陽性を示した腫瘍に対して治療を計画しております。

もう1つのテーマは、ACNUに対する耐性克服を考慮した化学療法であります。ACNUは、悪性脳腫瘍に対する薬剤として国内で認可されている数少ない抗癌剤のひとつであります。これに対する耐性機構がO^6－methylguanine－DNA methyltransferase (MGMT)と言われ、多くの脳腫瘍で発現しております。このMGMTを枯渇させる作用を持つprocarbazineで前処置をすることで、ACNUの効果を高めようという試みで、新しい治療計画を立てました。星細胞腫 grade 3、4に対し、摘出後procarbazineを10日間投与いたします。7日目にACNUを投与して、放射線治療を同時に開始いたします。1日2Gyで30回、総線量60Gyの照射を行い、その第6週目に再びprocarbazineを10日間、第7週目にACNUという形で治療化学放射線治療を行います。これを従来のACNU単剤併用による化学放射線治療の効果と比較します。

今回、我々のグループはJCOGに認可され、今後、本臨床試験はJCOG脳腫瘍グループの試験として行われます。評価項目といたしましては、まず安全性の確認、そしてprimary endpointとして生存期間、secondary endpointは無増悪生存期間、奏効率であり、その他MGMTと生存期間の関係、procarbazineによるMGMTの低下の程度などもあわせて検討する予定です。

原発性脳腫瘍の発生率は、10万人に11から12人とされています。脳腫瘍全国統計によりますと、今回対象としましたgrade 3、grade 4の星細胞腫はその14%であり、このような希少疾患での臨床研究は、多施設共同試験以外は困難であります。今まで脳腫瘍の分野ではしっかりした臨床試験の基盤が存在しなかったために、エビデンスとなりうるような研究結果に乏しかったと言えます。今回我々は、JCOG内に脳腫瘍グループを設立し、その管理下で臨床研究を行うことになり、脳神経外科領域での新しい研究の方向づけとなりうるのではないかと思っております。

以上です。

高嶋　ありがとうございました。

それではご討議をお願いいたします。

質問　2つの薬剤は、特にSS誘導体というのはもう海外では認められていますよね。

渋井　はい。

質問　procarbazineはどうですか。

渋井　procarbazineは国内国外とも悪性リンパ腫などでは認められていますが、脳腫瘍では未承認です。これについてはできれば承認を取るところまで持っていきたいと考えております。

質問　と言うことは、海外データ、GCP準拠はどうかわかりませんけど、海外データは本資料として使える状況ですね。

渋井　はい使えると思います。

質問　そうすると、ミニマムのデータで済むということですか。
渋井　そう思います。
質問　JCOGもそれは治験体制で組むということですね。
渋井　検討中です。
高嶋　このastrocytomaの現在の標準的治療法は手術ですか。
渋井　標準的な治療としては、手術および放射線の治療ですが、それに併用してACNUを投与するというところまでは、コンセンサスが得られていると思います。
高嶋　5年生存というのはどの程度ありますか。
渋井　astrocytoma grade 3の5年生存は30%ぐらいです。glioblastomaは10%以下です。
高嶋　先生が行う臨床試験で、どの程度これを引き上げるのが目標ですか。
渋井　海外からの報告で、奏効率がglioblastoma、astrocytomaそれぞれ30%以上あるという報告も出ておりますので、その位を期待しております。
質問　本研究の対象症例が6種類の疾患だったと思うんですが。最終的にはソマトスタチンレセプターのエクスプレッションの多いastrocytomaとglioblastomaが対象ですか。
渋井　免疫組織化学的染色ではglioblastoma、astrocytomaの陽性率が低く、他の腫瘍のほうがむしろ高かったため、glioma以外の再発腫瘍もよい適応になると考えます。また、本来の特異性という意味から外れてしまいますが、ソマトスタチンレセプターが陽性でなくても、gliomaなどでは血液脳関門が破壊されており、SPECTで陽性を示すこともあるようです。もしそういった形でpentetreotideが到達するのであれば、これらの疾患の治療も可能であると考えます。SPECT陽性の腫瘍であれば治療の対象と考えております。
質問　その6種類の対象疾患は全部集積するということですね。
渋井　全例ではないと思いますが集積する可能性があります。手術による摘出標本での免疫組織化学的染色で確認しながら、治療を進めていきたいと思います。
高嶋　この臨床試験のコンセプトは、JCOGで承認されたのですか。
渋井　今、コンセプトは検討している最中です。
高嶋　今年度中に始められるかどうかというところですね。
渋井　そのつもりでおります。
高嶋　ありがとうございました。

Neurosurg Rev (2003) 26:262–265
DOI 10.1007/s10143-003-0280-y

ORIGINAL ARTICLE

Masato Ohki · Kaori Sakurada · Yukihiko Sonoda ·
Shinya Sato · Shinjiro Saito · Takamasa Kayama

Analysis of the extent of astrocytic tumour resection evaluated by magnetic resonance images

Received: 14 January 2003 / Accepted: 3 April 2003 / Published online: 19 June 2003

Abstract One of the most important prognostic factors in brain tumours is the extent of tumour resection. Its evaluation has been difficult on computed tomography (CT); however, magnetic resonance imaging (MRI) can clearly determine the extent of tumour resection. Using MRI, we analyzed 77 patients with astrocytic tumours that were surgically treated at our department from 1994 to 2001. The tumours were classified into the following types: (a) well-circumscribed: single lesions that can be distinguished from normal brain; (b) localised: single lesions that are localised in one gyrus; and (c) diffuse: other tumours. Our treatment of glioma resection is to use sulcus opening and gyrectomy, a technique based on identification and dissection of the sulcus adjacent to the tumour followed by en bloc tumour resection. Almost total tumour resection (≥95% resection) was achieved in 76.8% in the well-circumscribed type, 100% in the localised type, and only 10.5% in the diffuse type. Nearly total resection was achieved in 61%, which is considerably more than in the literature of the CT era (10.4–23.5%). The sulcus opening and gyrectomy technique based on MRI achieved radical glioma removal in cases which could be identified by MRI, resulting in a better tumour removal rate than that based on CT.

Keywords Extent of tumour resection · Glioma · Gyrectomy · Magnetic resonance imaging

Introduction

The extent of tumour removal at surgery is one of the most important prognostic factors in the treatment of brain tumours. Planning for the maximum extent of removal can be based on magnetic resonance imaging (MRI), which provides more detailed anatomical information than computed tomography (CT), is very useful for selecting the optimum operative approach, and allows accurate estimation of the possible extent of tumour removal. This extent can also be assessed more correctly using pre- and postoperative MRI. However, safe and reliable methods of radical tumour removal should also prevent postoperative aggravation of neurological symptoms. Here we describe our experience with surgical removal of the brain tumour bulk using our sulcus opening and gyrectomy technique [2, 4] based on preoperative MRI findings.

M. Ohki · K. Sakurada · Y. Sonoda · S. Sato · S. Saito · T. Kayama (✉)
Department of Neurosurgery,
Yamagata University School of Medicine,
2-2-2 Iida-Nishi, Yamagata 990-9585, Japan
e-mail: nouge@med.id.yamagata-u.ac.jp
Tel.: +81-23-6285349
Fax: +81-23-6285351

Materials and methods

Subjects

Seventy-seven patients, 51 females and 26 males aged 1 to 80 years (mean 49.9) with astrocytic tumours, underwent surgery including biopsy in the neurosurgical department of Yamagata University School of Medicine from May 1994 to April 2001. Histological diagnosis was pilocytic astrocytoma in three cases, fibrillary astrocytoma in 22, protoplasmic astrocytoma in one, gemistocytic astrocytoma in two, pleomorphic xanthoastrocytoma in one, anaplastic astrocytoma in 18, glioblastoma multiforme in 28, and "other" in two.

Classification of brain tumours by magnetic resonance imaging

The brain tumour bulk was considered to be the area on T1-weighted MR images enhanced with gadolinium (Gd) or the area of abnormal intensity, if no enhancement was present. The tumours were classified into three types according to the appearance on MRI of the bulk portion as follows.

a. Well-circumscribed: the lesion boundaries were clear, even in cases showing abnormal intensity without Gd enhancement. The lesion did not extend outside the sulcus, and the boundary with the subcortex was also identifiable. The position and depth of the sulcus provided good indicators of tumour extent, and total tumour removal was achieved easily (Fig. 1).

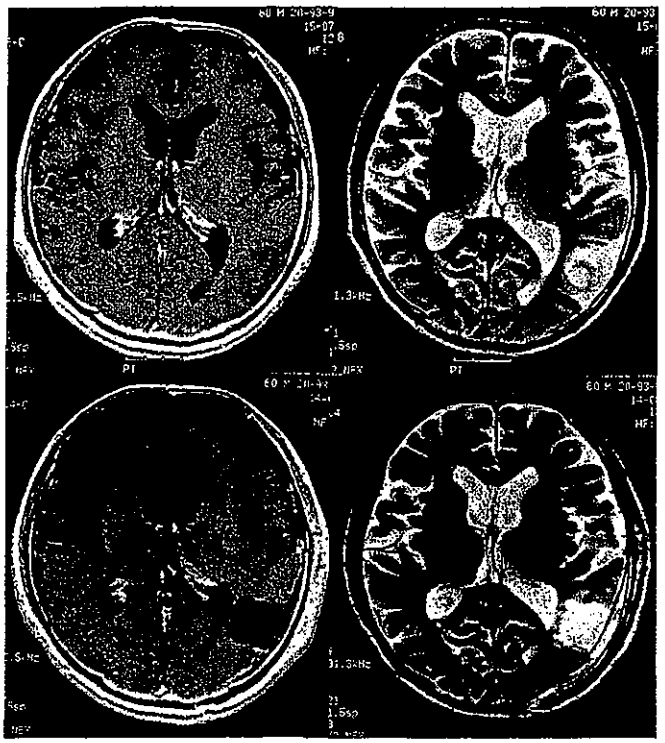

Fig. 1 The well-circumscribed type of astrocytic tumour. Preoperative (*upper*) and postoperative (*lower*) Gd-enhanced T1- and T2-weighted axial MRI scans. The margin of the tumour bulk can be distinguished from surrounding normal brain. Extent of tumour removal is over 95%

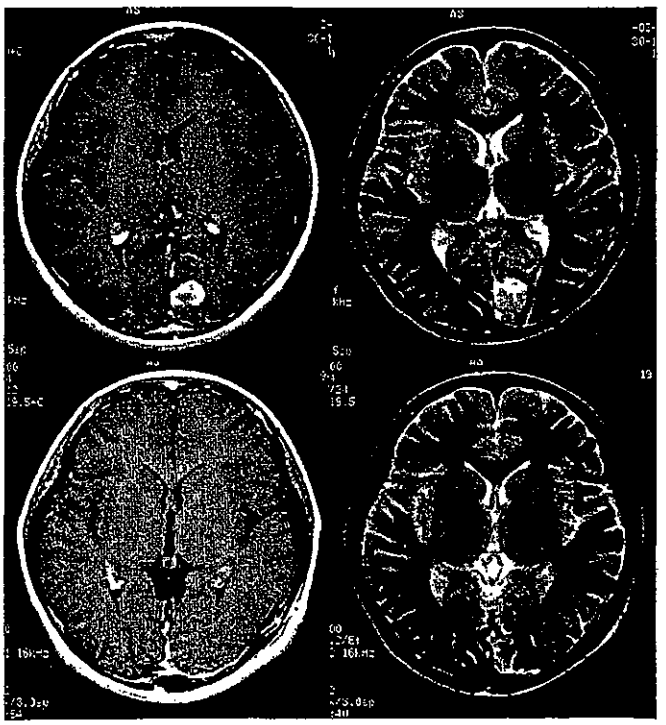

Fig. 2 The localised type of tumour. Preoperative (*upper*) and postoperative (*lower*) Gd-enhanced T1- and T2-weighted axial MRI scans. Tumour bulk is located in only one gyrus. Extent of tumour removal is over 95%

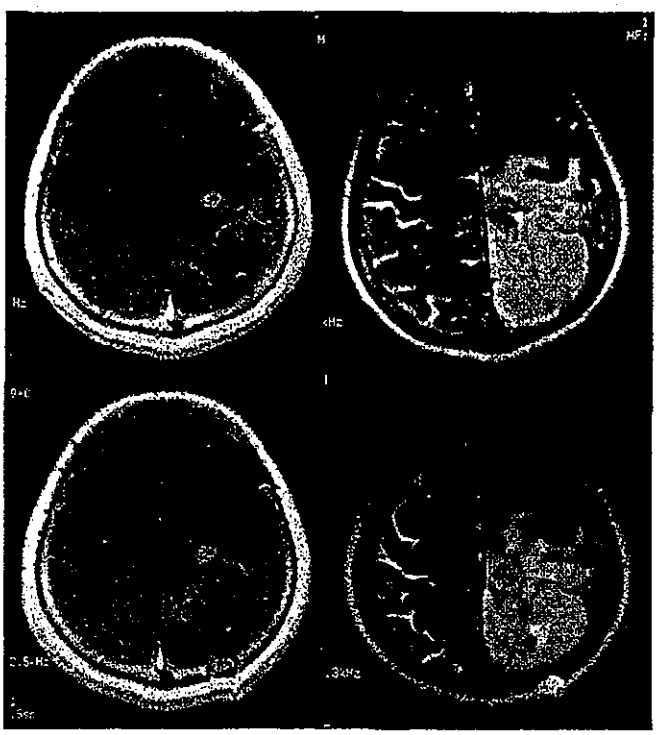

Fig. 3 The diffuse type of *tumour*. Preoperative (*upper*) and postoperative (*lower*) Gd-enhanced T1- and T2-weighted axial MRI scans. The margin of the tumour bulk is not clear, therefore only a biopsy was performed

b. Localised: the tumour was demarcated by the sulcus and localised in one gyrus. The boundary with the subcortex was also identifiable. Total tumour removal could be achieved by sulcotomy and en bloc removal of the gyrus including the lesion (Fig. 2).
c. Diffuse: the boundary of the enhanced tumour was unclear, or the area of abnormal intensity was extensive. Total tumour removal was rarely achieved (Fig. 3).

Assessment of brain tumour bulk removal

The maximum dimensions of the brain tumour bulk were measured in anteroposterior, lateral, and superioinferior directions on pre- and postoperative MR images, and the percentage of removal was calculated from them (Fig. 4). The extent of removal was divided into four categories: total, indicating ≧95% removal (including 100%); subtotal, indicating 65–94% removal; partial, indicating ≦64% removal; and biopsy [1]. Differentiation between 95% and 100% removal was impossible due to the presence of surrounding oedema or haematoma caused by the tumour or surgery.

Results

Classification of brain tumours

The well-circumscribed type was the most common, occurring in 56 patients (72.7%), followed by the diffuse type in 19 (24.7%) and the localised type in two (2.6%).

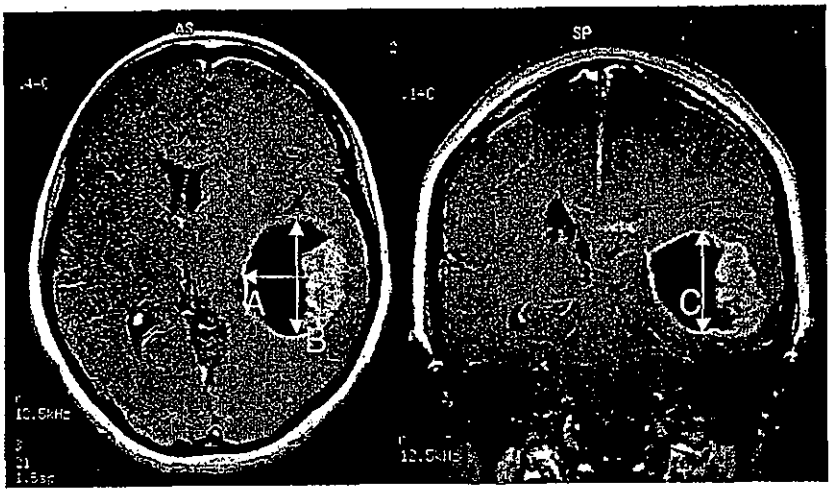

Fig. 4 Evaluation method of the extent of tumour resection in MRI scans. Tumour volume is calculated using horizontal (*A*), anteroposterior (*B*), and vertical (*C*) maximum tumour diameters. (ABC/2). (1–postoperative tumor volume/preoperative tumor volume)×100% is the formula for calculating the extent of tumour resection

Table 1 Tumour type and extent of tumour removal

	Well circumscribed	Diffuse	Localised	All
Total removal	43	2	2	47 (61%)
Subtotal removal	3	2	0	5 (6.5%)
Partial removal	2	2	0	4 (5.2%)
Biopsy	8	13	0	21 (27.3%)
Total	56 (72.7%)	19 (24.7%)	2 (2.6%)	77

Table 2 Summary of reported series. *BTRJ* Brain Tumour Registry of Japan

Author	Year	Total cases	Total removal cases	Ratio of total removal
Laws	1984	461	57	12.4
Soffietti	1989	81	19	23.5
Shaw	1988	126	23	18.3
North	1990	77	10.4	–
McCormack	1992	53	10	18.9
Philippon	1993	118	16	13.6
Suzuki	1988	503	106	21.1
BTRJ	1978–1987	3,092	441	14.3
Present study	1994–2001	77	47	61.0

Removal of the brain tumour bulk

Total removal was achieved in all of the localised and 43/56 of the well-circumscribed types, but in only two of 19 cases of the diffuse type. Partial and subtotal removal was performed in nine patients. Biopsy was performed in 21 patients, of which 13 were of the diffuse type. Eight of 56 cases of the well-circumscribed type underwent biopsy, since the brain tumour bulk was located in eloquent areas or the deep brain (Table 1).

Discussion

The study found that the well-circumscribed type, with an identifiable boundary between the brain tumour bulk and surrounding normal tissue on MRI, was easily removed at the high percentage of ≧95% in 43 of 56 cases. The overall rate of ≧95% removal in the present series assessed by MRI was 47 of 77 cases (61%), suggesting an improvement in total removal over series evaluated by CT, with rates of around 20% and a maximum of 23.5% [3, 5, 6, 7, 8, 9] (Table 2).

Conclusions

Our sulcus opening and gyrectomy technique can remove the gyrus and lesion safely and reliably only when the brain tumour bulk and adjacent sulcus can be identified by preoperative MRI [2, 4]. This method represents an improvement in the rate of brain tumour removal, achieving radical glioma removal and a higher tumour removal rate than that based on CT.

References

1. Kayama T, Kumabe T, Tominaga T, Yoshimoto T (1996) Prognostic value of complete response after the initial treatment for malignant astrocytoma. Neurol Res 18:321–324
2. Kokubo Y, Takamasa K, Saito S, Kuroki A, Saino M, Nakajima M (1997) A case of tuberous sclerosis presenting intractable

adversive seizure, successfully resected with the technique of "gyrectomy". No Shinkei Geka 25:959–964
3. Laws ER Jr, Taylor WF, Clifton MB, Okazaki H (1984) Neurosurgical management of low-grade astrocytoma of the cerebral hemisphere. J Neurosurg 61:665–673
4. Maruya J, Takamasa K, Kuchiki H, Ando T (1997) Localized glioma in the occipital lobe manifesting as scintillating scotoma: case report. No Shinkei Geka 25:265–269
5. McCormack BM, Miller DC, Budzilovich GN, Voorhees GJ, Ransohoff J (1992) Treatment and survival of low-grade astrocytoma in adults—1977–1988. Neurosurgery 31:636–642
6. North CA, North RB, Epstein JA, Piantadosi S, Wharam MD (1990) Low-grade cerebral astrocytomas. Survival and quality of life after radiation therapy. Cancer 66:6–14
7. Philippon JH, Clemenceau SH, Fauchon FH, Foncin JF (1993) Supratentorial low-grade astrocytomas in adults. Neurosurgery 32:554–559
8. Shaw EG, Daumas-Duport C, Scheithauer BW, Gilbertson DT, O'Fallon JR, Earle JD, Laws ER Jr, Okazaki H (1989) Radiation therapy in the management of low-grade supratentorial astrocytomas. J Neurosurg 70:853–861
9. Soffietti R, Chio A, Giordana MT, Vasario E, Schiffer D (1989) Prognostic factors in well-differentiated cerebral astrocytomas in the adult. Neurosurgery 24:686–692

中心前回に存在する神経膠腫の解剖学的所見による分類

隈部　俊宏[1]・中里　信和[2]・永松　謙一[1]
岩崎　真樹[1]・鈴木　匡子[3]・吉本　高志[1]

Abstract

In order to maximize tumor resection of gliomas at precentral gyrus while preserving motor function, these tumors should be classified according to the anatomical characteristics. They can be classified into 4 groups at the cortical level as follows: Group 1, The tumor is located medial to the inverted omega-shape (IOS) on the axial images of the central sulcus. The tumor also involves the central sulcus side of precentral gyrus; Group 2, The tumor is located medial to the IOS. The tumor does not involve the central sulcus side of precentral gyrus; Group 3, The tumor is located lateral to the IOS; Group 4, The tumor infiltrates diffusely at the precentral gyrus. In addition, these tumors can be classified into 3 groups at the subcortical level as follows: Group A, All the descending motor tracts originating from the hand-digit to the leg motor cortices are damaged; Group B, Some parts of them are damaged; Group C, All of them are preserved. Well circumscribed gliomas at the precentral gyrus classified as group 2C and 3C could be resected without permanent motor deficits.

Key words: central sulcus, descending motor pathway, glioma, precentral gyrus, primary motor cortex

はじめに

運動機能温存を図った上での積極的手術摘出を前提として，中心前回に存在する神経膠腫に関して解剖学的存在形式からの分類を行った．

方　法

1996年12月以降当科にて術前および術中機能マッピングを行って中心前回自体に可及的摘出操作を及ぼした神経膠腫23例を対象とし，画像所見，脳機能マッピング結果，摘出状態，摘出前後の神経学的所見を検討した．

結　果

中心前回に存在する神経膠腫は，皮質レベルでの存在形式から，Group 1：手指運動野より内側上方で中心溝側の皮質に腫瘍が及ぶもの，Group 2：手指運動野より内側上方で腫瘍がより前方に存在し中心溝側の皮質が正常な状態で残るもの，Group 3：顔面運動野より外側下方，Group 4：びまん性に中心前回に浸潤するもの，の4つに分類できると考えられた（Fig. 1, 2）．さらに白質レベルで錐体路との兼ね合いから，Group A：手指から下肢までの錐体路全体に浸潤するもの，

[1] 東北大学大学院神経外科学分野；[2] 広南病院脳神経外科；[3] 東北大学大学院医学系研究科高次機能障害学分野

Classification of the Gliomas at the Precentral Gyrus According to the Anatomical Characteristics

Toshihiro KUMABE [1], Nobukazu NAKASATO [2], Ken-ichi NAGAMATSU [1], Masaki IWASAKI [1], Kyoko SUZUKI [3], Takashi Yoshimoto [1]

[1] Department of Neurosurgery and [3] Division of Neuropsychology, Department of Disability Medicine, Tohoku University Graduate School of Medicine; [2] Department of Neurosurgery, Kohnan Hospital, Sendai

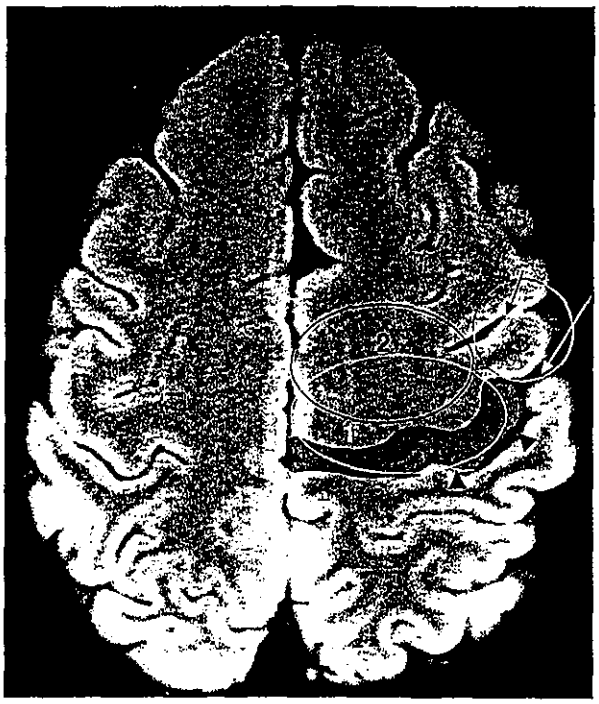

Fig. 1 Axial magnetic resonance image of a formalin fixed brain specimen. *White arrows, black arrows,* and *arrowheads* indicate the central sulcus, precentral sulcus, and the inverted omega-shaped sulcus. *Red shaded area* indicate the hand-digit, arm, and leg motor cortices. *Yellow, blue, and green circle* indicate the location of the tumor classified into the group 1, 2, and 3, respectively.

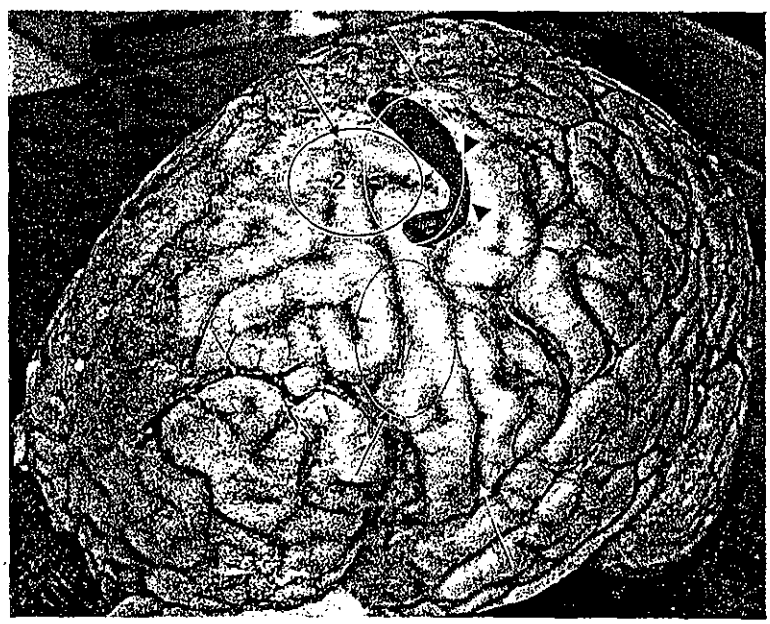

Fig. 2 Upper lateral oblique view of the same brain specimen used in Fig. 1. *White arrows, black arrows,* and *arrowheads* indicate the central sulcus, precentral sulcus, and the posteriorly directed middle genu corresponded to the inverted omega-shaped sulcus on the axial magnetic resonance image. *Red shaded area* indicate the hand-digit, arm, and leg motor cortices. *Yellow, blue, and green circle* indicate the location of the tumor classified into the group 1, 2, and 3, respectively.

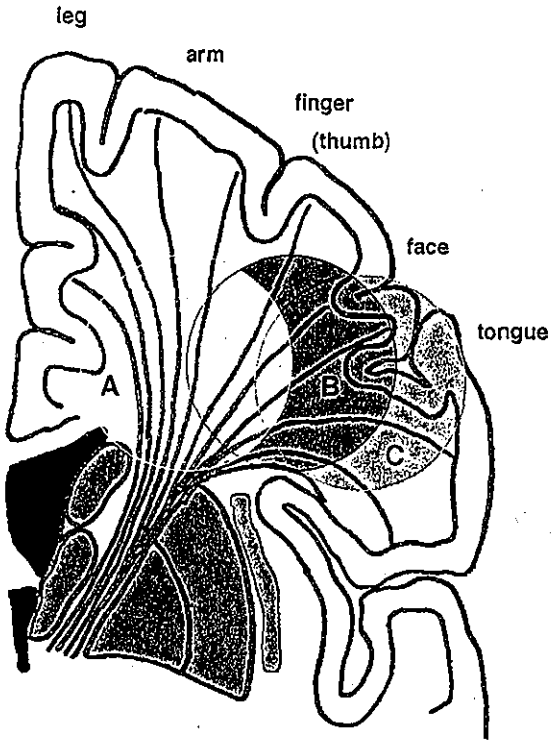

Fig. 3　Schematic drawings of the descending motor tract in the coronal plane. *Yellow, blue, and green shaded circle* indicate the location of the tumor classified into the group A, B, and C, respectively.

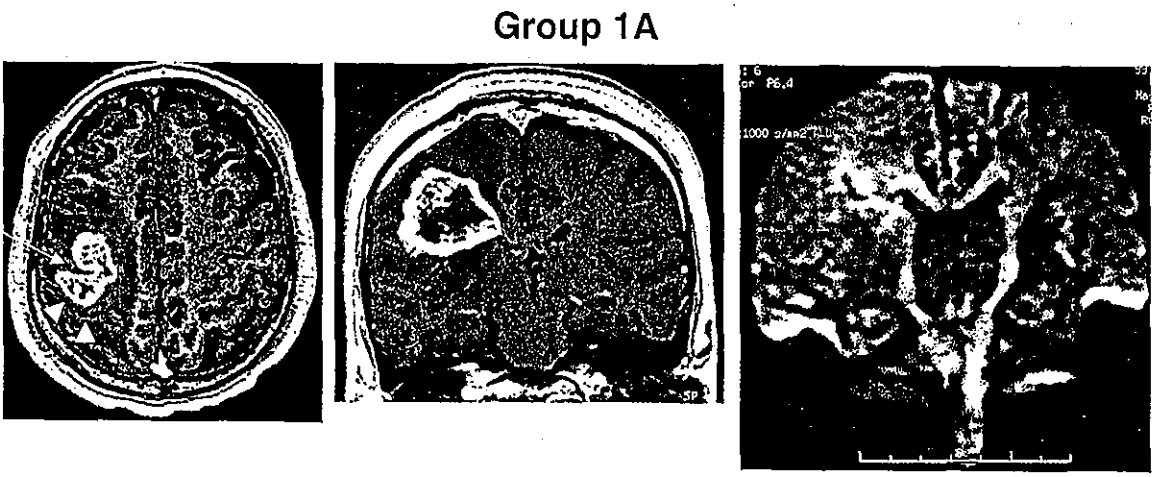

Fig. 4　Group 1A: Axial (*left*) and coronal (*center*) gadolinium enhanced T1-weighted and coronal diffusion-weighted (*right*) magnetic resonance images. *Arrow* and *arrowheads* indicate the central sulcus and the inverted omega-shaped sulcus, respectively.

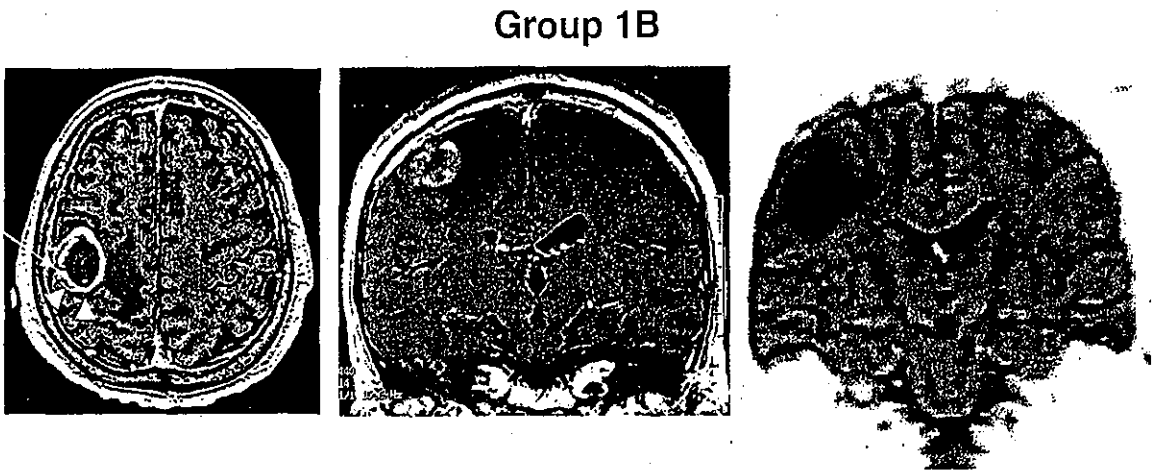

Fig. 5 Group 1B: Axial (*left*) and coronal (*center*) gadolinium enhanced T1-weighted magnetic resonance images and fiber mapping image (*right*). Fiber mapping images showing nerve fibers running in the superior-inferior, left-right, or anterior-posterior directions in red, green, or blue, respectively. *Arrow* and *arrowheads* indicate the central sulcus and the inverted omega-shaped sulcus, respectively.

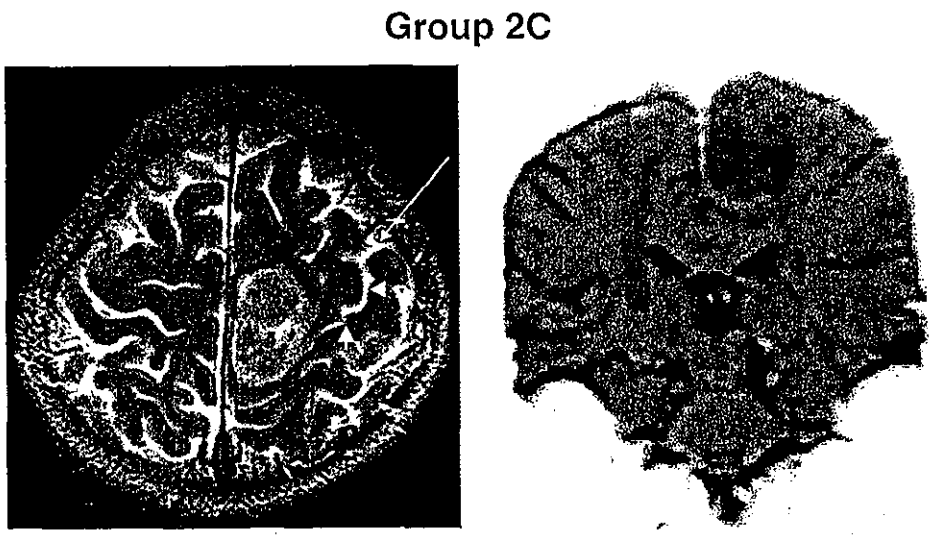

Fig. 6 Group 2C: Axial T2-weighted and fiber mapping image. *Arrows* and *arrowheads* indicate the central sulcus and the inverted omega-shaped sulcus, respectively (Modified from reference 4).

Group B：手指から下肢までの錐体路の一部に浸潤するもの，Group C：手指から下肢までの錐体路に浸潤しないもの，の３つに分類できると考えられた（Fig. 3）．

Group 1 では術前から麻痺症状が認められ，永続的な運動機能障害は回避できなかった（Fig. 4，5）．ただし Group 1B に分類される症例で，手指運動野に腫瘍が存在し，下肢運動野およびそこからの錐体路が温存できれば下肢運動機能は温存され，下肢運動野に腫瘍が存在し，上肢運動野およびそこからの錐体路が温存できれば，上肢と手指の運動機能を温存することが可能であった（Fig. 5）．Group 2 は後方の皮質と後方から回旋して下降する錐体路温存（Fig. 6），Group 3 は下降する錐体路温存（Fig. 7 ～ 9），が可能であれば，永続

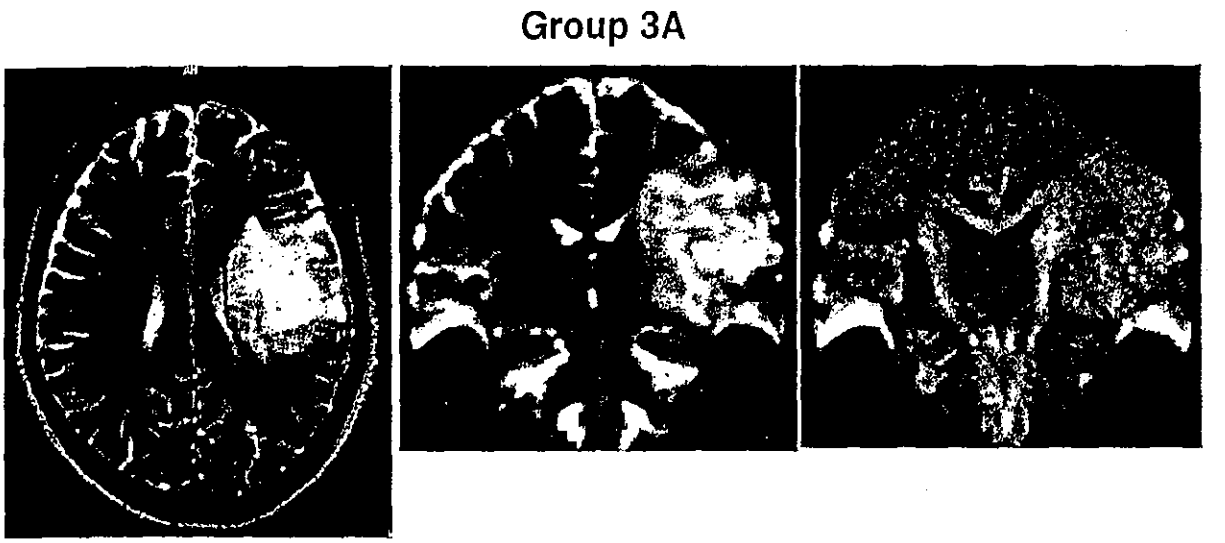

Fig. 7　Group 3A: Axial (*left*) and coronal (*center*) T2-weighted and coronal diffusion-weighted (*right*) magnetic resonance images.

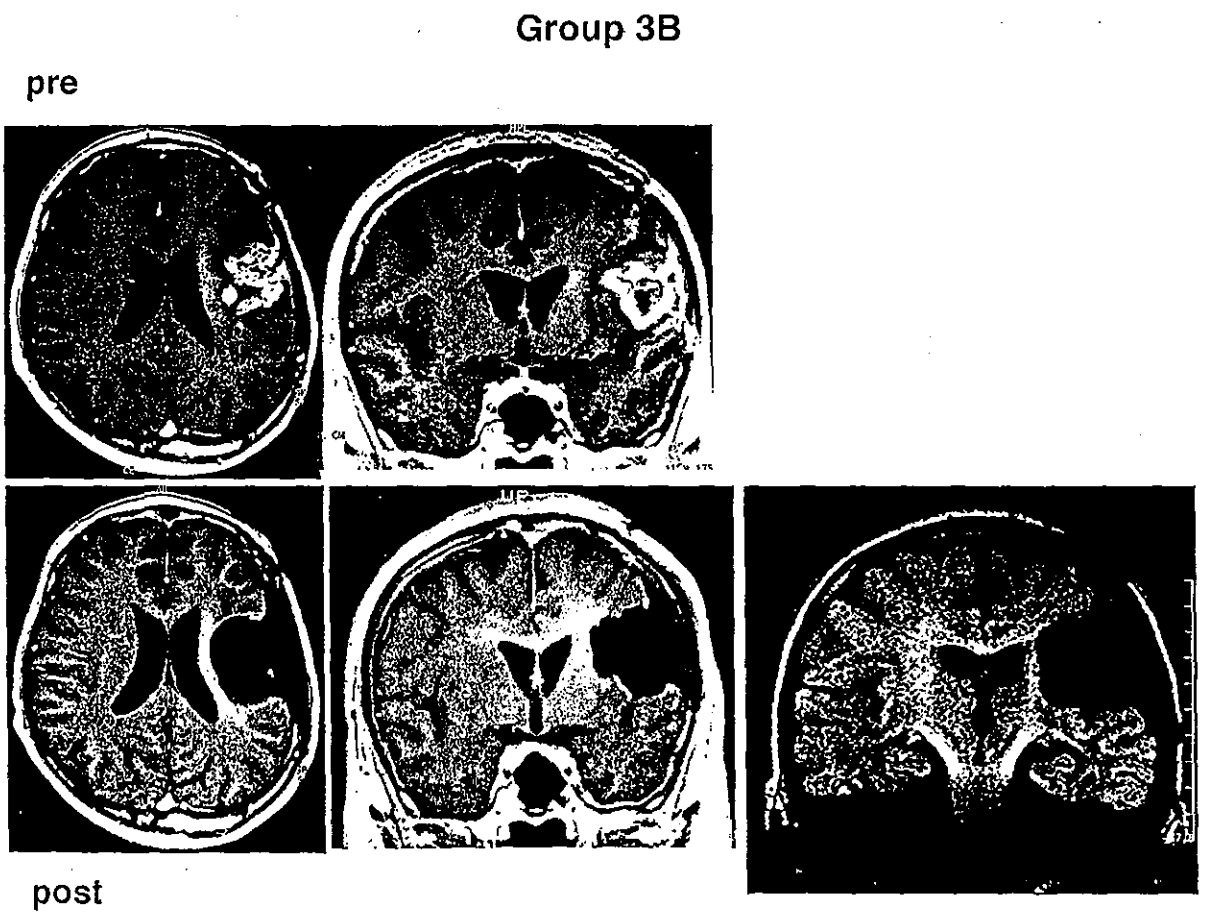

Fig. 8　Pre- (upper row) and postoperative (lower row) images of Group 3B: Axial (*left*) and coronal (*center*) gadolinium enhanced T1-weighted and coronal diffusion-weighted (lower right) magnetic resonance images.

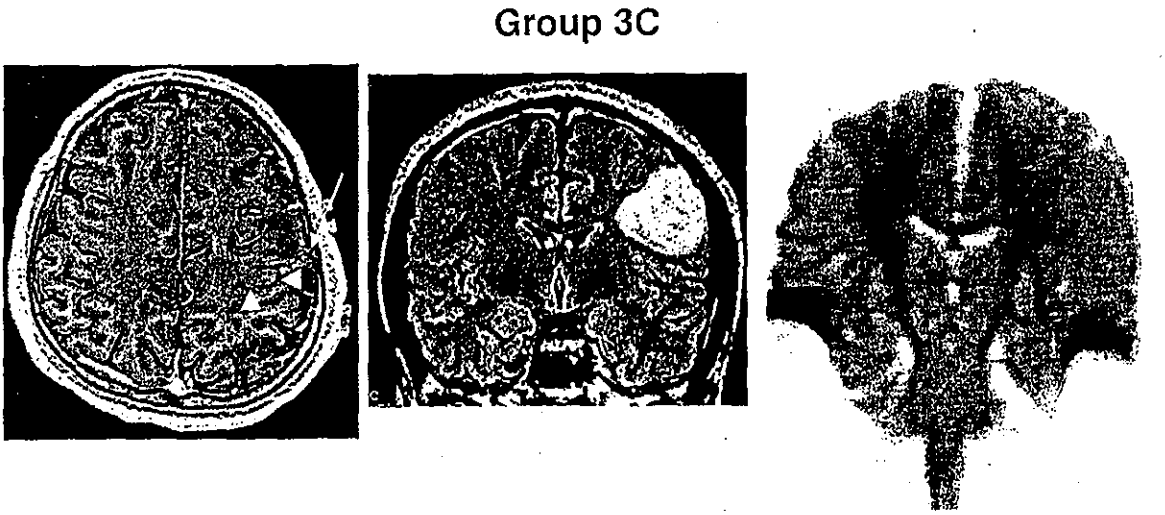

Fig. 9 Group 3C: Axial gadolinium enhanced T1-weighted (*left*) and coronal FLAIR (*center*) magnetic resonance images and fiber mapping image (*right*). *Arrow* and *arrowheads* indicate the central sulcus and the inverted omega-shaped sulcus, respectively (Modified from reference 3).

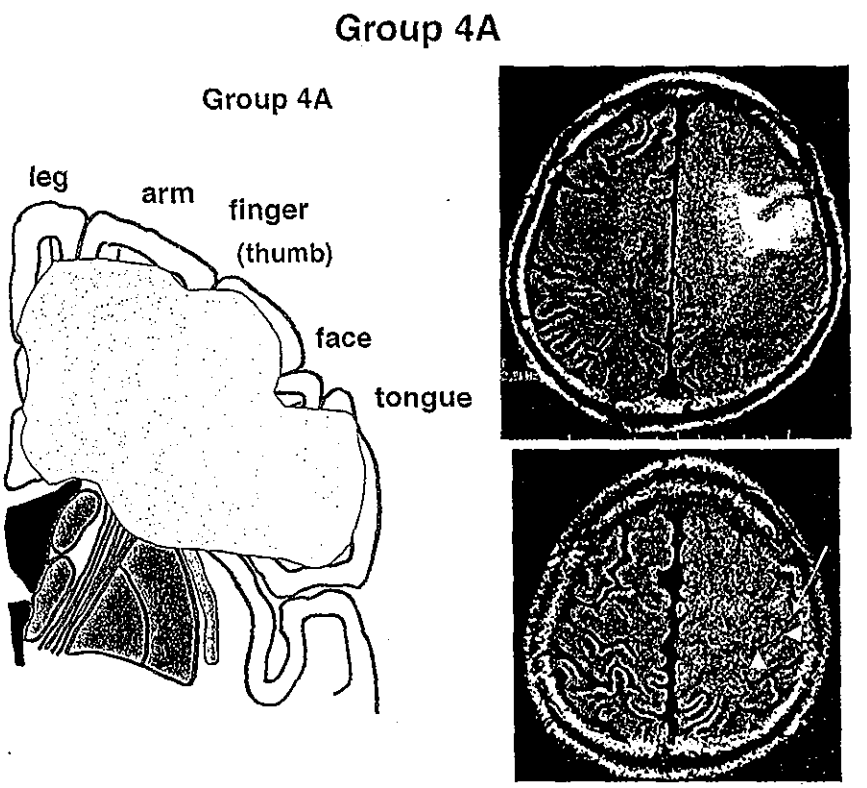

Fig. 10 Group 4A: Schematic drawings of the descending motor tract in the coronal plane (left column) and axial FLAIR magnetic resonance images (right column). The tumor is indicated by the shaded area. *Arrow* and *arrowheads* indicate the central sulcus and the inverted omega-shaped sulcus, respectively.

的な麻痺症状なく摘出可能であった．今回は言語機能に関しては言及しないが，Group 3の優位半球症例では失構音を特徴とする症状が残存した．Group 4は全摘出術の適応にはならなかった（Fig. 10）．

Group Aは，運動機能障害なしでの全摘出は不可能であった（Fig. 4, 7）．Group Bは浸潤された錐体路機能が障害されることは免れないが，前述のごとく，皮質および白質レベルで浸潤されていない運動機能は温存することが可能であった（Fig. 5, 8）．Group Cは運動機能温存をした上での積極的な摘出術の適応となった（Fig. 6, 9）．

以上より，中心前回に存在してもgroup 2Cと3Cの神経膠腫に対しては，運動機能を温存した上での全摘出術が可能であった．

考　察

近年脳機能マッピングが進み，ニューロナビゲーションシステムとの併用を行うことにより，これまで全てがeloquent areaと考えられていた中心前回に摘出操作を及ぼすことが可能となってきた．言語機能を含めた高次脳機能に関してはまだ究明すべき内容が多く残されているが，運動感覚野に関しては解剖学的特徴と機能が確からしく相関することが分かってきた．「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構造の前方のモprecentral knobモに存在する」というYousryらの1997年の報告[6]は，この事実を明確にした重要な報告である．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運動機能障害なく摘出することが可能であると報告されている[3,5]．すなわちprecentral knobよりも外下方の腫瘍は，錐体路への障害を回避できれば摘出可能であるということになる．一方precentral knobよりも内上方の腫瘍は永続的な麻痺症状なしでは全て摘出できないのであろうか．Brodmannの大脳領野を見るとarea 4は決して中心前回全てではなく，中心溝側に偏在している．Geyerらは猿の脳解剖より，Brodmannのarea 4にあたるベッツ錐体細胞を有する一次運動野は，中心溝の直前に存在すると報告している[2]．T1強調画像の低信号域とT2強調画像の高信号域が一致する境界鮮明な，すなわち膨張性拡大形式を主とした神経膠腫で，術前運動機能障害を認めないgroup 2Cに分類される神経膠腫は全摘出可能であった[4]．摘出術の際，中心前回前後方向のどの範囲まで運動誘発が生ずるか，すなわち中心溝側のどこまでを皮質機能として残す必要があるかを，皮質刺激による機能マッピングで決定することは重要な手技であった．また，拡散強調画像によって描出された錐体路を含めた皮質脊髄路をニューロナビゲーションシステムに導入し手術に役立てようという試みが行われている[1]が，現時点では錐体路を放線冠より上方の皮質下領域で非侵襲的に確実に同定することは難しく，術中皮質下刺激により下降する錐体路を同定することは必須であると考えられた．

結　論

中心前回に存在する神経膠腫の術中の注意点および術後に出現する可能性のある機能障害は，解剖学的特徴からかなりの所まで予測可能であり，手術適応とその方法を考慮する上で今回の分類は役立つものと考えられる．

文　献

1) Coenen VA, Krings T, Mayfrank L,et al: Three-dimensional visualization of the pyramidal tract in a neuronavigation system during brain tumor surgery: First experiences and technical note. Neurosurgery 49:86-93, 2001
2) Geyer S, Matelli M, Luppino G, et al: Functional neuroanatomy of the primate isocortical motor system. Anat Embryol (Berl) 202:443-474, 2000
3) 隈部俊宏，中里信和，鈴木匡子，他：運動領野周辺神経膠腫に対する手術ム術前解剖学的および機能的マッピングの融合とニューロナビゲーションシステムおよび術中マッピング併用による運動機能温存手術ム　脳外誌 10 ： 56-64, 2001

4) 隈部俊宏，中里信和，岩崎真樹，他：中心前回に存在する神経膠腫の手術.脳外誌 11 ： 271-277, 2002
5) LeRoux PD, Berger MS, Haglund MM, et al: Resection of intrinsic tumors from nondominant face motor cortex using stimulation mapping: report of two cases. Surg Neurol 36:44-48, 1991
6) Yousry TA, Schmid UD, Alkadhi H, et al: Localization of the motor hand area to a knob on the precentral gyrus. Brain 120:141-157, 1997

[連絡先：〒 980-8574　仙台市青葉区星陵町 1-1
東北大学大学院神経外科学分野　隈部俊宏]

[Frontiers in Bioscience 8, e281-288, May 1, 2003]

CORRELATION OF MOLECULAR GENETIC ANALYSIS OF P53, MDM2, P16, PTEN, AND EGFR AND SURVIVAL OF PATIENTS WITH ANAPLASTIC ASTROCYTOMA AND GLIOBLASTOMA

Yukitaka Ushio, Kenji Tada, Shoji Shiraishi, Takanori Kamiryo, Naoki Shinojima, Masato Kochi, Hideyuki Saya

Department of Neurosurgery and Department of Tumor Genetics and Biology, Kumamoto University Medical School, Kumamoto, Japan

TABLE OF CONTENTS

1. *Abstract*
2. *Introduction*
3. *p53 mutation*
4. *MDM2 amplification / overexpression*
5. *p16 alteration*
6. *PTEN alteration*
7. *EGFR amplification / overexpression*
8. *Combined genetic alterations*
9. *References*

1. ABSTRACT

This article reviews studies on the correlation between genetic abnormalities in malignant astrocytic tumors and patient survival. It is almost certain that alterations of *PTEN* on chromosome 10 represent a significant unfavorable prognostic factor in glioblastoma patients. The association of alterations in *p53*, *MDM2*, *p16* or *EGFR* with the survival of patients with anaplastic astrocytoma or glioblastoma remains controversial. It is possible that the *p16* alteration and *EGFR* amplification are associated with poor survival in certain groups of patients and that there might be a relationship with age. Malignant transformation of astrocytic cells are driven by the sequential acquisition of genetic alteration. Therefore, it is reasonable to subgroup gliomas by their patterns of genetic alterations. However the studies that correlated the multiple genetic alterations with survival are still limitted.

Further studies on large cohorts are necessary to elucidate the genetic factors that affect the prognosis and response to therapy of patients with malignant gliomas and to develop effective management strategies.

2. INTRODUCTION

Malignant gliomas are the most common primary malignant neoplasms of the central nervous system. Most of these are anaplastic astrocytomas or glioblastomas. As these tumors are highly resistant to current treatment modalities including surgery, radiotherapy and chemotherapy, their prognosis is dismal. Even after multidisciplinary treatment, the median survival of patients with anaplastic astrocytoma is only around 4-5 years; for patients with glioblastoma it is less than 2 years. In each histological group, there is a considerable difference in response to therapy and wide range in survival, and the tumors are thought to be highly heterogeneous. In an attempt to establish individualized treatments and to improve the clinical management of these tumors, efforts have been made to subgroup them genetically (1). One of the approaches to the goal is to identify genetic alteration(s) that are relevant to tumor sensitivity to the treatment. This article reviews studies that investigated the correlation between the survival of patients with anaplastic astrocytoma and glioblastoma and the presence of single or multiple alterations of major genes thought to be importantly involved in tumorigenesis and/or tumor progression.

3. *P53* MUTATION

Analyses of the *p53* tumor suppressor status in brain tumors have demonstrated frequent alterations of the *p53* gene as well as stabilization of mutant and intact p53 proteins in tumor cells. *P53* mutations are predominantly found in astrocytic tumors; approximately 50-60 % of anaplastic astrocytomas (2,3) and 25-30% of glioblastomas (4,5) manifested these mutations. Tumor cells carrying *p53* mutations are resistant to apoptosis induced by DNA damage; overexpression of wild-type p53 enhances the radiosensitivity of glioma cells. However, the effect of *p53* mutations on the radio- and chemosensitivity of gliomas, especially glioblastomas, remains controversial. There are reports that *p53* gene mutations were associated with poor outcomes in pediatric brain tumors (6) and adult gemistocytic astrocytomas (7). Other studies using single-strand conformation polymorphism (SSCP) analysis or yeast functional assay found that the presence of *p53* mutations was a positive predictor for a response to radiation therapy (8) and for a good prognosis in patients with glioblastomas (9). On the other hand, in patients with astrocytic tumors, *p53* mutations were reportedly not associated with survival (10,11). We employed yeast functional assay and DNA sequencing to study the *p53* gene status in glioblastoma patients and found no significant difference in the average age of patients with and without the *p53* mutation. Multivariate analysis

adjusted for prognostic factors including the age, gender, and the Karnofsky performance status (KPS) of glioblastoma patients revealed that the mutation did not affect their progression-free or overall survival (12). These conflicting data may be attributable to differences in the patient populations studied, variations in the accuracy of histological diagnoses or tumor sampling, and the reliability of methodologies used for study.

In gliomas, not only mutant but also wild-type p53 proteins (13) are often stabilized by mechanisms that are currently not entirely understood. The turnover is sustained by proteolytic degradation which takes place in the cytoplasm and involves targeting of p53 at proteosomal degradation by the murine double minute 2 (MDM2). The increased stability of wild-type proteins is partly due to an alternatively spliced MDM2 which cannot degrade the target p53 protein (14,15). On the other hand, MDM2 overexpression with or without gene amplification(s) is observed mainly in glioblastomas without *p53* gene mutations (9,16). This is suggestive of p53 dysfunctional mechanisms other than mutation. $P14^{ARF}$ is an inhibitor of p53 proteolysis by MDM2 encoded on the *INK4a* locus that is frequently deleted in human tumors. *P53* mutations and $p14^{ARF}$ deletions have been shown as mutually exclusive in human glioblastoma (17). Ichimura et al. (2) reported that 76% of glioblastomas, 72% of anaplastic astrocytomas, and 67% of low-grade diffuse astrocytomas manifested deregulation of the p53 pathway either by *p53* gene mutation, *MDM2* amplification, or homozygous deletion/mutation of $p14^{ARF}$. This leads to the speculation that the p53 tumor suppression pathway is inactivated not only by *p53* mutation in the early stages of astrocytic tumorigenesis, and that most glioblastomas have acquired impairment of tumor suppression functions. Our preliminary data showed that anaplastic astrocytomas and glioblastomas differed in the distribution of the *p53* mutation locus, suggesting the existence of different mechanisms for the *p53* mutation in these two types of high-grade astrocytic tumors (9).

4. *MDM2* AMPLIFICATION / OVEREXPRESSION

MDM2 oncoprotein promotes cell survival and cell-cycle progression by inhibiting the p53 tumor suppressor protein (18). The *MDM2* gene which locates on 12q13-14 was found to be amplified in about 10-15% of high-grade gliomas, thus it represents the second most frequently amplified gene after the *EGFR* gene in these tumor types (19,20). Previous attempts to correlate *MDM2* gene amplification with survival produced contradictory results. There is a report that immunohistochemical positivity for MDM2 protein was associated with longer survival in patients with malignant astrocytic tumors(21); in other studies, immunohistochemical positivity for MDM2 protein or *MDM2* gene amplification was associated with shorter survival time (9,22). Others (23-25) found *MDM2* gene amplification to have no significant relevance to overall patient survival.

5. *P16* ALTERATION

The *p16* gene, which maps to chromosome 9p21, is a tumor suppressor gene that has been investigated in many human cancers including gliomas (26,27). In high-grade astrocytic tumors, its inactivation is the most common alteration in the p16-cdk4-cyclinD1-Rb pathway and occurs mainly through homozygous deletion (28-30). Homozygous deletion of the p16 gene has been reported in approximately 10-50 % of anaplastic astrocytomas and 30-70 % of glioblastomas. These divergent results may at least partly be attributable to the different methods employed for the detection of homozygous deletions in clinical tumor samples (2,29,31-38). As the p16 protein regulates cell-cycle control at the G1-S transition, its inactivation leads to loss of cell-cycle control and consequently, increased proliferation. High-grade astrocytic tumors with *p16* homozygous deletion had higher Ki-67 indices than those without the deletion (34). At the same chromosome 9p21 (*INK4a* locus), the $p14^{ARF}$ gene is known to be located and shares exon 2 and 3 with *p16* gene, but encodes a distinct protein which participated in p14-MDM2-p53 pathway (39,40). Recently, it has been elucidated that in astrocytic tumors, homozygous deletion of the *p16* gene is associated with co-deletion of the $p14^{ARF}$ gene (2,17,36). Although no studies have addressed the correlation between $p14^{ARF}$ alteration alone and survival in patients with high-grade astrocytic tumors, *p16* alteration has been investigated in relation to survival. While the correlation between *p16* inactivation and survival in patients with high-grade astrocytic tumors has been investigated, the prognostic value of *p16* abrogation remains controversial. Studies using multiplex polymerase chain reaction (PCR) assay (37,41), Western analysis (42), or immunohistochemical methods (43) detected no significant correlation between homozygous deletions of the *p16* gene or loss of p16 protein expression, and survival in patients with high-grade astrocytic tumors (37,41-43). On the other hand, Newcomb et al. (11) found that in glioblastoma patients older than 61 years, p16 immunonegativity tended to signal a poor prognosis. In high-grade astrocytic tumors, p16 immunonegativity was an independent indicator of a poor prognosis according to univariate and multivariate analyses adjusted for age, tumor histology, extent of surgery, and the Ki-67 labeling index (LI) (44). Using quantitative real-time PCR assay of 105 primary gliomas, Labuhn et al. (45) recently demonstrated that deletion of the *INK4a* locus, where *p16*- and $p14^{ARF}$ gene are located at the 9p21 locus, affected both genes. They separated glioblastoma patients into those with homozygous deletion at the *INK4a* locus and those with retention of both copies of the locus and compared their survival. The only significant difference in survival was recorded for patients older than 50 years. We studied patients with supratentorial glioblastoma who had been treated with surgery and postoperative radio- and chemotherapy and examined homozygous deletion of the *p16* gene (exon 2) using multiplex PCR (38). We chose this assay because in glioma tissue samples, the results of multiplex PCR were highly concordant with data obtained by fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) (46). When we subjected primary tumor tissue to multiplex PCR, we performed titration experiments to confirm the validity of the results (38). We detected *p16* homozygous deletion in 30.4% of all patients; this was true in 30.8% male and

30.0% female patients. Cox proportional hazard regression analysis, adjusted for age at surgery, the KPS score, the extent of surgical resection, and the MIB-1 LI revealed that homozygous deletion of the *p16* gene was associated with neither overall- nor progression-free survival in our glioblastoma patients. However, in male patients, this deletion was significantly correlated with poor prognosis. The results of both Labuhn et al. (45) and our study suggest that homozygous deletion of the *INK4a* locus is an unfavorable prognostic factor in certain subgroups of glioblastoma patients (38,45). The gender difference revealed by our study concerning the effect of homozygous deletion of the *p16* gene on survival requires further investigation.

The mechanisms of *p16* inactivation other than homozygous deletion include mutation of the gene and transcriptional repression due to hypermethylation of the 5'CpG island in the promoter region (47-50). However, these abrogations are rare in astrocytic tumors (51-53) and to our knowledge, no studies have addressed the correlation between these abrogations and the survival of patients with high-grade astrocytic tumors. Most glioblastomas manifest molecular genetic alteration of at least one component in each of the Rb and p53 pathways (2,17,29,30,33). In addition, alterations of individual components appear inversely correlated in each of these pathways (17,29,30,33,35). To understand their possible effects on the survival of patients with high-grade astrocytic tumors, systematic investigation of these pathways is necessary (54). Presumably, homozygous deletion of the *INK4a* locus in high-grade astrocytic tumors is the most effective means of abrogating both the Rb and p53 pathways.

6. *PTEN* ALTERATION

The most common chromosomal abnormality in high-grade gliomas is the loss of all or part of chromosome 10. This is true in approximately 30-70% of anaplastic astrocytomas and 60-95% of glioblastomas (55-58). Candidate tumor suppressor genes on chromosome 10 that may be involved in tumorigenesis and/or tumor progression have been identified. They include *PTEN* at 10q23.3 (59), *FGFR2* at 10q25.3-26 (60), *DMBT1* at 10q25.3-26.1 (61), *LGI1* at 10q24 (62), and "*h-neu*" at 10q25.1 (63). The most important tumor suppressor among them is the *PTEN* (also known as *MMAC1* (64) / *TEP1* (65)) gene. PTEN negatively regulates the phosphatidylinositol 3-kinase (PI3K)-Akt pathway, and thereby affects control of the cell-cycle and cell survival (66,67). Additionally, PTEN inhibits focal adhesion, spreading, and migration by dephosphorylating focal adhesion kinase, and also regulates tumor-induced angiogenesis (68,69).

The correlation between loss of heterozygosity (LOH) for chromosome 10 and the survival of glioma patients has been examined. Using microsatellite analysis, Lin et al. (70) studied two loci including *MMAC/PTEN* and *DMBT1* for LOH in patients with various histological types of glioma. They found that LOH around *MMAC/PTEN* was a significant unfavorable prognostic factor in patients with anaplastic astrocytoma and glioblastoma; their survival was not significantly affected by the presence or absence of LOH in the *DMBT1* region. In their reverse transcription PCR (RT-PCR) study, Sano et al. (71) examined the expression of *MMAC/PTEN* in high-grade glioma patients and found that those whose tumors expressed high levels of *MMAC/PTEN* had a significantly better prognosis. We used microsatellite analysis to investigate the correlation between survival and LOH for several loci on chromosome 10 where candidate tumor suppressor genes for the oncogenesis of high-grade astrocytomas are thought to reside (72). We found that in patients with glioblastoma, the only statistically significant predictor of overall survival, identified by both univariate and multivariate analyses, was LOH for *PTEN/MMAC1*. The LOH frequency at each locus manifested a different pattern in anaplastic astrocytomas and glioblastomas. In anaplastic astrocytomas, telomeric regions deleted more frequently than *PTEN/MMAC1*. This finding coincides with the speculation (70,73) that LOH at these loci may represent an early genetic event in the progression of astrocytic tumors. In our study, the frequency of LOH was significantly higher in older than younger patients with anaplastic astrocytomas. This result too was consistent with that of Lin et al. (70) who demonstrated that the frequency of LOH for *MMAC/PTEN* significantly increased with age in patients with anaplastic astrocytoma as well as those with glioblastoma.

PTEN is altered by mechanisms other than LOH, including mutations, homozygous deletion, and methylation defects. In all informative cases, *PTEN* mutations occured in tumors with LOH on chromosome 10q, suggesting the inactivation of this gene by a 2-hit mechanism. The mutations were reported in approximately 20-40% of glioblastomas (55,56,74). Among studies investigating whether the *PTEN* mutation is associated with survival in patients with high-grade astrocytic tumors, a small cohort of pediatric patients suggested the correlation of *PTEN* mutation and survival (75). Using denaturing gradient gel electrophoresis (DGGE) followed by DNA sequencing, Zhou et al. (76) found no significant correlation between the *PTEN* mutation and survival in glioblastoma patients. Other studies showed the same results (41,76).

7. *EGFR* AMPLIFICATION / OVEREXPRESSION

The most frequent oncogenic alteration in glioblastomas is *EGFR* gene amplification resulting in the overexpression of EGFR, a transmembrane tyrosine kinase receptor (78-81). *EGFR* amplification is thought to be present in 30-50% of all glioblastomas and to occur more frequently in primary (de novo) glioblastomas (24,78,81-89). It confers to cells advantages of growth and invasiveness, and radio- and chemo-resistance (90-93). Furthermore, glioblastomas with *EGFR* amplification frequently exhibit a variety of *EGFR* alterations and mutations, especially *EGFRv•* (also known as *del2-7EGFR* and *•EGFR*), whose extracellular ligand-binding domain is truncated; *EGFRv•* is constitutively activated and enhances tumorigenicity in vivo (90,93-98). While there is evidence that *EGFR* amplification may signal a less favorable

prognosis in glioblastoma patients, the results of clinical studies were not conclusive.

In a study using differential PCR assay, multivariate analysis assigned no statistical value to *EGFR* amplification with respect to the survival of patients with astrocytic gliomas (85). Simmons et al. (99), who assessed EGFR overexpression in glioblastoma patients by immunohistochemical- and *p53* mutation by immunopositivity studies as well as by SSCP found no association of these alterations with survival. However, when they separated patients according to age (younger vs. older than median age), univariate analysis revealed that EGFR overexpression was associated with better prognosis in the older group; there was no such association in the younger patient group. Their multivariate analysis demonstrated that EGFR overexpression was an independent unfavorable prognostic factor in younger patients whose *p53* statuses was normal. Barker et al. (100) found positive EGFR immunoreactivity to be an independent significant predictor of a poor radiation response in glioblastoma patients; their multivariate analysis detected no significant correlation between the immunoreactivity or mutation status of *p53* and tumor radiation sensitivity. Feldkamp et al. (101), who assessed *EGFR* and *EGFRv•* expression by RT-PCR, Western blotting, and immunohistochemistry in a small cohort of glioma patients, reported that those with EGFRv••-positive tumors survived for shorter periods than did patients with EGFRv••-negative tumors, although the difference was not statistically significant by the Log-rank test.

8. COMBINED GENETIC ALTERATIONS

There is general consensus that malignant transformation of astrocytic cells is a multistep process driven by the sequential acquisition of genetic alterations. Therefore, it is reasonable to sub-categorize gliomas by their patterns of genetic alterations. The observed different combinations of *p53* mutations, LOH on chromosomes 17p or 10, and *EGFR* amplification, have led to the hypothesis that there exist subsets of glioblastomas with distinct genetic alterations; i.e. primary (de novo) and secondary glioblastomas (87,89). The former are seen more frequently in elderly patients and are characterized by *EGFR* amplification and LOH on chromosome 10 without *p53* mutation; the latter occur more frequently in younger patients and are characterized by *p53* mutation and LOH of chromosome 17p. While there was general agreement regarding the proposed sub-classification of gliomas, there are only a few reports that studied survival significance related to the two subsets.

An extensive study on 80 glioblastoma patients showed no significant association of altered expression of *p16*, *p53*, *EGFR*, *MDM2* or *Bcl-2* with survival (11). Leenstra et al. (102) studied *EGFR* alteration by dual-probe FISH and differential PCR, *PTEN* abnormality by FISH and PCR-based analysis, and *p53* by PCR-based analysis. They classified their 75 patients with high-grade astrocytoma into 4 groups according to genetic changes in the tumors: *p53* gene alterations without complete LOH of chromosome 10 (Group 1); complete LOH of chromosome 10 only (Group 2); *p53* gene alterations plus complete LOH of chromosome 10 (Group 3); complete LOH for chromosome 10 plus *EGFR* gene amplification (Group 4). Multivariate analysis adjusted for age and gender revealed that complete LOH for chromosome 10 plus *EGFR* gene amplification (Group 4) was a significantly more unfavorable prognostic factor than *p53* gene alteration alone. Simmons et al. (99), who investigated EGFR overexpression in glioblastoma patients by immunohistochemical methods, p53 immunopositivity, and *p53* mutation by SSCP analysis, found a statistically significant association of EGFR overexpression with worse survival in younger patients with wild-type *p53* but not those with *p53* alterations.

Investigations to correlate gene alterations in malignant gliomas with survival have produced contradictory results. Although the reasons for these divergent findings remain unclear, they reflect different methodologies and differences among the patient populations studied. Systematic large-scale studies are needed to obtain important information regarding the various genetic factors that affect the prognosis and response to therapy of patients with malignant gliomas. Such studies will also facilitate the development of better management strategies.

9. REFERENCES

1. Louis D. N, E. C. Holland & J. G. Cairncross: Glioma classification: a molecular reappraisal. *Am J Pathol* 159, 779-86 (2001)

2. Ichimura K, M. B. Bolin, H. M. Goike, E. E. Schmidt, A. Moshref & V. P. Collins: Deregulation of the p14ARF/MDM2/p53 pathway is a prerequisite for human astrocytic gliomas with G1-S transition control gene abnormalities. *Cancer Res* 60, 417-424 (2000)

3. Rasheed B. K, R. E. McLendon, J. E. Herndon, H. S. Friedman, A. H. Friedman, D. D. Bigner & S. H. Bigner: Alterations of the TP53 gene in human gliomas. *Cancer Res* 54, 1324-1330 (1994)

4. Louis D. N: The p53 gene and protein in human brain tumors. *J Neuropathol Exp Neurol* 53, 11-21 (1994)

5. Ohgaki H, B. Schauble, A. zur Hausen, K. von Ammon & P. Kleihues: Genetic alterations associated with the evolution and progression of astrocytic brain tumours. *Virchows Arch* 427, 113-118 (1995)

6. Pollack I. F, R. L. Hamilton, S. D. Finkelstein, J. W. Campbell, A. J. Martinez, R. N. Sherwin, M. E. Bozik & S. M. Gollin: The relationship between TP53 mutations and overexpression of p53 and prognosis in malignant gliomas of childhood. *Cancer Res* 57, 304-309 (1997)

7. Watanabe K, O. Tachibana, Y. Yonekawa, P. Kleihues & H. Ohgaki: Role of gemistocytes in astrocytoma progression. *Lab Invest* 76, 277-284 (1997)

8. Tada M, R. Matsumoto, R. D. Iggo, R. Onimaru, H. Shirato, Y. Sawamura & Y. Shinohe: Selective sensitivity to radiation of cerebral glioblastomas harboring p53 mutations. *Cancer Res* 58, 1793-1797 (1998)

9. Schiebe M, P. Ohneseit, W. Hoffmann, R. Meyermann, H. P. Rodemann & M. Bamberg: Analysis of mdm2 and

p53 gene alterations in glioblastomas and its correlation with clinical factors. *J Neurooncol* 49, 197-203 (2000)
10. Kraus J. A, M. Wenghoefer, N. Glesmann, S. Mohr, M. Beck, M. C. Schmidt, R. Schroder, U. Berweiler, W. Roggendorf, S. Diete, K. Dietzmann, K. Heuser, B. Muller, R. Fimmers, A. von Deimling & U. Schlegel: TP53 gene mutations, nuclear p53 accumulation, expression of Waf/p21, Bcl-2, and CD95 (APO-1/Fas) proteins are not prognostic factors in de novo glioblastoma multiforme. *J Neurooncol* 52, 263-272 (2001)
11. Newcomb E. W, H. Cohen, S. R. Lee, S. K. Bhalla, J. Bloom, R. L. Hayes & D. C. Miller: Survival of patients with glioblastoma multiforme is not influenced by altered expression of p16, p53, EGFR, MDM2 or Bcl-2 genes. *Brain Pathol* 8, 655-667 (1998)
12. Shiraishi S, K. Tada, H. Nakamura, K. Makino, M. Kochi, H. Saya, J. Kuratsu & Y. Ushio: Influence of p53 mutations on prognosis of patients with glioblastoma. *Cancer*, (in press)
13. Rubio M. P, A. von Deimling, D. W. Yandell, O. D. Wiestler, J. F. Gusella & D. N. Louis: Accumulation of wild type p53 protein in human astrocytomas. *Cancer Res* 53, 3465-3467 (1993)
14. Matsumoto R, M. Tada, M. Nozaki, C. L. Zhang, Y. Sawamura & H. Abe: Short alternative splice transcripts of the mdm2 oncogene correlate to malignancy in human astrocytic neoplasms. *Cancer Res* 58, 609-613 (1998)
15. Kraus A, F. Neff, M. Behn, M. Schuermann, K. Muenkel & J. Schlegel: Expression of alternatively spliced mdm2 transcripts correlates with stabilized wild-type p53 protein in human glioblastoma cells. *Int J Cancer* 80, 930-934 (1999)
16. Biernat W, P. Kleihues, Y. Yonekawa & H. Ohgaki: Amplification and overexpression of MDM2 in primary (de novo) glioblastomas. *J Neuropathol Exp Neurol* 56, 180-185 (1997)
17. Fulci G, M. Labuhn, D. Maier, Y. Lachat, O. Hausmann, M. E. Hegi, R. C. Janzer, A. Merlo & E. G. Van Meir: p53 gene mutation and ink4a-arf deletion appear to be two mutually exclusive events in human glioblastoma. *Oncogene* 19, 3816-3822 (2000)
18. Freedman D. A, L. Wu & A. J. Levine: Functions of the MDM2 oncoprotein. *Cell Mol Life Sci* 55, 96-107 (1999)
19. Reifenberger G, L. Liu, K. Ichimura, E. E. Schmidt & V. P. Collins: Amplification and overexpression of the MDM2 gene in a subset of human malignant gliomas without p53 mutations. *Cancer Res* 53, 2736-2739 (1993)
20. Reifenberger G, J. Reifenberger, K. Ichimura & V. P. Collins: Amplification at 12q13-14 in human malignant gliomas is frequently accompanied by loss of heterozygosity at loci proximal and distal to the amplification site. *Cancer Res* 55, 731-734 (1995)
21. Korkolopoulou P. P, Christodoulou, K. Kouzelis, M. Hadjiyannakis, A. Priftis, G. Stamoulis, A. Seretis & E. Thomas-Tsagli: MDM2 and p53 expression in gliomas: a multivariate survival analysis including proliferation markers and epidermal growth factor receptor. *Br J Cancer* 75, 1269-1278 (1997)
22. Rainov N. G, K. U. Dobberstein, H. Bahn, H. J. Holzhausen, C. Lautenschlager, V. Heidecke & W. Burkert: Prognostic factors in malignant glioma: influence of the overexpression of oncogene and tumor-suppressor gene products on survival. *J Neurooncol* 35, 13-28 (1997)
23. Hunter S. B, K. Abbott, V. A. Varma, J. J. Olson, D. W. Barnett & C. D. James: Reliability of differential PCR for the detection of EGFR and MDM2 gene amplification in DNA extracted from FFPE glioma tissue. *J Neuropathol Exp Neurol* 54, 57-64 (1995)
24. Galanis E, J. Buckner, D. Kimmel, R. Jenkins, B. Alderete, J. O'Fallon, C. H. Wang, B. W. Scheithauer & C. D. James: Gene amplification as a prognostic factor in primary and secondary high-grade malignant gliomas. *Int J Oncol* 13, 717-724 (1998)
25. Olson J. J, D. Barnett, J. Yang, R. Assietti, G. Cotsonis & C. D. James: Gene amplification as a prognostic factor in primary brain tumors. *Clin Cancer Res* 4, 215-222 (1998)
26. Kamb A, N. A. Gruis, J. Weaver-Feldhaus, Q. Liu, K. Harshman, S. V. Tavtigian, E. Stockert, R. S. Day, 3rd, B. E. Johnson & M. H. Skolnick: A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 264, 436-440 (1994)
27. Nobori T, K. Miura, D. J. Wu, A. Lois, K. Takabayashi & D. A. Carson: Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature* 368, 753-756 (1994)
28. Giani C & G. Finocchiaro: Mutation rate of the CDKN2 gene in malignant gliomas. *Cancer Res* 54, 6338-6339 (1994)
29. Schmidt E. E, K. Ichimura, G. Reifenberger & V. P. Collins: CDKN2 (p16/MTS1) gene deletion or CDK4 amplification occurs in the majority of glioblastomas. *Cancer Res* 54, 6321-6324 (1994)
30. Ichimura K, E. E. Schmidt, H. M. Goike & V. P. Collins: Human glioblastomas with no alterations of the CDKN2A (p16INK4A, MTS1) and CDK4 genes have frequent mutations of the retinoblastoma gene. *Oncogene* 13, 1065-1072 (1996)
31. Walker D. G, W. Duan, E. A. Popovic, A. H. Kaye, F. H. Tomlinson & M. Lavin: Homozygous deletions of the multiple tumor suppressor gene 1 in the progression of human astrocytomas. *Cancer Res* 55, 20-23 (1995)
32. Moulton T, G. Samara, W. Y. Chung, L. Yuan, R. Desai, M. Sisti, J. Bruce & B. Tycko: MTS1/p16/CDKN2 lesions in primary glioblastoma multiforme. *Am J Pathol* 146, 613-619 (1995)
33. Ueki K, Y. Ono, J. W. Henson, J. T. Efird, A. von Deimling & D. N. Louis: CDKN2/p16 or RB alterations occur in the majority of glioblastomas and are inversely correlated. *Cancer Res* 56, 150-153 (1996)
34. Ono Y, T. Tamiya, T. Ichikawa, K. Kunishio, K. Matsumoto, T. Furuta, T. Ohmoto, K. Ueki & D. N. Louis: Malignant astrocytomas with homozygous CDKN2/p16 gene deletions have higher Ki-67 proliferation indices. *J Neuropathol Exp Neurol* 55, 1026-1031 (1996)
35. Burns K. L, K. Ueki, S. L. Jhung, J. Koh & D. N. Louis: Molecular genetic correlates of p16, cdk4, and pRb immunohistochemistry in glioblastomas. *J Neuropathol Exp Neurol* 57, 122-130 (1998)
36. Newcomb E. W, M. Alonso, T. Sung & D. C. Miller: Incidence of p14ARF gene deletion in high-grade adult and pediatric astrocytomas. *Hum Pathol* 31, 115-119 (2000)
37. James C. D, E. Galanis, L. Frederick, D. W. Kimmel, J. M. Cunningham, P. J. Atherton-Skaff, J. R. O'Fallon, R.

B. Jenkins, J. C. Buckner, S. B. Hunter, J. J. Olson & B. W. Scheithauer: Tumor suppressor gene alterations in malignant gliomas: histopathological associations and prognostic evaluation. *Int J Oncol* 15, 547-553 (1999)
38. Kamiryo T, K. Tada, S. Shiraishi, N. Shinojima, H. Nakamura, M. Kochi, J. Kuratsu, H. Saya & Y. Ushio: Analysis of homozygous deletion of the p16 gene and correlation with survival in patients with glioblastoma multiforme. *J Neurosurg* 96, 815-822 (2002)
39. Kamijo T, F. Zindy, M. F. Roussel, D. E. Quelle, J. R. Downing, R. A. Ashmun, G. Grosveld & C. J. Sherr: Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell* 91, 649-659 (1997)
40. Zhang Y, Y. Xiong & W. G. Yarbrough: ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell* 92, 725-734 (1998)
41. Kraus J. A, N. Glesmann, M. Beck, D. Krex, T. Klockgether, G. Schackert & U. Schlegel: Molecular analysis of the PTEN, TP53 and CDKN2A tumor suppressor genes in long-term survivors of glioblastoma multiforme. *J Neurooncol* 48, 89-94 (2000)
42. Alleyne C. H, Jr., J. He, J. Yang, S. B. Hunter, G. Cotsonis, C. D. James & J. J. Olson: Analysis of cyclin dependent kinase inhibitors in malignant astrocytomas. *Int J Oncol* 14, 1111-1116 (1999)
43. Puduvalli V. K, A. P. Kyritsis, K. R. Hess, M. L. Bondy, G. N. Fuller, G. P. Kouraklis, V. A. Levin & J. M. Bruner: Patterns of expression of Rb and p16 in astrocytic gliomas, and correlation with survival. *Int J Oncol* 17, 963-969 (2000)
44. Kirla R. E. Salminen, S. Huhtala, J. Nuutinen, L. Talve, H. Haapasalo & H. Kalim: Prognostic value of the expression of tumor suppressor genes p53, p21, p16 and prb, and Ki-67 labelling in high grade astrocytomas treated with radiotherapy. *J Neurooncol* 46, 71-80 (2000)
45. Labuhn M, G. Jones, E. J. Speel, D. Maier, C. Zweifel, O. Gratzl, E. G. Van Meir, M. E. Hegi & A. Merlo: Quantitative real-time PCR does not show selective targeting of p14(ARF) but concomitant inactivation of both p16(INK4A) and p14(ARF) in 105 human primary gliomas. *Oncogene*,20, 1103-1109 (2001)
46. Perry A, T. Nobori, N. Ru, K. Anderl, T. J. Borell, G. Mohapatra, B. G. Feuerstein, R. B. Jenkins & D. A. Carson: Detection of p16 gene deletions in gliomas: a comparison of fluorescence in situ hybridization (FISH) versus quantitative PCR. *J Neuropathol Exp Neurol* 56, 999-1008 (1997)
47. Merlo A, J. G. Herman, L. Mao, D. J. Lee, E. Gabrielson, P. C. Burger, S. B. Baylin & D. Sidransky: 5' CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. *Nat Med* 1, 686-692 (1995)
48. Costello J. F, M. S. Berger, H. S. Huang & W. K. Cavenee: Silencing of p16/CDKN2 expression in human gliomas by methylation and chromatin condensation. *Cancer Res* 56, 2405-2410 (1996)
49. Fueyo J, C. Gomez-Manzano, J. M. Bruner, Y. Saito, B. Zhang, W. Zhang, V. A. Levin, W. K. Yung & A. P. Kyritsis: Hypermethylation of the CpG island of p16/CDKN2 correlates with gene inactivation in gliomas. *Oncogene* 13, 1615-1619 (1996)
50. Kyritsis A. P, B. Zhang, W. Zhang, M. Xiao, H. Takeshima, M. L. Bondy, J. E. Cunningham, V. A. Levin & J. Bruner: Mutations of the p16 gene in gliomas. *Oncogene* 12, 63-67 (1996)
51. Li Y. J, K. Hoang-Xuan, J. Y. Delattre, M. Poisson, G. Thomas & R. Hamelin: Frequent loss of heterozygosity on chromosome 9, and low incidence of mutations of cyclin-dependent kinase inhibitors p15 (MTS2) and p16 (MTS1) genes in gliomas. *Oncogene* 11, 597-600 (1995)
52. Schmidt E. E, K. Ichimura, K. R. Messerle, H. M. Goike & V. P. Collins: Infrequent methylation of CDKN2A(MTS1/p16) and rare mutation of both CDKN2A and CDKN2B(MTS2/p15) in primary astrocytic tumours. *Br J Cancer* 75, 2-8 (1997)
53. Hegi M. E, A. zur Hausen, D. Ruedi, G. Malin & P. Kleihues: Hemizygous or homozygous deletion of the chromosomal region containing the p16INK4a gene is associated with amplification of the EGF receptor gene in glioblastomas. *Int J Cancer* 73, 57-63 (1997)
54. Perry A, K. Anderl, T. J. Borell, D. W. Kimmel, C. H. Wang, J. R. O'Fallon, B. G. Feuerstein, B. W. Scheithauer & R. B. Jenkins: Detection of p16, RB, CDK4, and p53 gene deletion and amplification by fluorescence in situ hybridization in 96 gliomas. *Am J Clin Pathol* 112, 801-809 (1999)
55. Wang S. I, J. Puc, J. Li, J. N. Bruce, P. Cairns, D. Sidransky & R. Parsons: Somatic mutations of PTEN in glioblastoma multiforme. *Cancer Res* 57, 4183-4186 (1997)
56. Rasheed B. K, T. T. Stenzel, R. E. McLendon, R. Parsons, A. H. Friedman, H. S. Friedman, D. D. Bigner & S. H. Bigner: PTEN gene mutations are seen in high-grade but not in low-grade gliomas. *Cancer Res* 57, 4187-4190 (1997)
57. Ichimura K, E. E. Schmidt, A. Miyakawa, H. M. Goike & V. P. Collins: Distinct patterns of deletion on 10p and 10q suggest involvement of multiple tumor suppressor genes in the development of astrocytic gliomas of different malignancy grades. *Genes Chromosomes Cancer* 22, 9-15 (1998)
58. Schmidt E. E, K. Ichimura, H. M. Goike, A. Moshref, L. Liu & V. P. Collins: Mutational profile of the PTEN gene in primary human astrocytic tumors and cultivated xenografts. *J Neuropathol Exp Neurol* 58, 1170-1183 (1999)
59. Li J, C. Yen, D. Liaw, K. Podsypanina, S. Bose, S. I. Wang, J. Puc, C. Miliaresis, L. Rodgers, R. McCombie, S. H. Bigner, B. C. Giovanella, M. Ittmann, B. Tycko, H. Hibshoosh, M. H. Wigler & R. Parsons: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275, 1943-1947 (1997)
60. Yamaguchi F, H. Saya, J. M. Bruner & R. S. Morrison: Differential expression of two fibroblast growth factor-receptor genes is associated with malignant progression in human astrocytomas. *Proc Natl Acad Sci U S A* 91, 484-488 (1994)
61. Mollenhauer J, S. Wiemann, W. Scheurlen, B. Korn, Y. Hayashi, K. K. Wilgenbus, A. von Deimling & A. Poustka: DMBT1, a new member of the SRCR