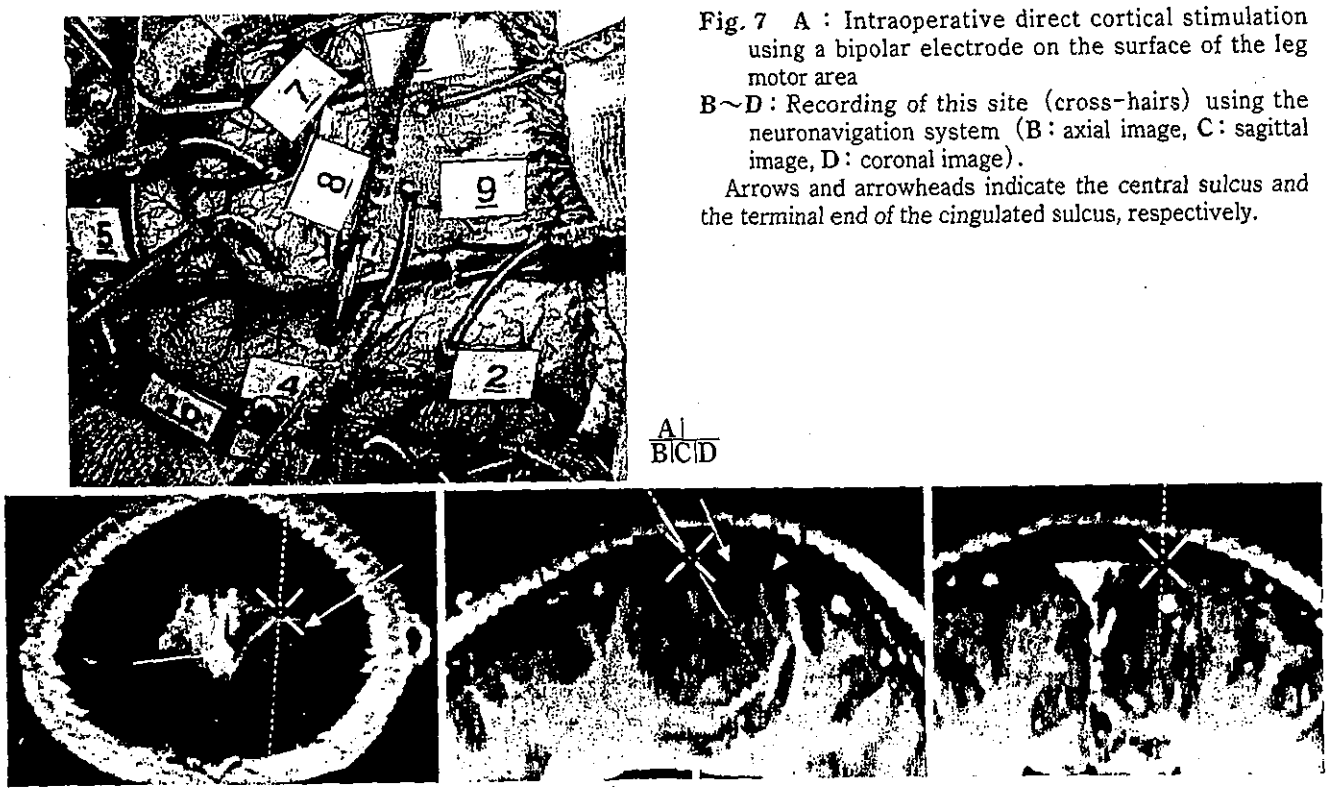


Fig. 7 A : Intraoperative direct cortical stimulation using a bipolar electrode on the surface of the leg motor area
B～D : Recording of this site (cross-hairs) using the neuronavigation system (B : axial image, C : sagittal image, D : coronal image).
Arrows and arrowheads indicate the central sulcus and the terminal end of the cingulated sulcus, respectively.