また下垂体腺腫，聴神経鞘腫などの脳実質外腫瘍に対しては，独自の分類がそれぞれ提唱されている[5)13)]．

一方，グリオーマ等の脳実質内腫瘍における病期ステージ分類の試みは髄芽腫，脳幹グリオーマ，視床グリオーマで認められているが，一般的な広義のグリオーマにおいてはステージ分類は認められていない[4)8)15)]．その理由として，グリオーマは浸潤性に存在するとの考えから tumor bulk を同定することが，CT 時代には困難であったからである．Burger[3)]の報告によれば，グリオーマは CT で造影される領域の周辺 3 cm まで浸潤しているとされている．したがって CT の造影領域より判定される摘出度は，予後との関連があるという報告は散見されるものの，正確な摘出度を反映しているとはいいがたかった[2)]．しかし MRI 出現後，その空間分解能の向上とともに，たとえグリオーマといえども，ある程度 Tumor bulk の同定が可能になった．そこでわれわれは，Tumor bulk を同定したところ，グリオーマの約半数にて Tumor bulk の同定が MRI 上可能であることがわかった[12)]．この事実が今回の手術ステージ分類を可能にしたと考えている．

今回，考案したグリオーマの手術ステージ分類と手術摘出率の検討から，大きく分ければ Stage 3 以下は全摘出が可能であり，Stage 4 以上は全摘出が困難であるということが示唆された．それをふまえて Stage 3 以下で部分摘出以下になった症例を検討すると，次のようなことが原因になっていると考えられた．まず，診断目的で biopsy を行っている症例に関しては，解析対象から除外できるものと考えられた．次に non-eloquent area に存在し，理論的には subtotal removal 以上は可能ではあるが partial removal になっている症例が，Stage 2 で 10 例（50%），Stage 3 で 34 例（55%）存在した．これに関しては，eloquent area 近傍であった可能性などさまざまな問題点があり，今後の検討の必要がある．また，eloquent area に存在しながら，total removal となっている症例が Stage 3 で 2 例（3%），Stage 4 において 11 例（73%）存在した．これに関しては eloquent area の定義がきちんとなされていない可能性もあるが，年齢，組織学的悪性度，症例の KPS など，各施設間における治療方針の違いなども考慮しなくてはならない．

手術ステージ分類と予後に相関があるという多変量解析の結果は，手術ステージ分類の当初の目的とは異なるものであるが，今後グリオーマの治療成績を議論するうえでも，ステージ分類が重要な因子になっていく可能性が示唆された．

今回の手術ステージ分類は，手術の難易度に関係する腫瘍の大きさ，存在部位に焦点を当てたものであるが，全国的な多施設における調査でも，手術ステージと摘出率には一定の傾向がみられた．今後さらに eloquent area 等に関する詳細な基準を設定することで，より有用な分類法になる可能性がある．このようなグローバルスタンダードが，今後は必要になってくるものと思われた．

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要　旨

テント上グリオーマの手術ステージ分類と手術方針

嘉山　孝正　園田　順彦　佐藤　慎哉　藤巻　高光　渋井壮一郎　野村　和弘

神経膠腫の予後を決定する因子として，手術の摘出度は最も重要な因子であり，最大限の摘出が望まれる．その一方で，腫瘍が eloquent area にある場合は，その部位の摘出は不可能とされる．したがって病変の摘出が可能であるかを判断する標準ガイドラインが必要である．そこで，腫瘍の存在部位，大きさから手術ステージ分類を作成し，国内の施設において実際に手術治療された神経膠腫をそれに基づき分類し，摘出率との関係を検討した．結果としてわれわれの作成した手術ステージ分類のステージが上がるにつれ，摘出率が下がる傾向が認められた．またステージ分類は患者の予後とも相関していた．神経膠腫の手術方針を決定するうえで，この手術ステージ分類が有用なグローバルスタンダードとなる可能性が示唆された．

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Randomized Controlled Trial on Malignant Brain Tumors

—Activities of the Japan Clinical Oncology Group-Brain Tumor Study Group—

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Abstract

The Japan Clinical Oncology Group (JCOG)-Brain Tumor Study Group was organized with the support of the Health and Labour Sciences Research Grants of the Ministry of Health, Labour and Welfare. The group is now preparing a multi-institutional randomized controlled phase II/III study of chemoradiotherapy using ACNU versus procarbazine and ACNU for astrocytoma grades 3 and 4. The overall survival and response rates will be compared between the patients treated with ACNU and those treated with ACNU plus procarbazine. This study, under the surveillance of the JCOG, aims to set a standard protocol for treating patients with malignant glioma. Moreover, the study will establish a proper methodology for performing randomized studies in the field of neuro-oncology.

Key words: Japan Clinical Oncology Group, randomized controlled trial, malignant glioma, ACNU, procarbazine, O^6-methylguanine deoxyribonucleic acid-methyltransferase

Introduction

The Japan Clinical Oncology Group (JCOG) is a multi-institutional cooperative oncology group conducting clinical research for cancer and related problems.[2] JCOG consists of 13 oncology groups as of 2003. The Brain Tumor Study Group (JCOG-BTSG) was organized in April 2002 with support from the Health and Labour Research Grants of the Ministry of Health, Labour and Welfare in order to establish a standard therapy for malignant brain tumors.

This study describes a randomized controlled phase II/III study of chemoradiotherapy using ACNU versus procarbazine and ACNU for astrocytoma grades 3 and 4.

Materials and Methods

Patients with newly diagnosed supratentorial astrocytoma grade 3 or 4 will be enrolled and randomly divided into two groups. Patients in Group A will be treated with ACNU (80 mg/m^2 iv) during the postoperative radiotherapy (60 Gy local), whereas patients in Group B with procarbazine (80 mg/m^2 for 10 days per os) preceding and in addition to the administration of ACNU. Each regimen will be repeated every 8 weeks for 2 years if tolerated by the patients. The primary endpoint is the overall survival rate and the secondary endpoints are the response rate on magnetic resonance imaging and the frequency of adverse events. This study starts as a randomized phase II trial and proceeds to the phase III study if the efficacy of the Group B regimen in phase II warrants a study continuation.

The study protocol was developed under guidance of the JCOG and approved by the institutional review board of the institution to which each JCOG-BTSG member belongs. The study will be performed under surveillance by the JCOG.

Results

This study starts at the beginning of 2004. The expected number of patient enrollments is 310 in 5 years. The collected data will be monitored and statistical analyses carried out by the JCOG Data Center. The results will be evaluated by the Steering Committee.

Discussion

A standard therapy for malignant gliomas has not been established and various trials have been carried out. In most neurosurgical institutes in Japan, nimustine hydrochloride (ACNU) is administered in conjunction with conventional radiotherapy after surgical removal of the tumor. However, this common treatment regimen has never been scientifically justified by a randomized controlled study, and so should be considered "community standard."

The efficacy of ACNU in malignant glioma patients was evaluated in a group who received postoperative administration of ACNU in conjunction with radiation therapy and another group was received only radiation therapy.[4] This controlled study revealed an improved response rate for the patients treated with ACNU, however, no significant difference in overall survival was observed between the two groups.

ACNU is one of the most effective chemotherapeutic agents to date for malignant gliomas. ACNU passes through the intact blood-brain barrier and alkylates deoxyribonucleic acid (DNA) causing the anti-tumor effect. Most malignant gliomas nevertheless recur after ACNU chemotherapy and radiotherapy. Malignant gliomas frequently express high activities of O^6-methylguanine DNA-methyltransferase (MGMT), a DNA repair enzyme, which is considered to be one of the causes of the chemoresistance to ACNU. Procarbazine is another alkylating agent that yields O^6-alkylguanine.[3] If procarbazine is administered prior to ACNU as in our current

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protocol, we expect the abundant O^6-alkylguanine to deprive MGMT, leading to increased efficacy of ANCU.[5] A similar treatment protocol was applied using BCNU, procarbazine, and vincristine to 58 patients with recurrent glioblastoma and reported a high response rate of 29% (complete response 10.3%, partial response 19%).[1]

In order to establish a standard therapy for a certain clinical entity, strict randomized controlled studies are essential. Few such studies in the neuro-oncological field have been carried out in Japan. Brain tumor is one of the so-called orphan diseases. Hence, multi-institutional cooperation is essential to accomplish randomized trials that require a large number of patient enrollment. JCOG is a group of oncologists that conduct cooperative studies on various cancers in Japan. The BTSG was newly organized in JCOG and is now preparing this randomized trial in an unprecedented organized manner. Upon completion, this study should provide a scientific basis for the standard therapy for malignant gliomas. Moreover, we hope to establish a proper methodology for performing randomized studies in the field of neuro-oncology.

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Appendix: Members of the Japan Clinical Oncology Group-Brain Tumor Study Group

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悪性脳腫瘍の標準的治療法の確立に関する研究

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「悪性脳腫瘍の標準的治療法の確立に関する研究」について報告させていただきます。

私たちの研究グループを示します(図１)。全国の16の脳神経外科施設を中心に構成されております。

研究テーマとしては二つございます。一つはソマトスタチン誘導体を使用しました診断治療のこと、もう一つはJCOGスタディとして開始されます悪性神経膠腫に対する初期治療及び維持療法ということです。

最初にソマトスタチン誘導体に関する報告をいたします。ソマトスタチン受容体は多くの腫瘍組織に発現していると言われております。ペンテトレオタイドは合成ソマトスタチンアナログでソマトスタチン受容体、その主要なサブタイプであるタイプ２の受容体と高い親和性を持つと言われています。このペプチドを放射性同位元素で標識しましたインジウムペンテトレオタイド、MP1727はすでに神経内分泌系の腫瘍の局在診断に用いられておりますが、これをさらに脳腫瘍に応用しようという研究であります。またその後、さらに高エネルギーの放射性同位元素を標識することで、これを治療に応用しようと考えております。

対象としましては初発時に病理学的に脳腫瘍（神経膠腫、髄膜種、下垂体腺腫、悪性リンパ腫、転移性脳腫瘍、神経線維腫）と診断され、手術、放射線照射など標準的治療を受けたのちに画像的に再発あるいは増悪した症例、あるいは初発例で今後生検あるいは手術を予定している症例です。

方法といたしまして試験薬MP1727の111MBqを静脈内投与し、24時間後にガンマカメラによって全身像及び頭部SPECTを撮影します。そしてこの結果と手術によって得られたソマトスタチン受容体の免疫染色との結果を比較いたします。次の段階といたしましては、SPECT陽性例について高エネルギーMP1727による治療を検討いたしております。現在この研究につきましてはプロトコルが完成いたしまして、各施設でIRBも通過しましたので、薬剤が入手できた時点で開始できる段階に至っています。

もう一つは星細胞腫、グレード３、４に対する化学放射線治療としてのACNU単独療法とProcarbazine＋ACNU併用療法とのランダム化比較試験、第Ⅱ、Ⅲ相試験であります。ACNUは悪性脳腫瘍の治療薬として広く使われておりますが、このACNUに対する耐性機構がO^6－methylguanine DNA－methyltransferase（MGMT）であると言われています。Procarbazineも同じくO^6－methylguanineを形成することから、Procarbazineで前処置をすることでMGMTを枯渇させ、ACNUの効果を上げることが期待できます。このことを応用しましてProcarbazine＋ACNUという治療法を計画いたしました。

対照群といたしましてはグレード3、グレード4の星細胞腫に対しまして、術後の放射線治療として、60グレイの照射を行い、その第1週及び第6週にACNUを投与します。そしてさらに維持療法として8週ごとに12コース、ACNUを静注いたします（図2）。

それに対してProcarbazine＋ACNU群は放射線照射と同時にProcarbazineを10日間投与し、その7日目にACNUを投与いたします。そして同じく第6週目にProcarbazine＋ACNUを行います。さらに維持療法といたしまして8週ごとに12コース、Procarbazine＋ACNUを投与いたします（図3）。この両者を比較する形をとりました。

Procarbazine＋ACNUにつきましてはまだ第Ⅱ相試験が行なわれておりませんので、今回第Ⅱ、Ⅲ相試験という形をとりました。第Ⅱ相段階でのプライマリーエンドポイントは6カ月生存率にしました。これにつきましては脳腫瘍全国統計の6カ月生存率を参考にいたしまして、Procarbazine＋ACNU群について56例集まった段階で評価して、その生存率が80％を超えれば、第Ⅲ相試験に進むという形になります。

第Ⅲ相のプライマリーエンドポイントは生存期間、セカンダリーエンドポイントは無増悪生存期間、奏効割合、完全奏効割合、有害事象をとりました。5年間で310例を予定症例数といたしまして、2年間のフォローアップを考えております。

さらにまた予後に影響すると思われる組織診断、グレード3かグレード4か、年齢60歳未満か60歳以上か、術後MRIで残存腫瘍があるかないかを割り付け因子といたしましてランダム化いたしております。

現段階ではこれもプロトコルがほぼ完成いたしまして、3月中にはおそらく登録が開始できるのではないかと考えています。原発性脳腫瘍の発生率は10万人に11人から12人とされております。脳腫瘍全国統計によりますと、今回対象としました星細胞腫グレード3、4はそのうちの14％にすぎません。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が、今まで国内ではそのような基盤が存在せず、エビデンスとなり得る研究結果に乏しかったと言えます。今回初めてJCOG内に脳腫瘍グループが設立されたことで、今後の脳腫瘍の臨床研究に方向付けとなることと思われます。ありがとうございました。

野村　脳腫瘍はオーファンディジーズの中に入るのですが、これに企業があまり取り組んでくれない面があります。そういったところを突いているわけですが、ご質問、ご意見をどうぞ。どうぞ塚本先生。

質問　前半のほうですけれども、これはフェーズⅠスタディですか。計画研究の論文を見てもこれに関する論文が全く載ってないようで、アニマルスタディか何かで実際にこういうことをやって、あるいはパイロットスタディである程度やられていることなんでしょうか。

渋井　すでに内科のほうで第Ⅲ相試験まで進んでおります。それがまだ脳腫瘍に使われておりませんので、まず診断薬として用います。

質問　腫瘍のスタディに関して、内科ではある程度進んでいるということですか。
渋井　方法論自体は同じなので、毒性試験その他は済んでおります。
質問　それが脳腫瘍にどれだけ集積するとか、患者さんにどうやって説明し、納得させるデータがあるかが非常に気になっています。
渋井　特にオランダのKrenningらがこの研究をやっておりまして、すでに脳腫瘍についてもいくつか論文が出ています。
野村　よろしいでしょうか、他に　はい、どうぞ。
質問　やはり前半についての質問ですけれども、もともと脳腫瘍自身がそう数がないと思うのですが、治療の対象は非手術、インオペラブルケースですか。
渋井　手術例です。手術例で組織が確認できていることで、それに免疫染色を行いまして、その結果とSPECTの結果を対比したいと思います。
質問　潜在的な転移に対しての治療という意味ですか。
渋井　ということではなくて、あくまで画像上の脳腫瘍とわかっているものが対象です。
質問　手術前にやるということですか。診断ではなくて治療とおっしゃいましたよね。
渋井　治療についてはすべて再発例を考えておりますので、通常の標準治療で効果のなかったものについて考えています。
野村　はい、どうぞ。
質問　二つあります。最初はソマトスタチンのスタディは適応拡大の可能性があるのかないのか。それから後半の研究で300例という膨大な症例が本当に集積可能なのか。
渋井　ソマトスタチンは現在、内科の診断薬として適応申請をしているところであります。ですからそれが通りますと、おそらく脳腫瘍にも応用可能になると考えております。
　後半の症例数ですが、我々のグループで登録可能症例数を確認いたしまして、各施設年間4、5例登録できれば5年間で300例いくと考えております。
野村　よろしいでしょうか。それではどうもありがとうございました。

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図　1

対象・方法

【対象】Grade III または IV の星細胞腫（退形成性星細胞腫または膠芽腫）初発例

【A群】 Day 1　ACNU 80mg/m² *iv*
Day 1-40　Radiotherapy 60Gy/30f
Day 36　ACNU 80mg/m² *iv*
（その後，8週間ごとに12コース ACNU *iv*）

図　2

対象・方法

【対象】Grade III または IV の星細胞腫（退形成性星細胞腫または膠芽腫）初発例

【B群】 Day 1-10　Procarbazine 80m/m²/day *po*
Day 8　ACNU 80mg/m² *iv*
Day 8-47　Radiotherapy 60Gy/30f
Day 36-45　Procarbazine 80mg/m²/day *po*
Day 43　ACNU 80mg/m² *iv*
（その後，8週間ごとに12コース PCZ *po*, ACNU *iv*）

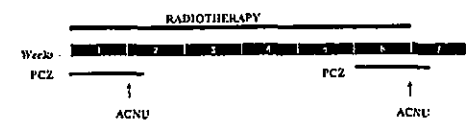

図　3

Selective Expression of a Subset of Neuronal Genes in Oligodendroglioma with Chromosome 1p Loss

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Gliomas are classified based mainly on microscopic resemblance to their presumed glial origin such as astrocyte and oligodendrocyte. However, more objective diagnostic criteria are indispensable for the precise treatment of patients. For instance, loss of the short arm of chromosome 1 (1p) in oligodendrogliomas is recognized as an important marker for better response to chemotherapy and longer survival of the patients. To gain insight into their molecular biological background and to identify genes characterizing each subgroup, we investigated gene expression profile of the 4 glioma subsets, oligodendroglioma with and without 1p loss, diffuse astrocytoma and glioblastoma using DNA microarray. Remarkably, most of the genes showing distinctive expression in oligodendroglioma with 1p loss were also highly expressed in normal brain tissues and had neuron-related function, which included *MYT1L*, *INA*, *RIMS2*, *SNAP91* and *SNCB*. Histological analysis also demonstrated that *MYT1L*, which were abundantly expressed in normal neuron, were certainly present in tumor cells. These results suggest that oligodendroglioma, especially with 1p loss, has more or less neuronal characteristics although oligodendroglioma is thought to originate form glial lineage cell. With further pathological studies, those neuron-related genes might be good diagnostic markers for oligodendroglioma of better prognosis as well.

Brain Pathol 2004;14:34-42.

INTRODUCTION

Gliomas are a major type of brain tumors, which constitute approximately one third of all primary brain tumors (11). Most gliomas have diffuse infiltrative trait, rendering surgical cure impossible and recurrence inevitable despite aggressive adjuvant treatment including radiotherapy and chemotherapy. Prognosis of each patient is determined primarily by the biological characteristics of tumor cells including response to treatment and rate of growth. Prediction of such biological characteristics of gliomas has been based on histological diagnosis which mainly relied on the morphological features of the tumor, and on the classification referring to the presumed origin of the tumor cells such as astrocytes, oligodendrocytes and ependymal cells. However, recent development in molecular genetic analysis have shown that even gliomas in a single histological entity can be divided into different subsets and may sometimes show different clinical features. A prominent example is that allelic loss of the short arm of chromosome 1 (1p), which is found in 60% to 80% of oligodendrogliomas, is closely associated with the chemosensitivity and longer survival (1, 8). Molecular biological background of such differences should be important information to be investigated which potentially leads to better management of gliomas, and one of powerful tools to do so is DNA microarray technology (5). Several studies have successfully demonstrated subtype specific genes in diffuse gliomas based on the expression profile analysis, and also showed that such molecular profiles could indeed help accurate prediction of clinical outcome (4, 6, 7, 17, 20, 21).

In a previous work, we have demonstrated that expression profiles of oligodendrogliomas with 1p loss are significantly different from other oligodendrogliomas, and numerous genes presumed to be related to neuronal cells are preferentially expressed in this specific subset (16). In this study, we asked whether this trait would still hold within a wider range of gliomas including astrocytic tumors. Such findings may not only be of diagnostic significance, but also would bring a new insight into glioma classification based on gene expression.

MATERIALS AND METHODS

Sample preparation. Tumor samples and paired blood samples were obtained at surgery after written informed consents. Consensus histological diagnoses were made on formalin-fixed paraffin-embedded tissues by four independent neuropathologists following the WHO classification (11). Loss of heterozygosity (LOH) assay on chromosomes 1p and 19q using microsatellite markers were performed as described previously (24). The frozen tumor sample was homogenized in Trizol (Invitrogen, Corp., Carlsbad, Calif) and total RNA was isolated following manufacturer's instructions. RNA was quantitated by ultraviolet absorbance at 260 and 280 nm and its quality was assessed by agarose gel electrophoresis.

GeneChip experiment. In addition to 2 normal brains, 6 oligodendrogliomas with 1pLOH (4 WHO grade II and 2 grade III cases), and 5 oligodendrogliomas without 1pLOH (4 grade II and 1 grade III cases) which were all reported in our previous study (16), 6 diffuse astrocytomas (grade II) and 5 glioblastomas (grade IV) were subjected to gene expression profile analysis. The high-density oligonucleotide arrays (GeneChip Human U95A array, Affymetrix, Santa Clara, Calif), which contain probe sets for approximately 12 626 human genes and ESTs, were used. Biotin-labeled cRNA was synthesized from aliquots (5 μg) of total RNA from each sample, and hybridization, washing, and detection of signals were carried out as described previously (9, 16). The Microarray Analysis Suite (MAS) 4.0 software (Affymetrix) was used to calculate the gene expression levels. The average background and noise (Raw Q) value calculated by MAS 4.0 were less than 241 (199 ± 26) and 7.13 (6.07 ± 0.78), respectively under 100% PMT setting. To allow comparison among multiple arrays, gene expression levels were normalized for each array by assigning the average of overall expression levels to be 100. The signal values of β-actin as an internal control showed <2-fold variation (4488 ± 576). The scaling factor used for all samples was 0.68 ± 0.18. The percentage of probe sets scored as detected ("Present") in each sample ranged from 48 ± 3% (42%-55%). These metrics demonstrate that the quality of each array is comparable. A value of 10 was assigned to every expression value below 10, because such low values are vulnerable to noise and artifacts.

Selection of subtype-specific genes. All glioma samples analyzed by GeneChip (N = 22) fall into 4 groups: oligodendroglial tumors with 1pLOH (n = 6), without 1pLOH (n = 5), low-grade astrocytomas (n = 6), and glioblastomas (n = 5). An ideal subtype-specific gene should have higher expression in samples of this subgroup and lower expression in samples of the other 3 types. For the selection of such genes, we used public software called Significant Analysis of Microarrays (SAM 1.21) (23), which is one of the methods to solve the statistical problem occurring in the analysis of large numbers of genes with small numbers of experiments. Basically, a score assigned by SAM is signal-to-noise (S/N) ratio called relative difference $d(i)$, which is calculated by $\{\mu_I(i)-\mu_U(i)\}/\{s(i)+s_0\}$ when $\mu_I(i)$ and $\mu_U(i)$ denote the average levels of expression for gene(i) in group I and U, respectively, and $s(i)$ is defined as the standard deviation of repeated expression measurements. Then, taking gene-specific fluctuations into account, SAM estimates the percentage of genes identified by chance as the false discovery rate (FDR) using permutations of the repeated measurements. SAM also identifies genes with statistically significant changes and score q-value, which is similar to the familiar "p-value."

Before SAM was applied, the control probes and genes called absent (not detected) by the expression algorithm in MAS 4.0 software or less than 100 in all 24 samples were excluded because of low confidence of scarcely expressed genes. Then by the pre-filtering, the 2756 probe sets whose maximum and minimum expression levels among 22 tumor samples differed by more than 100, and had more than 5-fold difference, were selected for the following statistical analysis.

Comparison with normal brain tissue data. To see expressions of the selected genes in normal brain tissues, we used Affymetrix U95A array expression data in the Gene Expression Atlas on the website of Genomics Institute of the Novartis Research Foundation (22), in addition to the data obtained from our two normal whole brain samples. This database contains 2 whole brain, a cerebral cortex, 2 cerebellum, 2 caudate nucleus, 2 amygdala, 2 thalamus, 3 corpus callosum and 2 spinal cord. These data were linearly scaled to the same target signal (100) as in our own expression data. After this conversion, the expression levels of internal control genes such as β-actin (4425 ± 1122) in these normal tissues were similar to our data. The average gene expression levels in our normal brains and those in downloaded samples (whole brain) were also well correlated (coefficient r = 0.88) among pre-filtered genes. Hierarchical clustering was carried out by the programs Cluster and TreeView using selected 80 subtype-specific genes (3).

Quantitative real-time PCR. Quantitative real-time PCR (qPCR) was performed using iCycler (Bio-Rad, Hercules, Calif). cDNA was synthesized with oligo-dT primer from 2 μg total RNA using SuperScript Preamplification System (Invitrogen). The aliquot of cDNA were amplified by Taq polymerase for 40 cycles, consisted of 15 seconds of denaturing at 94°C, 15 seconds of annealing at 63-70°C, and 30 seconds of extension at 72°C with monitoring of the SYBR Green I dye intercalation signal. Each PCR reaction was done in triplicate. For each sample, relative expression of a gene to the expression in reference cDNA mixture of several cell lines and tissues was calculated, and the expression of each gene was then normalized using β-*actin* expression of the same sample as an internal control. The following primer sets and annealing temperature (Tm) were used: forward (F) 5′-AGAAG GAGAT CACTG CCCTG GCACC-3′, reverse (R) 5′-CCTGC TTGCT GATCC ACATC TGCTG-3′ and Tm 65°C for β-*actin*; F 5′-AATTA TTTCG GGGCT CTGCG GAACC-3′, R 5′-GCACC TTGCT TCAGC TCTCA AAACG-3′ and Tm 67°C for *MYT1L*; F 5′-TACCA CCCGG TCCCC ACTTT ATTGC-3′, R 5′-TTCGG GCCAC CCCTA CTTCT TCTCC-3′ and Tm 63°C for *L1CAM*; F 5′-CAGAC TCTGG CAACA CCTGC AATGG-3′, R 5′-CACGG GCCCG GATGA TTTCT ACCTC-3′ and Tm 70°C for *RIMS2*; F 5′-GAAGT GGCCC AGGAA GCTGC TGAAG-3′, R 5′-CAGGG ACAGA ATTGT GCTGC TGGTG-3′ and Tm 70°C for *SNCB*; F 5′-GAGGA CCGTC ATCAG GCCGA CATTG-3′, R 5′-GGCCA TCTCC CACTT GGTGT TCCTC-3′ and Tm 70°C for *NEFH*; F 5′-CCCTT TCCCC AAAAG TAGCG TAACC-3′, R 5′-TTGAC AGGAC GGCGA CTGTG AGAC-3′ and Tm 68°C for *OLIG1*. The specificity of the amplification products was validated using post-amplification melt curve analysis. Differences of gene expression in oligodendroglioma with 1pLOH and the other gliomas were tested by Kruskal-Wallis analysis.

In situ hybridization. Tumor samples and adjacent normal brain tissue stored at -80°C were embedded into Tissue-Tek OCT compound (Sakura Finetek, Torrance, Calif) and cryosectioned (7-μm thick), and then fixed in 4% paraformaldehyde. For the detection of *MYT1L* mRNA, the sections were treated with proteinase K

GeneChip probe number	Genbank accession number	Gene	Symbol	Expression level[1]					Locus
				Oligodendroglioma		Astrocytoma	GBM	Normal whole brain	
				1pLOH (+)	1pLOH (-)				
32712_at	AB029029	Myelin transcription factor 1-like *[2]	MYT1L	125±70	13±5	20±19	10±0	203±102	2p
37210_at	S78296	Internexin neuronal intermediate filament protein α	INA	622±182	177±54	235±102	136±18	816±85	10q
34526_s_at	AF052108	Hypothetical protein LOC157627	LOC157627	154±77	30±15	39±50	11±2	272±120	8p
38551_at	U52112	L1 cell adhesion molecule *	L1CAM	479±273	58±62	44±46	73±43	620±167	Xq
41589_at	Y15065	Potassium voltage-gated channel, KQT-like	KCNQ2	119±86	32±25	10±0	10±0	325±146	20q
40234_at	X96484	DiGeorge syndrome critical region gene 6	DGCR6	443±209	258±167	19±21	59±60	439±175	22q
33426_at	Y00064	Chromogranin B (secretogranin 1)	CHGB	233±139	85±44	49±44	16±9	282±148	20p
32709_at	L39833	Potassium voltage-gated channel, shaker-related	KCNAB1	81±36	23±10	27±15	26±20	180±33	3q
37568_at	U79242	Clone 24816 mRNA sequence		97±33	20±10	44±15	30±11	140±22	
38163_at	AB018294	Regulating synaptic membrane exocytosis 2 *	RIMS2	133±58	29±14	39±33	11±2	140±58	8q
34520_at	AJ012582	Potassium voltage-gated channel, brain 2	HCN2	79±28	39±16	25±13	11±2	144±26	19p
36965_at	U13616	Ankyrin 3, node of Ranvier (ankyrin G)	ANK3	196±83	78±70	63±35	44±19	490±215	10q
38699_at	X00734	Tubulin beta 5	TUBB5	500±152	217±115	138±76	94±90	1785±282	19p
37580_at	AF036271	SH3-domain GRB2-like 3	SH3GL3	127±59	50±25	45±37	12±4	377±132	15q
39095_at	M58018	Myosin, heavy polypeptide 7, cardiac muscle, β	MYH7	95±79	20±13	14±8	18±10	10±0	14q
40733_f_at	D89377	Msh homeo box homolog 2	MSX2	79±33	32±5	30±5	36±6	31±24	5q
41675_at	AB014556	Synaptosomal-associated protein, 91kDa homolog	SNAP91	341±151	167±118	74±85	10±0	950±127	6q
38174_at	X99688	Pleckstrin and Sec7 domain protein	PSD	374±198	164±45	133±70	65±16	1068±259	10q
33947_at	U18550	G protein-coupled receptor 3	GPR3	112±20	50±24	32±23	38±15	133±39	1p
34788_at	AL049365	cDNA DKFZp586A0618		194±72	82±80	42±27	58±60	120±8	
37060_at	U79289	Clone 23695 mRNA sequence		72±35	22±2	32±16	27±8	215±30	
38855_s_at	D82343	Olfactomedin 1	OLFM1	762±599	236±56	222±215	143±114	2059±155	9q
180_at	S82470	Leukocyte receptor cluster (LRC) member 4	LENG4	74±33	29±17	23±15	22±8	121±74	19q
40653_at	U32439	Regulator of G-protein signaling 7	RGS7	133±32	43±15	59±32	61±37	371±61	1q
34527_r_at	AF052108	Hypothetical protein LOC157627	LOC157627	154±30	61±22	88±42	45±11	213±59	
41792_at	L78207	Sulfonylurea receptor	ABCC8	417±233	172±78	148±91	88±16	205±30	11p
37857_at	AL080188	MT-protocadherin	KIAA1775	141±61	68±22	48±33	17±9	48±15	10q
1998_i_at	U19599	BCL2-associated X protein	BAX	92±14	61±7	28±10	34±6	160±108	19q
40753_at	AF053136	Synuclein β*	SNCB	146±146	11±2	26±25	41±17	953±263	5q

Table 1. Highly expressed genes in oligodendroglioma with 1pLOH.
[1] Expression level of each gene was demonstrated as Mean±S.E.M in each subgroup.
[2] The genes examined by qPCR are indicated by *.

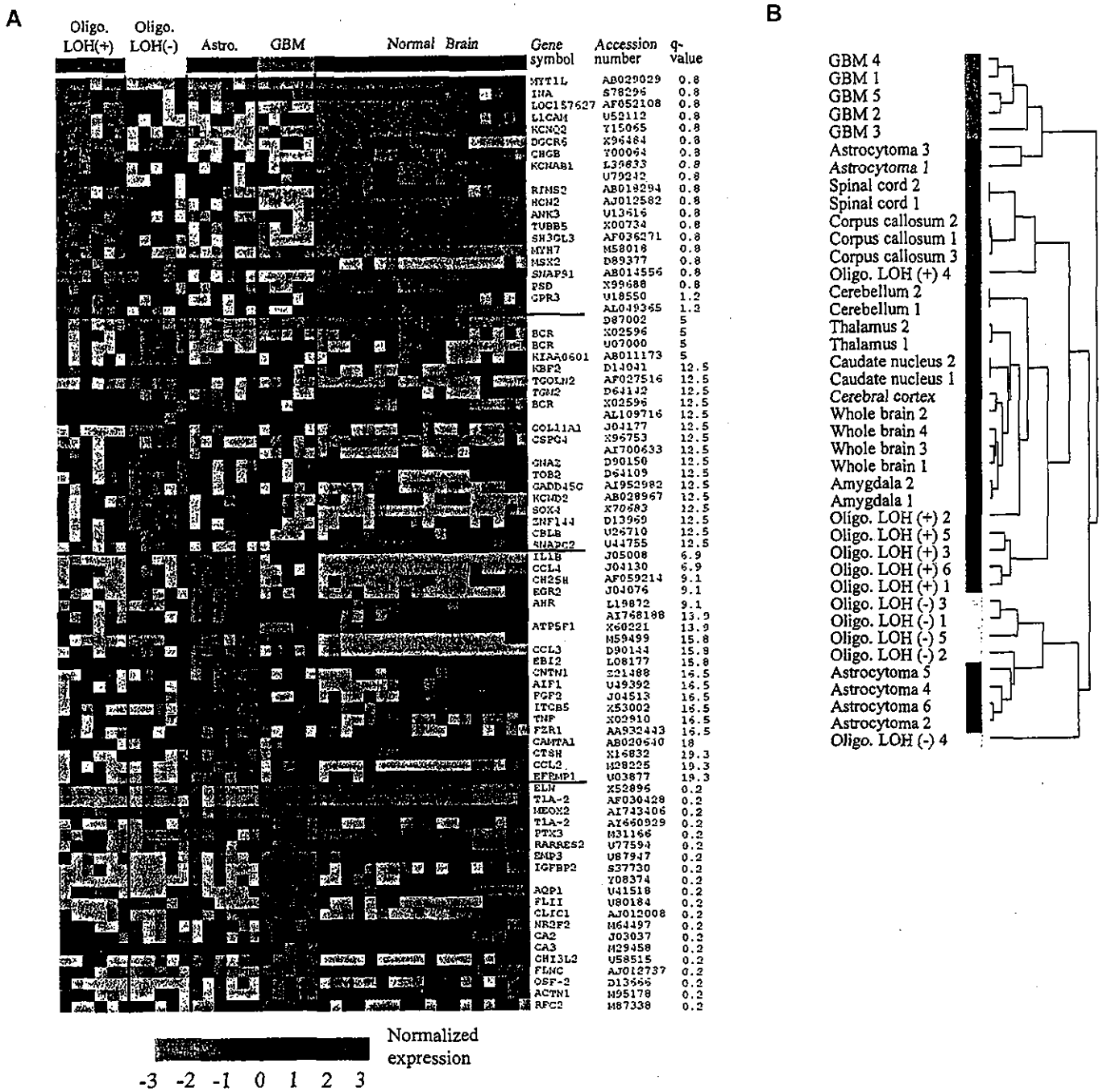

Figure 1. A. The genes characterizing 4 subgroups of diffuse gliomas. Each column represents a sample and each row represents a gene. Expression of each gene in 22 gliomas, together with 18 normal brain tissues was demonstrated in color gradation after normalization. Red indicates increased expression, and green indicates decreased gene expression. The order of samples are oligodendroglioma with 1pLOH (1-6), without 1pLOH (1-5), astrocytoma (1-6), glioblastoma (1-5), our whole brain (1, 2), downloaded whole brain (3, 4), cerebral cortex (1), cerebellum (1, 2), caudate nucleus (1, 2), amygdala (1, 2), thalamus (1, 2), corpus callosum (1-3) and spinal cord (1, 2). Note that the genes showing higher expression in oligodendroglioma with 1pLOH were also highly expressed in normal brain, except for corpus callosum and spinal cord. **B.** The result of hierarchical clustering using selected 80 subtype-specific genes. The oligodendrogliomas with 1pLOH were clustered into the same group with the normal brains, and were more similar to whole brain, cerebral cortex, cerebellum, caudate nucleus, amygdala and thalamus than corpus callosum and spinal cord. Two astrocytomas were clustered together with glioblastomas.

(code S3004; Dako, Glostrup, Denmark) diluted to 1:5000 at room temperature for 10 minutes, and 2 ng/μl of biotin-labeled oligonucleotide probe (antisense of 5′-ACATG GCTGT CACTG GATTT AGGCT TTCTG TCCTC C-3′ and *sense of* 5′-GGAGG ACAGA AAGCC TAAAT CCAGT GACAG CCATG T-3′) was hybridized at 37°C overnight. A GenPoint catalyzed signal amplification system (Dako) was used following manufacturer's instructions, and DAB substrate (Dako) was used to visualize amplified signal. The tissues were counterstained with hematoxylin. Furthermore, to test the quality of the mRNA in samples, positive and negative fluorescein-conjugated peptide nucleic acid (PNA) probes *against glyceraldehyde*

3-phosphate dehydrogenase, and PNA in situ hybridization detection kit (code K5201; Dako) were used according to the manufacturer's instruction.

Immunohistochemistry. Normal brain slides prepared simultaneously for in situ hybridization were immunolabeled. The slides were pretreated with microwave for total 20 minutes in citrate buffer pH 6.0, and then incubated with mouse anti-neuronal nuclei (NeuN) monoclonal antibody (Chemicon, Temecula, Calif) at 1:100 dilution for one hour at room temperature. A LSAB kit and a DAB substrate (DAKO) were used to visualize the antibody bidding, and tissues were counterstained with hematoxylin.

RESULTS

The subtype-specific genes. SAM identified 29, 0, 0, and 247 subtype-specific genes with statistical significance of q-value <1.25% for oligodendroglioma with 1pLOH, without 1pLOH, astrocytoma and glioblastoma, respectively (highly expressed genes in oligodendroglioma with 1pLOH were listed in Table 1). Since SAM is applied 4 times to each subtype, the overall statistical significance for these genes is 5% after Benjamini correction for multiple testing. Then, we tried to select the same number of genes from each subtype of glioma for the subsequent clustering analysis, though SAM identified different number of genes as statistically significant. In this manuscript, main focus of our analysis was oligodendrogliomas with 1pLOH, in which 29 genes were identified as significantly highly expressed by SAM. On the other hand, genes list more than 20 in oligodendrogliomas without 1pLOH and astrocytoma had higher q-value and FDR. Therefore, we decided to select each 20 genes, which were sufficiently specific for oligodendroglioma with 1pLOH and glioblastoma, and were still acceptable for oligodendroglioma without 1pLOH and astrocytoma. Accordingly, we selected each 20 probe sets which showed lower q-value in each subgroup, as subtype-specific genes of oligodendroglioma with 1pLOH, without 1pLOH, astrocytoma and glioblastoma, of which median FDR were within 1.2, 12.5, 19 and 1.9 %, respectively (Figure 1A). Some of those genes showed consistency with other studies, such as insulin-like growth factor binding protein 2 (*IGFBP2*) whose higher expressions in glioblastoma were reported in the previous microarray studies (4, 20, 21).

Most of the genes that showed distinctively higher expression in oligodendroglioma with 1pLOH also showed similarly high expression in the normal brain, while the genes showing higher expression in other glioma subgroups did not have such a trend (Table 1, Figure 1A). Notably, many of those genes were considered to have neuron-related function. For example, myelin transcription factor 1-like (MYT1L) is thought to be a neuron specific transcription factor (10); internexin neuronal intermediate filament protein α (INA) may act as a neuron-specific intermediate filament protein (15); regulating synaptic membrane exocytosis 2 (RIMS2) and synaptosomal-associated protein 91kDa homolog (SNAP91) are supposed to be synapse related molecules; β-synuclein (SNCB) may play a role in neuronal plasticity and abundant in neurofibrillary lesions (2). L1 cell adhesion molecule (L1CAM), chromogranin B (CHGB), ankyrin 3 (ANK3), tubulin β5 (TUBB5), SH3-domain GRB2-like 3 (SH3GL3), pleckstrin and Sec7 domein protein (PSD), olfactomedin 1 (OLFM1), regulator of G-protein signaling 7 (RGS7), potassium voltage-gated channels such as KCNQ2, KCNAB1 and HCN2 are all thought to be expressed in neuronal cells. Besides those known genes, ESTs such as hypothetical protein LOC157627 and clone 23695 also seem to be abundantly expressed in the brain and neural tissue according to the public database such as UniGene, though their functions in the nervous system are not yet proven.

Using these 80 subtype-specific genes in total, clustering analysis was performed on the 22 tumors and 18 normal brain and spinal cord tissues (Figure 1B). The oligodendroglial tumors with 1pLOH were clustered into the same group with the normal brains, indicating their similarity in the expression pattern of the selected genes. Furthermore, oligodendroglioma with 1pLOH were more similar to whole brain, cerebral cortex, cerebellum, caudate nucleus, amygdala and thalamus than corpus callosum and spinal cord, possibly because corpus callosum and spinal cord consist mostly of glial cells than neurons. Two astrocytomas were clustered together with glioblastomas.

The validation studies using quantitative real-time PCR. Of the 29 genes that showed significantly higher expression in oligodendroglioma with 1pLOH, we selected four known genes for further validation study using qPCR. We also analyzed a gene for neurofilament heavy polypeptide (NEFH) which is known to be expressed in normal brain, and OLIG1 gene that is reported to be expressed specifically in oligodendrogliomas (12, 14). Forty-seven samples including 24 samples used in the microarray experiment and 23 additional gliomas were analyzed. The relative expression levels in qPCR of each six gene, *MYT1L*, *L1CAM*, *RIMS2*, *SNCB*, *NEFH* and *OLIG1* were shown in Figure 2. *MYT1L*, *L1CAM*, *RIMS2* and *SNCB* showed significantly higher expression in oligodendrogliomas with 1pLOH than other gliomas ($p<0.0001$, <0.005, <0.0001 and <0.001, respectively), and normal brains also had higher expression as expected from GeneChip data (Note that Y-axis in Figure 2 represents the relative gene expression level to the average expression in normal brain). We recognized, however, some exceptional cases (one of them was indicated by circle in Figure 2) that had higher expression of these genes in other glioma subgroups. Including such cases, gliomas showing higher expression in one of the 4 genes usually had similarly higher expression in other 3 genes as well. The expression levels of *NEFH* in gliomas were much lower than normal brain. *OLIG1* were highly expressed in gliomas comparing to normal brain, though it was not specific to oligodendrogliomas.

The results of qPCR corresponded well to the GeneChip data, and the correlation between the data from the qPCR and the GeneChip were 0.88 for *MYT1L*, 0.77 for *L1CAM*, 0.87 for *RIMS2*, and 0.98 for *SNCB* respectively, using Pearson correlation coefficient.

The expression of neuron-related molecules in oligodendroglioma with 1pLOH. To exclude the possibility of contaminated normal neurons as the source of the higher expression of neuronal genes in oligodendroglioma with 1pLOH, we performed in

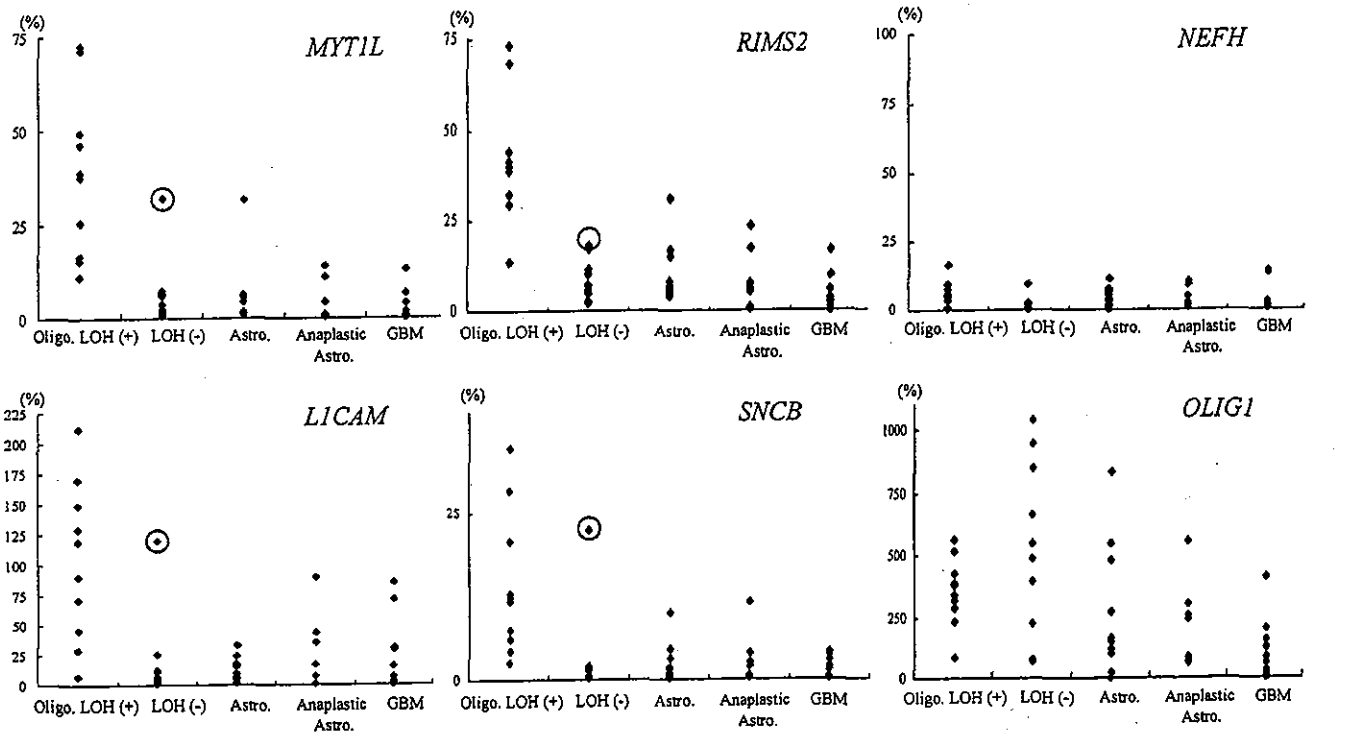

Figure 2. Quantitative real-time PCR analysis on oligodendrogliomas with 1pLOH (n=10), without 1pLOH (n=10), low-grade diffuse astrocytomas (n=9), anaplastic astrocytomas (n=7), glioblastomas (n=9) and normal brains (n =2). Note that Y-axis represents the relative gene expression level to the average expression in normal brains. *MYT1L, L1CAM, RIMS2* and *SNCB* showed significantly higher expression in oligodendrogliomas with 1pLOH than other gliomas ($p<0.0001$, <0.005, <0.0001 *and* <0.001, respectively) using the Kruskal-Wallis test. There were two exceptional cases; one oligodendrogliomas without 1p loss (indicated by circle) and one astrocytoma showed higher expression in those four genes. *NEFH* showed consistent lower expression in all glioma samples comparing with normal brain (<20%). Most of glioma samples had higher *OLIG1* expressions.

situ hybridization for *MYT1L* transcripts. Eleven samples containing good quality of mRNA confirmed by in situ hybridization using PNA probe as a positive control were evaluated; 2 normal brain tissues, 3 oligodendrogliomas with 1pLOH, 3 oligodendrogliomas without 1pLOH, 2 astrocytomas and 1 glioblastoma (Figure 3). *MYT1L* expressions were detected in 2 normal brain tissues and were highly expressed in cells containing large nuclei (Figure 3A, C). The cells containing large nuclei were also immunostained with anti-neuronal nuclei (NeuN) antibody and were assumed to be neurons (Figure 3A, inset). Expression of *MYT1L* transcripts was clearly demonstrated in 2 of 3 oligodendrogliomas with 1pLOH (Figure 3B, D), but not in oligodendrogliomas without LOH (Figure 3E), astrocytomas (Figure 3F) nor glioblastoma (Figure 3G).

DISCUSSION

In this study, we demonstrated that some of the genes showing higher expression in oligodendroglioma with 1p loss compared to the other major subtypes of gliomas were functionally neuron-related genes, with the expression at the similar levels in normal neurons. Although it was rather unexpected that neuron-related genes were expressed in gliomas, contamination of normal neurons in the samples of oligodendroglioma with 1p loss was not likely, because *i)* allelic losses observed on the microsatellite analysis were almost complete in all cases, indicating that the examined tissues consisted mostly of tumor cells, and *ii)* our in situ hybridization for *MYT1L transcripts* demonstrated that these genes were indeed expressed in the tumor cells. Furthermore, *iii)* expressions of other neuron specific genes expressed in normal brain tissues, such as gene encoding neurofilament subunit (*NEFH*, *NEFM* and *NEFL*), were much lower in oligodendrogliomas with 1p loss than those in normal brain. Therefore, the microarray analysis represented expression profile of the tumor cells, not normal neurons.

The qPCR analysis on several genes confirmed the microarray analysis results, and validated them on additional 23 gliomas samples. The results were mostly consistent, showing similar levels of higher expression in oligodendrogliomas with 1p loss but not in other gliomas. However, there were 2 exceptional cases; one oligodendroglioma without 1p loss and one astrocytoma showed higher expression in those genes. On re-reviewing the histology of those 2 tumors, we noticed that the oligodendroglioma case had occasional ependymoma-like portion, but the astrocytoma case was typical astrocytoma without any unusual morphology. We consider that these exceptional cases may reflect the heterogeneity of yet unknown background. To be noted was that the changes of expression levels of the genes were always to the same trend in all gliomas including the exceptional cases, suggesting a possible functional link among those genes.

We also compared WHO grade II (n=8) and grade III (n=3) oligodendrogliomas using Mann-Whitney test with cut-off p-values of 0.05, and 368 genes were detected as differentially expressed by grade (whole list of the selected genes would be available on request). Downregulated genes in grade III tumors included genes for CD44, alpha 1 syntrophin, connexin43 gap junction protein, CCAAT/enhancer binding protein delta, and chemokine receptor 4. Genes upregulated in grade III tumors included

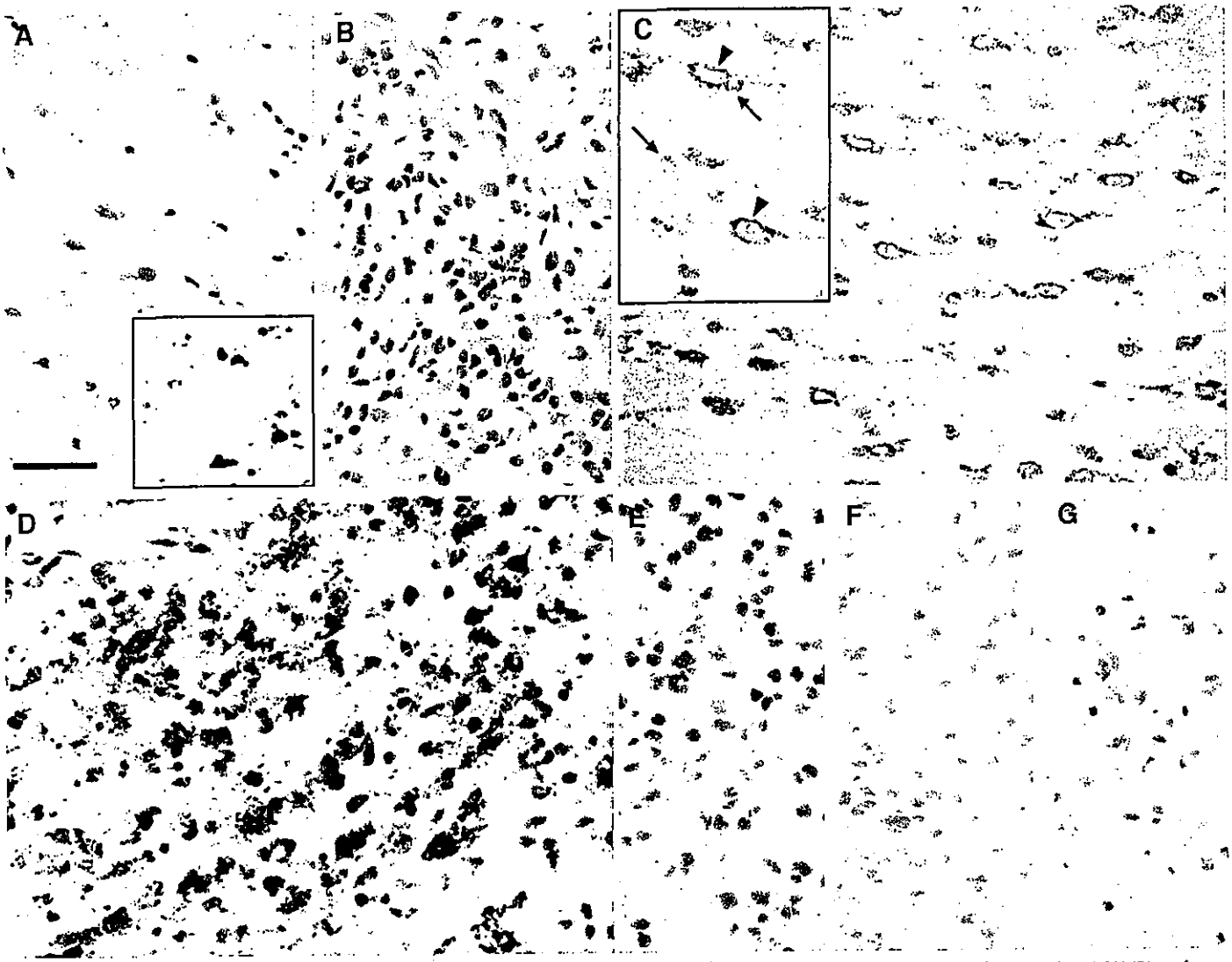

Figure 3. In situ hybridization for *MYT1L* transcripts, with corresponding H&E staining of normal brain (**A**) and oligodendroglioma with 1pLOH (**B**) on frozen section. *MYT1L* expressions were strongly observed in neurons (arrowheads), which contain large nuclei, comparing with the surrounding cells with small, round nuclei which were putatively considered as oligodendrocytes (arrows) (**C**). The cells containing large nuclei were also immunostained with anti-neuronal nuclei (NeuN) antibody (inset, **A**). Oligodendroglioma with 1pLOH actually expressed MYT1L transcripts (**D**), though oligodendroglioma without 1pLOH (**E**), astrocytoma (**F**) and glioblastoma (**G**) did not. (Bar = 50 μm [A, B]; 30 μm [C, D, E, F, G]).

genes for myosin and interferon induced proteins. These genes listed above were concordant with previous report (25), suggesting the consistency of this DNA array analysis. However, further analysis of differentially expressed genes by tumor grade was not performed in this study, because statistical confidence for this data might be limited by small number of our grade III tumors.

Both Myt1 and its homologue Myt1l are zinc finger proteins of CCHC class that are expressed in neurons at early stages of differentiation. While Myt1 is expressed in cells of glial lineage, Myt1l is not detected in glial cells but co-expressed with Tuj1 in neurons around terminal mitosis (10). Therefore, Myt1l is supposed to play a role in the development of neurons. In our study, *MYT1L* was also expressed in neuron of normal adult human brain. The fact that oligodendroglioma with 1p loss express a subset of neuron specific genes like *MYT1L* would raise a question whether these tumor originate from the same glial progenitor cells as the other gliomas. Neurocytic differentiation and variable degrees of neuronal marker expression have been reported in oligodendrogliomas (19, 26, 27), and neuron-like physiological properties of oligodendroglioma cells have been observed as well (18). On the other hand, *OLIG1* and *OLIG2* genes, which are crucial in maturation of oligodendrocyte and its progenitor, were strongly expressed in oligodendrogliomas as previously reported 12, 14), although the expression of those 2 genes were not specific to oligodendrogliomas in our data. Therefore, these results together may suggest that oligodendroglioma with 1p loss have both neuronal and glial differentiation patterns at least on a certain group of genes. In line with such a still hypothetical proposition, recent studies indicated that some oligodendrocytes might share the same progenitor cells with neurons (13, 28).

Since the era of Baily and Cushing's inaugural works, classification of gliomas has been based upon hypothetical origins of the tumor, which were assigned to each tumor type according to their morphological features. With the rapid advancement in developmental biology of nervous system at the molecular level, such classifications could be reorganized using molecular markers related to neural development. Our

observations suggest that genetic subsets in gliomas may well be one of the subjects for such possible redefinition in the future. A clinically important question is whether these neuronal genes such as *MYT1L* could be used to identify more favorable subset of gliomas as diagnostic markers, independently to 1p loss. A recent report showed that in certain malignant gliomas, the expression profiling using microarray was successful in identifying a set of genes more accurate in predicting prognosis of patients than histological diagnosis (17). Notably, 2 patients of astrocytoma grade II which were clustered together with glioblastomas in our series (Figure 1B), indeed showed the clinical course equivalent to glioblastomas, suggesting usefulness of microarray for predicting patients' prognosis. However, almost all oligodendroglioma patients in the current study are still alive and therefore we do not have sufficient prognostic data for analysis at this point. Further investigation with more data, both in number of cases and length of follow-up, would certainly answer such question in the future.

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原著

一次運動野近傍病変の手術における運動誘発電位モニタリングの意義について

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原 著

一次運動野近傍病変の手術における運動誘発電位モニタリングの意義について

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要旨 運動野近傍髄内病変の摘出手術に際して術中錐体路誘発電位(MEP)を記録し，術中MEPの変化から術後運動機能を予測し得るか否かにつき検討した。一次運動野および錐体路近傍占拠性病変45例を対象とし，術中に一次運動野を電気刺激して頸髄硬膜外に挿入した電極からMEPを記録した。術前後の運動機能はDeJong分類で評価し，摘出終了時のMEP D-responseの振幅変化と比較検討した。

術前運動機能がDeJong 3以上の症例では全例MEPが記録できたが，DeJong 2以下の高度運動機能障害例では記録は困難であった。摘出終了時D-responseの振幅が開始時のそれの50%以上に保たれれば，術後運動機能は温存され，50%以下に低下すると術後運動機能の悪化が認められた。

運動野近傍腫瘍摘出術に際し，術中MEPが運動機能温存の指標となり，運動野近傍病変の治療成績向上の一助となり得る。

Key words：motor evoked potential, intraoperative monitoring, cortical stimulation

はじめに

神経膠腫の治療においては，摘出術による腫瘍容積の減少が生命予後に大きく関わる[2,7,8,19]。一方で，術後神経障害は患者のquality of life(QOL)に大きく影響を与えるため，病変に近接する神経機能温存に最大限の努力を払いつつ摘出することが要求される[22]。

近年，magnetic resonance imaging (MRI)，functional MRI，magnetoencephalography (MEG)などの画像診断法の導入により，病変と正常脳の位置関係のみならず，一次運動野や錐体路も画像上推定できるようになった[4,10,20]。しかし，占拠性病変による圧排偏位がある場合，画像のみで運動野や錐体路を同定し，温存を図ることは必ずしも容易ではなく，術後に予想外の運動麻痺の増悪をきたす可能性がある[15]。

錐体路運動誘発電位(corticospinal motor evoked potential：MEP)は，一次運動野を直接電気刺激することによって誘発される対側四肢運動を筋電図で，または脊髄錐体路において発生する第一ニューロンの活動電位として記録するものである[1,2,16,18]。MEPを頸髄硬膜外から記録する場合，direct-response (D-response)とそれに続く波indirect-response (I-response)が得られるが，これまで報告されているようにD-responseは直接一次運動ニューロンの興奮の伝達を表し，錐体路機能を示すものである[12,18]。

われわれは，種々の術前画像診断に加え，このMEPのD-responseを術中にモニタリングすることで頭蓋内占拠性病変，特に神経膠腫の摘出率の向上および運動機能の温存を図ってきた[14,15,22]。今回，脳腫瘍摘出手術における術中MEPのD-response検出率と術中D-responseの変化から，術後運動機能が予測し得るか否かにつき検討したので報告する。

I. 対象および方法

1. 対象

1992年11月～2002年12月までの10年間に開頭摘出手術を施行した頭蓋内占拠性病変で，画像所見から病変が一次運動野またはその近傍か錐体路近傍に存在すると考えられた64症例に対し，術中MEPモニタリングを試みた。電極の破損や硬膜外腔への刺入

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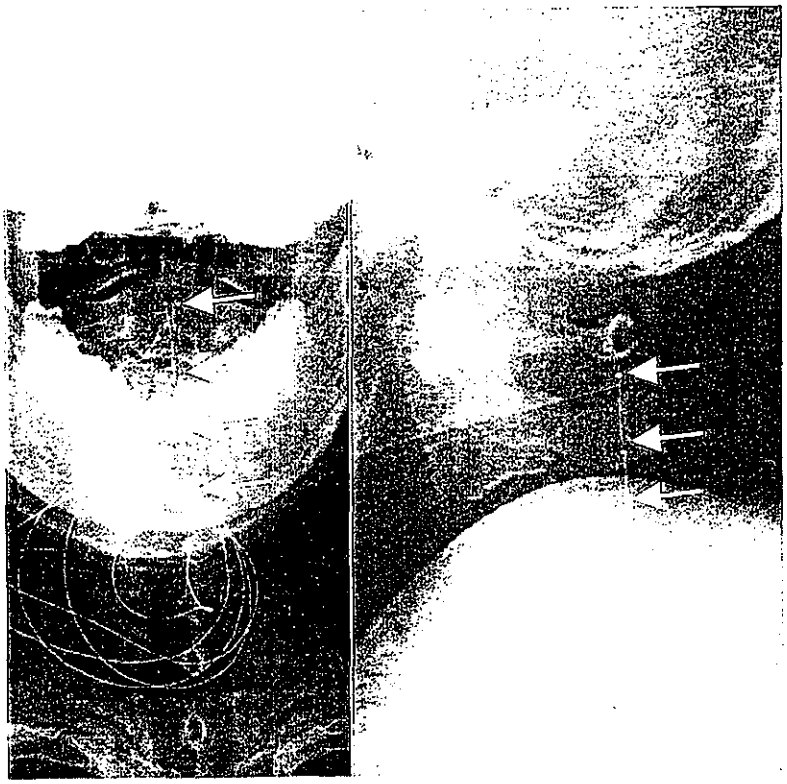

Fig. 1 The catheter-electrode setting.
A-P and lateral X-ray views.
The electrode (arrows) is transcutaneously inserted into the cervical epidural space as high as the rostral edge of the second cervical spine.

成功などの技術的な問題で記録できなかった6例を除く58例の中から，髄膜腫などの髄外病変を除いた占拠性病変45例を対象とした。男性22例，女性23例で年齢は13～77歳(平均49.1歳)であった。病変の病理像は，神経膠腫が31例，海綿状血管腫が6例，転移性脳腫瘍が6例，dysembryoplastic neuroepithelial tumorが1例，悪性リンパ腫が1例であった。病変の局在は，中心前回に接しないもののその近傍の前頭葉13例，中心前回に接した前頭葉10例，中心前回を含む前頭葉あるいは頭頂葉7例，中心前回に接した頭頂葉4例，中心前回に接しないものの近傍の頭頂葉5例，中心前回近傍の側頭葉6例であった。

2. 方法

1)MEP記録電極の留置

術前に，X線透視下に，下位頸椎棘間から頭髄硬膜外腔にカテーテル電極(インターメディカル社，IMC-KG-102 R)を経皮的に挿入し，可能な限り第2頸椎上縁の脊柱管後方正中まで上行させ留置した(Fig. 1)。

2)MEP記録法

使用した全身麻酔の内訳は，静脈内麻酔(propofol)14例，吸入麻酔27例，吸入麻酔とNLAの併用4例であった。麻酔薬の選択は覚醒下手術を行う場合は静脈内麻酔とし，その他の場合には基本的に吸入麻酔剤(isoflurane あるいは sevoflurane)を使用した。

開頭，硬膜切開後，まず中心溝と推定される脳溝の前後にまたがるように4連円板電極(インターメディカル社)をおき，対側正中神経電気刺激によるsomatosensory evoked potential (SEP) を記録した。N20/P20の位相逆転が観察された電極間の脳溝を中心溝，その直前の脳回を一次運動野を含む中心前回と同定した。

次いで，上述の4連円板電極を用いて，一次運動野を双極電気刺激し，頭髄硬膜外に留置した電極よりMEPを記録した。刺激条件は，持続時間0.2 msec，頻度5 c/s，強度10～25 mAであり，10～30回の加算平均波形を記録した。

摘出操作前のMEPをコントロールとし，手術操作中のMEPのD-responseの振幅変化を観察した。

II. 検討項目

1. MEPの検出率

摘出操作前に安定したMEPが記録可能であった例と，刺激強度や電極位置を種々変更してもMEPが記録できなかった例とでそれぞれの術前運動機能につき検討した。

2. 術中MEPの変化と術前後における運動機能の変化の相関について

摘出操作前のD-responseの振幅をコントロールとして摘出操作終了時のD-responseの振幅を比較し，その変化率と術前後における運動機能の変化について