ホウ素中性子捕捉療法(Boron neutron capture therapy；BNCT)

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はじめに

悪性神経膠腫の治療において最大の難題となるのは，正常脳組織に浸潤性に存在する腫瘍細胞であり，これを治療の標的としなければ再発は免れない．腫瘍の画像上の造影域を手術で全て摘出し得ても，周囲脳の浸潤細胞からの再発が必発で，治療には放射線・化学療法を組み合わせた集学的治療が必須となる．放射線治療は非常に有効な治療法であるが，画像診断をもとに治療医が治療計画を行う定位的照射は，開頭手術における治療計画と同様，浸潤部からの再発や正常脳の損傷が問題となる．ホウ素中性子捕捉療法(BNCT；boron neutron capture therapy)は，局所高線量による放射線治療という性格を有しながら，腫瘍を細胞レベルで生物学的に標的とし，正常脳に浸潤した腫瘍細胞をも選択的に治療できるという“細胞選択的粒子線治療”であり，画期的な治療法として注目される[1,2]．

BNCTの原理・背景

ホウ素の同位体である^{10}B(boron-10)が中性子と核反応を生じ，そこから生じたヘリウム原子核(アルファ粒子)とリチウム反跳核により腫瘍細胞を破壊する“ホウ素中性子捕捉療法(BNCT)”の理論が提唱されたのは，中性子発見から間もない1936年のことである．この反応は非常に弱いエネルギーの中性子で得られ，しかも生じるこれらの粒子の飛程がほぼ腫瘍細胞一つ分に相当するため，腫瘍細胞のみが選択的に破壊されるわけである．大量の中性子線や腫瘍選択性のホウ素化合物を必要とし，当時は夢のような治療であった．

BNCTの骨子は腫瘍細胞に^{10}B化合物を取り込ませ中性子を照射することにより，高LET(linear energy transfer；線エネルギー付与)のアルファ粒子が腫瘍細胞一つ分に相当する飛程で放出されることによって，ホウ素が集積した腫瘍細胞のみを選択的に破壊するという細胞生物学的な標的手法にある．特筆すべきは，投与するホウ素化合物および照射する中性子が各々単一では無害であることで，両者が相まって初めて殺細胞効果を示すbinary approach(バイナリーアプローチ)であることである．この反応を中性子捕獲反応といい，中性子捕獲反応は下記の式で表される．

$$n+{}^{10}B\rightarrow{}^{11}B\rightarrow{}^{4}He+{}^{7}LI \text{ あるいは } {}^{10}B(n,\ \alpha){}^{7}Li$$

この反応によって生じるヘリウム原子核，リチウム反跳核は分裂後それぞれ9 μm，4 μmと腫瘍細胞1個の大きさ以下の距離(飛程)を動き停止し，その間に全運動エネルギーを放出する高LET放射線であり，殺細胞効果が非常に高いというわけである(図1)．

脳実質内に浸潤性に発育する悪性脳腫瘍は浸潤領域の脳細胞が機能していると考えられ，腫瘍を細胞レベルで標的とする選択的治療は理想となる．BNCTは，浸潤性発育を特徴とする外科的治癒切除が不能な腫瘍に対する治療効果が期待され，臨床試験・研究が行われてきた．あらゆる治療に抵抗性を示し，有効な治療手段の限られる悪性グリオーマ(神経膠腫)，特にグリオブラストーマ(神経膠芽腫，WHOグレードⅣ)においては期待が高く，これまでにBNCTで治療がなされた症例の多くは悪性グリオーマである[2]．

BNCTによるグリオーマの治療成績

BNCTの原理が提唱されて以後，臨床応用へ向けた開発研究が進められてきた．その結果，米国では1951年には医療照射用原子炉〔ブルックヘブン国立研究所(BNL)研究炉〕が作られ，1953年から脳腫瘍患者に対するBNCTが開始された．BNLおよびその後のマサチューセッツ工科大学炉(MITR)での臨床研究は1961年に終了したが，当時のホウ素化合物が腫瘍選択性に乏しかったこと，試験に用いられた熱中性子線は組織深達性が悪かったことなどの課題があり，そのため血中ホウ素濃度は高く，正常組織の障害も高頻度であった[1]．その後改良が行われ，米国では単剤のホウ素化合物(BPA；boronophenylalanine)を用い，組織深達性で勝る熱外中性子を用いた非開頭照射が1999年まで行われたが，生存期間は13～15ヵ月と治療効果はわずかであり，中性子照射線量の増加試験では生存期間が延長したが深刻な中枢神経合併症が生じ[3]，現在米国でのBNCTは困難となっている．欧州においては，これまでにオランダ，チェコでのBSH(sodium borocaptate)を用いた臨床試験，スウェーデン，フィンランドでのBPAを用いた臨床試験などがある[2]．スウェーデンのグループは，BPAの投与量増量・長時間持続投薬(BPA 900 mg/kg)によるプロトコールを導入し，これによってより均一かつ高濃度のホウ素を腫瘍に集積させ，治療成績の向上を示している．2001～2003年に本手法で新規診断膠芽腫を治療し，生存期間中央値(MST)が17.7ヵ月と，BNLの成績〔BPA(250～330 mg/kg)，MST 12.8ヵ月〕[3]に比較して有意に良好な成績であったと報告し，同一のホウ素化合物でも投薬プロトコールにより異なった治療成績となることが示された[12]．また同時に，BNCTにおいてもテモゾロミドを併用

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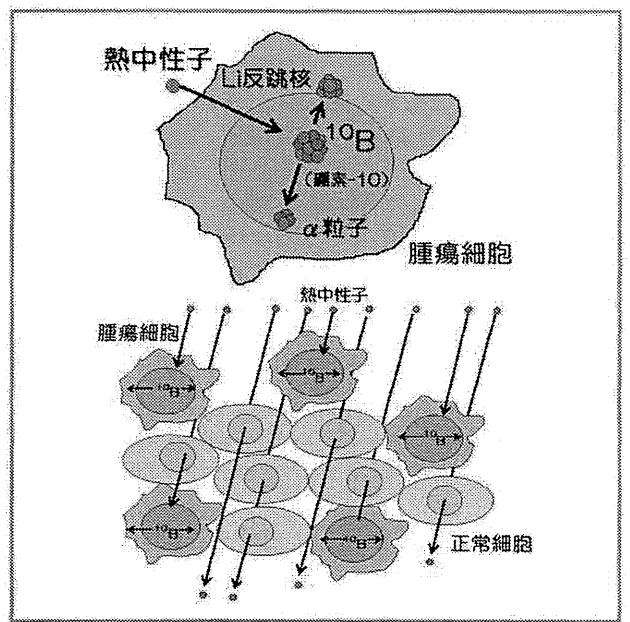

図 1　ホウ素中性子捕捉療法の原理図

BNCT では腫瘍選択性を有するホウ素(^{10}B)化合物を投与し，低エネルギーの中性子を照射することで^{10}B が中性子と核反応を生じ，そこから生じたヘリウム原子核(アルファ粒子)とリチウム反跳核で腫瘍細胞の選択的破壊を実現する．ホウ素化合物が選択的に腫瘍に充分集積し，かつ正常脳・血中の濃度が低下した時点で患部に中性子を照射すれば，腫瘍のみが浸潤部においても選択的に破壊される．

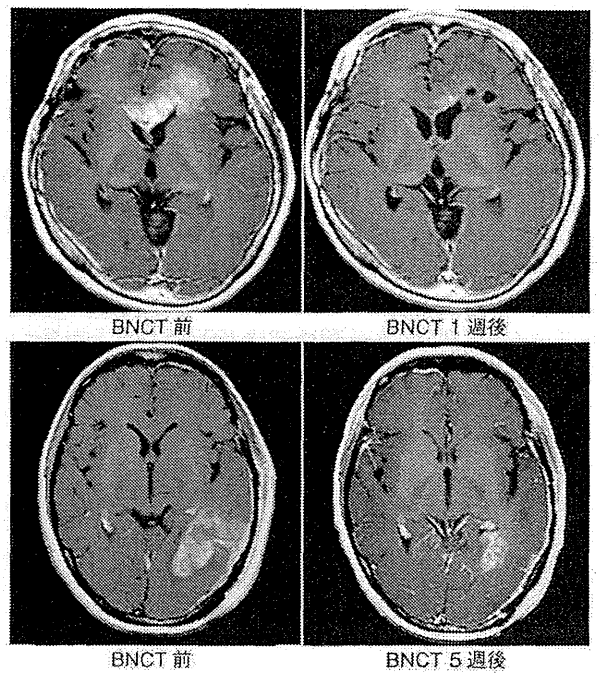

図 2　ホウ素中性子捕捉療法の治療効果

熱外中性子を用いた非開頭 BNCT に，集積機序の異なる 2 種類のホウ素化合物(BPA，BSH)を併用したプロトコールでの治療例．治療後早期から造影 MRI 画像上増強効果を示す腫瘍の縮小効果を認め，非常に良好な局所制御が得られた．

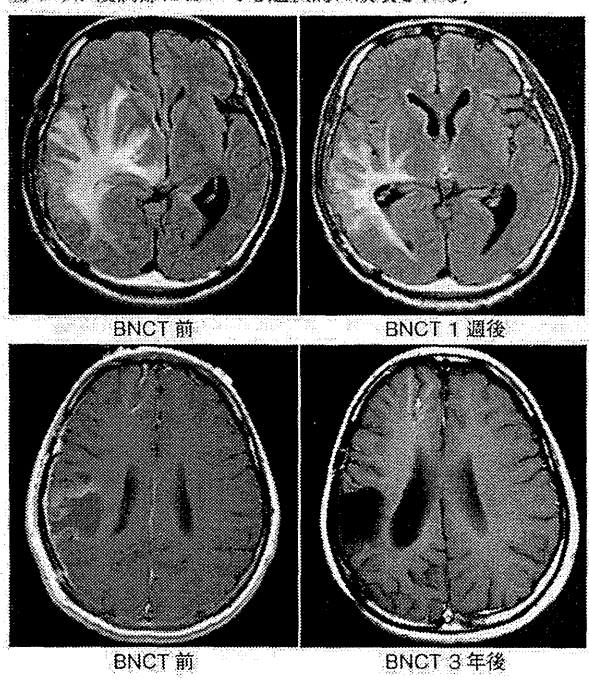

図 3　ホウ素中性子捕捉療法後の画像変化

BNCT 後早期から，ステロイド剤や浸透圧利尿剤等を用いなかったが，MRI(FLAIR)画像上の高信号病変は縮小し，それに伴って神経脱落症状の改善が得られた(上段)．また，外科的全摘出を実施した例(下段)でも長期間局所再発はなく，照射後の周囲脳の変化はほとんどみられない．

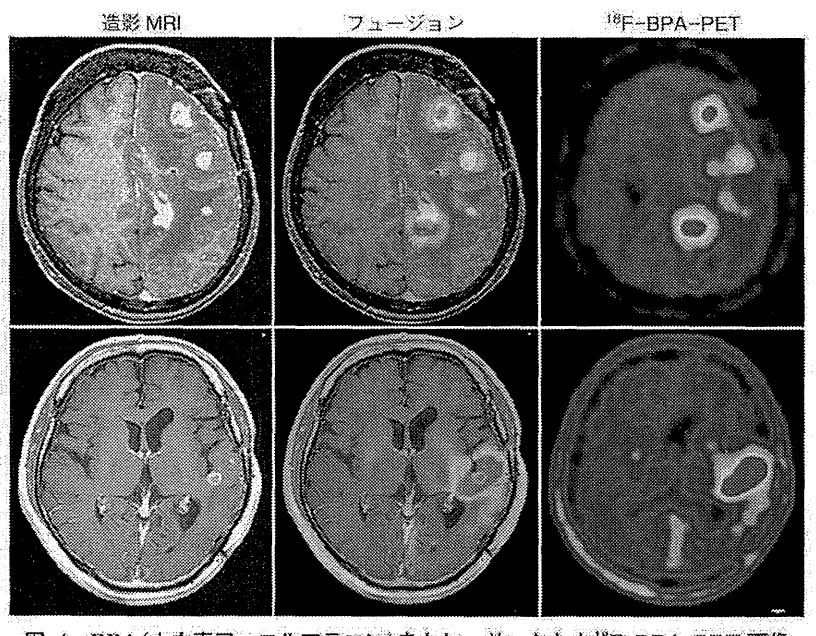

図 4　BPA(ホウ素フェニルアラニン)をトレーサーとした^{18}F-BPA-PET 画像

多発性の病変においても，病変部を含めた中性子照射野を設定することで，ホウ素化合物の集積に合わせて 1 回の治療ですべてに高線量が照射できる(上段)．また，MRI でのガドリニウム増強を受ける腫瘍塊を超えて^{18}F-BPA-PET で周辺の非造影部にも高集積がみられる例を経験するが，このような例においてもホウ素化合物の分布によって高線量が照射され，この部を含めた照射野を設定する以外，特別な工夫は不要である(下段)．

することで治療成績が向上することが示されたが，実際にはテモゾロミド抵抗性を示す患者群におけるBNCTの有用性が期待されるものの，テモゾロミド反応性の患者群においては積極的な併用療法が推奨される[13]．

本邦でも1968年から日本原子力研究開発機構等でBNCTが行われるようになり，Nakagawaらは149例の神経膠腫に対しBSH単剤による開頭術中，中性子照射を行い，膠芽腫のMSTは21.3ヵ月と高い効果を報告している[10]．京都大学原子炉実験所では1990年から悪性神経膠腫に対する治療が行われたが，多くの再発例を含みながらも，3年生存率で20%以上と従来の治療に比べ約2倍に向上したが，欧米同様多くに課題も残った[15]．現在までに臨床研究で用いられてきたホウ素化合物はBSHとBPAのみであり，これまでの臨床研究では各々が単剤で使用されてきた．BSHには1分子あたりのホウ素含有量が多く，多量のホウ素を送達できるが，分子量が大きく血液脳関門を通過しにくいという問題がある．また，BPAは必須アミノ酸であるフェニルアラニン骨格を有し，アミノ酸代謝の活発な腫瘍細胞に高集積を示すが，腫瘍とコントラストがあるものの正常脳にも分布する点，および細胞周期に依存しやすい点など一長一短がある．われわれは2002年から，熱外中性子を用いた非開頭BNCTにこれら2種類のホウ素化合物を併用し両者の欠点を克服する試みを開始し[6]，新規診断膠芽腫のMSTは15.6ヵ月と，それまでの施設コントロールを有意に上回った．2004年以降われわれは，BPAの増量(700 mg/kg)およびX線分割外照射をBNCTに組み合わせ，MSTが23.5ヵ月と延長することを示した(図2)[7]．X線分割外照射併用プロトコールは他グループでも採用され，最近YamamotoらがBNCTの自験例から，新規診断膠芽腫における開頭・術中照射群と非開頭・外照射群の治療成績を比較し報告している．これによれば，開頭・術中照射群ではBSH単剤を用い，非開頭・外照射群ではBSH，BPA(250 mg/kg)の併用にX線分割外照射を組み合わせ，MSTがそれぞれ23.3ヵ月(N=7)，27.1ヵ月(N=8)と非常に良好である[5,7,14]．これら熱外中性子を用いた国内外の臨床試験の詳細については最近行なったレビューを参照いただきたい[2]．

悪性神経膠腫の再発例となるとさらに難治となり，特に既放射線治療例では治療法の選択に難渋する．新規診断例の標準治療と同様，これまでに手術や放射線の追加照射も行われてきたが，生存期間は約6ヵ月である．再発例においてもBNCTの治療効果は強力で，他の治療法では得られ難い画像上の縮小効果も多くの例で経験する(図3)．最近の再発膠芽腫を対象とした報告によると，BNCT後の生存期間は8.7ヵ月であり[11]，われわれの10.8ヵ月と同等，良好である[9]．

近年の放射線治療装置・技術の進歩は目覚ましいが，画像誘導下に照射計画を行い，それによって計画された領域内すべての組織が照射を受ける点は共通した問題点である．BNCTでは画像上造影効果を示した病変を照射野内に含めたとしても，ホウ素化合物が高集積しなければ線量が付与されず，例えば治療後の画像に混在する壊死巣やその周囲の正常脳への再照射・被曝は最小限にとどまることになる．これを効率よく視覚化するのがわれわれが積極的に導入する^{18}F-BPA-PET(図4)ということになる[4,6,8]．局所高線量による再発例の治療で問題となる十分な治療効果(線量付与)と周囲や内部に存在するリスク組織への線量低減といったジレンマが，BNCTではその生物学的選択性により解消されると考えられ，他の治療法と比べても安全性を維持しつつ治療効果が発揮できる理由と思われる(図2，3)．

加速器を中性子源とするBNCTの可能性

BNCTを取り巻く最近の話題としては加速器を中性子源としたBNCTの開発である．BNCTが医療として認知されるには原子炉から脱却しなければならない．最近，脳腫瘍での成績の向上や他臓器への応用など多方面からの注目もあり，加速器中性子源の開発研究に拍車がかかっている．

医療照射に対応可能な加速器中性子源の開発は既に実現しており，医療機器としての申請も不可能ではなくなっている．これによりBNCTはようやく医療承認を目指す治験という枠組みに参入できるようになり，本邦においては世界に先駆けBNCT用加速器中性子源を用い，再発悪性神経膠腫を対象にした第一相臨床試験が現在進行中である．

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A surgical loupe system for observing protoporphyrin IX fluorescence in high-grade gliomas after administering 5-aminolevulinic acid

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KEYWORDS
5-Aminolevulinic acid;
Glioma;
Surgery;
Surgical loupes

Summary
Background: We recently developed a surgical loupe system for observing the fluorescence emitted by protoporphyrin IX (PpIX), a metabolite of 5-aminolevulinic acid.
Methods: This system used a semiconductor laser as the excitation light source. A compact, transparent, and ultraviolet cut-off filter was mounted on an eyepiece lens, which did not require filter on–off manipulation.
Results: Good quality protoporphyrin IX fluorescence was acquired using the surgical loupe system during glioblastoma resection, which was nearly identical to that acquired by fluorescent microscopy. In addition, surgeons can perform ordinary surgical procedures using this surgical loupe system under white light.
Conclusion: This surgical loupe system enables the detection of PpIX fluorescence during resection of high-grade glioma. Further evaluations of this system are required to determine the extent of surgical resection before its practical application.

Introduction

Surgical microscopes with integrated protoporphyrin IX (PpIX) fluorescence systems are marketed by several manufacturers. However, they involve relatively large and complicated equipment. Because surgical microscopes use xenon light that passes through a filter, the excitation light has a broad wavelength range (approximately 405 nm). Therefore, this excitation light requires attenuation, which is usually accomplished by using a band-pass or a low-cut filter that allows the passage of light with a wavelength of approximately 635 nm. Consequently, filter on–off manipulation is required and the filter is mounted on the system only during observations.

When a laser is used as the excitation light source, the light exhibits a sharp peak at approximately 405 nm. In this case, filter on–off manipulation during observation is not required if an ultraviolet (UV) cut-off filter is mounted on

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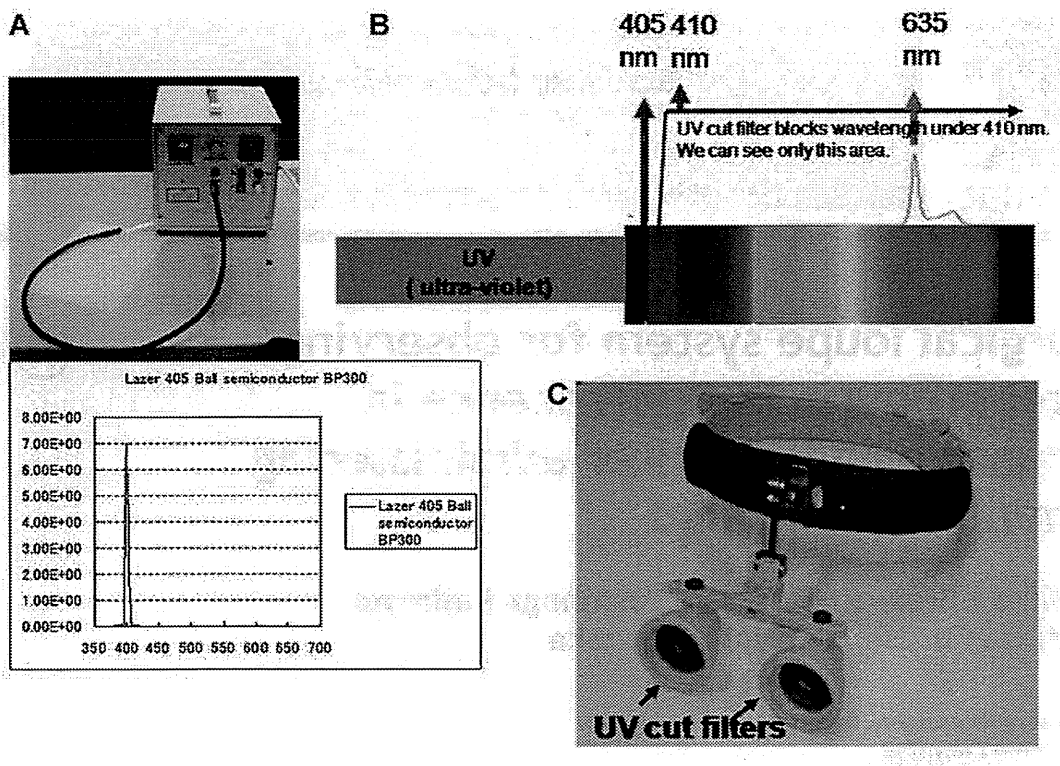

Figure 1 (A) Laser device with 405 nm irradiation and its wavelength distribution. The output at the fiber end is 300 mW. Irradiation of a 6-cm diameter area with light of 6 mW/cm^2 brightness is possible. (B) 405 nm excitation light, 635 nm fluorescence emitted by protoporphyrin IX, and their relationship to an ultraviolet cut-off filter that blocks wavelengths less than 410 nm. (C) The surgical loupes are integrated with ultraviolet cut-off filters (arrows).

the eyepiece lens. We previously reported a neuroendoscope that used this type of system [1]. We have developed a surgical loupe system using an excitation laser that does not require filter on–off manipulation during PpIX fluorescence observations.

Materials and methods

Head-mounted ×4 magnification loupes (HEINE HR® binocular loupes, Heine Optotechnik, Herrsching, Germany) and a semiconductor laser exhibiting a sharp peak at approximately 405 nm (Fig. 1A) were used as the excitation light source. A UV cut-off filter that attenuated light with wavelengths less than 410 nm was used to observe the fluorescence at 635 nm (Fig. 1B). The surgical loupe system integrated with the UV cut-off filter (Fig. 1C) did not require filter on–off manipulation because the filter was transparent.

When this system was used under an ordinary light source, surgical manipulations were possible without being affected by the UV cut-off filter. When the ordinary light source was turned off and the laser was used, PpIX fluorescence at 635 nm could be observed.

We have used this system clinically after it was approved by our institutional ethics committee. This laser was rated lower than an FDA class IIIb by The Center for Devices and Radiological Health 21 Code of Federal Regulations. Therefore, it is recommended that laser eye protection should be provided to all staff members in the operating room when using this laser.

Results

The proposed laser system was used during glioma surgery for a patient with a glioblastoma. A microscope (OPMI Pentero, Carl Zeiss Meditec, Germany) and our surgical loupe system were used during tumor resection. Silicone fence-post tubes were inserted around the tumor, and the tumor surrounded by the fence posts was resected. Fig. 2A shows an intraoperative post-resection image under white light. Fig. 2B shows the same image captured through a 410 nm-low pass filter. This image simulated the field of vision that surgeons would observe with the surgical loupes.

Fig. 2C and D show the images obtained with fluorescence microscopy (BLUE 400, Carl Zeiss Meditec, Germany) without (Fig. 2C) and with the 405 nm laser (Fig. 2D). PpIX fluorescence was more distinct when the laser was used. Similar PpIX fluorescence intensity was obtained in an image when the laser light was passed through the 410 nm-low pass filter (Fig. 2E). The residual fluorescent tumor was resected.

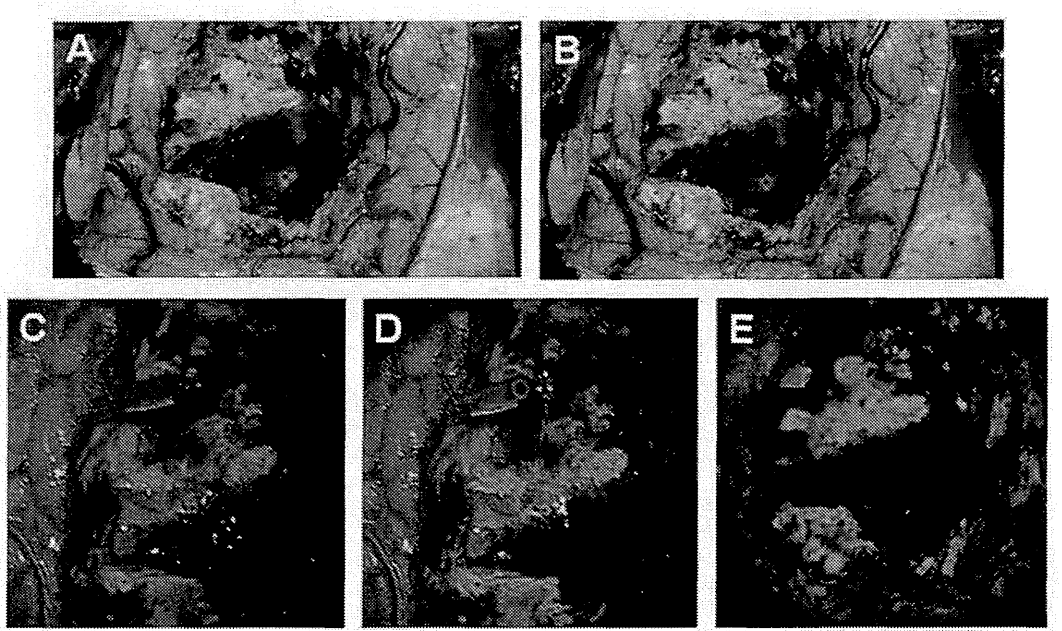

Figure 2 (A) Intraoperative image under white light during a glioblastoma resection. (B) Image obtained using a transparent ultraviolet (UV) cut-off filter, which simulates the surgeon's view using the surgical loupes. (C) Fluorescence image using a microscope. (D) Fluorescence image with a laser using a microscope. (E) Fluorescence image using a laser and a transparent UV cut-off filter.

Discussion

Fluorescence-guided glioma surgery initially used fluorescein sodium [2–5]. Recently, 5-aminolevulinic acid (5-ALA) has become widely used because PpIX has a theoretically higher selectivity than fluorescein sodium for tumor fluorescence. A randomized controlled trial using 5-ALA revealed a higher rate of complete resections and extended progression-free survival for newly diagnosed malignant gliomas [6]. Pathological examinations showed that 5-ALA fluorescence had a higher predictive value for tumor cells than white light [4].

With our system, the use of a laser as the excitation light source, which exhibits a sharp peak at 405 nm, does not require filter on–off manipulation during observations. When a laser is used with a surgical microscope, filter on–off manipulation is unnecessary as long as a UV cut-off filter is mounted on the eyepiece lens. Because the UV cut-off filter needs to be mounted only on the eyepiece lens, it can be used not only with a head-mounted loupe but also with a glasses frame-type or a lens insertion-type loupe. In addition, ordinary surgical procedures can also be performed using the surgical loupe under white light because a low pass UV cut-off filter at 410 nm does not alter the field of vision, particularly the color tone.

This system enables more accurate glioma resection than a regular surgical loupe because of the features of PpIX fluorescence technology. However, it is desirable that a video camera is mounted on the loupes to display operative images on a monitor for recording and education. Further evaluations of this system are required to determine the extent of surgical resection before its practical application for high-grade glioma.

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Phase II clinical study on intraoperative photodynamic therapy with talaporfin sodium and semiconductor laser in patients with malignant brain tumors

Clinical article

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Object. The objective of the present study was to perform a prospective evaluation of the potential efficacy and safety of intraoperative photodynamic therapy (PDT) using talaporfin sodium and irradiation using a 664-nm semiconductor laser in patients with primary malignant parenchymal brain tumors.

Methods. In 27 patients with suspected newly diagnosed or recurrent primary malignant parenchymal brain tumors, a single intravenous injection of talaporfin sodium (40 mg/m^2) was administered 1 day before resection of the neoplasm. The next day after completion of the tumor removal, the residual lesion and/or resection cavity were irradiated using a 664-nm semiconductor laser with a radiation power density of 150 mW/cm^2 and a radiation energy density of 27 J/cm^2. The procedure was performed 22–27 hours after drug administration. The study cohort included 22 patients with a histopathologically confirmed diagnosis of primary malignant parenchymal brain tumor. Thirteen of these neoplasms (59.1%) were newly diagnosed glioblastomas multiforme (GBM).

Results. Among all 22 patients included in the study cohort, the 12-month overall survival (OS), 6-month progression-free survival (PFS), and 6-month local PFS rates after surgery and PDT were 95.5%, 91%, and 91%, respectively. Among patients with newly diagnosed GBMs, all these parameters were 100%. Side effects on the skin, which could be attributable to the administration of talaporfin sodium, were noted in 7.4% of patients and included rash (2 cases), blister (1 case), and erythema (1 case). Skin photosensitivity test results were relatively mild and fully disappeared within 15 days after administration of photosensitizer in all patients.

Conclusions. Intraoperative PDT using talaporfin sodium and a semiconductor laser may be considered as a potentially effective and sufficiently safe option for adjuvant management of primary malignant parenchymal brain tumors. The inclusion of intraoperative PDT in a combined treatment strategy may have a positive impact on OS and local tumor control, particularly in patients with newly diagnosed GBMs. Clinical trial registration no.: JMA-IIA00026 (https://dbcentre3.jmacct.med.or.jp/jmactr/App/JMACTRS06/JMACTRS06.aspx?seqno=862).
(http://thejns.org/doi/abs/10.3171/2013.7.JNS13415)

Key Words • **malignant brain tumor** • **malignant glioma** • **oncology** • **photodynamic therapy** • **talaporfin sodium** • **outcome**

Malignant brain tumors are characterized by invasive growth into adjacent normal neuronal tissue. Therefore, it is crucial that their man-

This article contains some figures that are displayed in color online but in black-and-white in the print edition.

Abbreviations used in this paper: GBM = glioblastoma multiforme; OS = overall survival; PDT = photodynamic therapy; PFS = progression-free survival; PS = performance status; 5-ALA = 5-aminolevulinic acid.

agement is directed not only to maximal possible resection (while ensuring preservation of the functionally important anatomical structures), but on suppressing the growth of the residual infiltrative tumor cells. Despite aggressive surgical removal followed by postoperative radiotherapy and chemotherapy, between 50% and 85% of WHO Grade IV gliomas recur locally.[9,16] This emphasizes the need for additional options to improve their growth control.

Photodynamic therapy (PDT) is a treatment method that involves administration of a photosensitizer that accumulates in tumor tissue and newly formed neoplastic vessels. During subsequent irradiation with a laser beam of a specific wavelength, the photosensitizer undergoes a photochemical reaction that produces singlet oxygen possessing strong oxidation properties that cause alteration of the cells. Because singlet oxygen has a short lifetime (0.04–4 μsec), the PDT-induced cell death is realized only locally in the areas irradiated by the laser beam.[2,7,8,15]

Talaporfin sodium (mono-L-aspartyl chlorine e6, or NPe6) is a relatively novel photosensitizer for PDT. Its administration in combination with a semiconductor laser has been approved in Japan for clinical use in cases of early stage lung cancer. Nonclinical pharmacological studies directed to its possible application for management of malignant brain tumors were initiated starting in 2001.[12–14] Experiments with glioblastoma cell lines demonstrated that such therapy induces mitochondrial apoptotic cell loss accompanied by tumor necrosis.[13,14] Our recent single-center pilot clinical study on the use of talaporfin sodium and a semiconductor laser in patients with malignant gliomas demonstrated promising results with regard to tumor response rates and treatment safety.[1] Therefore, the present open-label, prospective, multicenter clinical trial was initiated for evaluation of the potential efficacy and safety of such therapy. This study was the first investigator-initiated clinical trial in Japan that planned to assess the use of talaporfin sodium and a semiconductor laser for intraoperative PDT as part of a combined management of primary malignant parenchymal brain tumors.

Methods

Patients with suspected primary malignant parenchymal brain tumors, either newly diagnosed or recurrent, which according to preoperative neuroimaging corresponded to a WHO histopathological grade of III or IV,[11] were enrolled in this study. The recruitment of patients and analysis of treatment efficacy were mainly focused on newly diagnosed glioblastoma multiforme (GBM). The main inclusion criteria included agreement of the patient to provide written informed consent to participate in the study; age between 20 and 69 years at the time of informed consent; performance status (PS) score of 0, 1, 2, or 3 according to Eastern Cooperative Oncology Group PS scale (a PS score of 3 was accepted only when the score was attributable to neurological symptoms caused by the tumor); supratentorial location of the tumor not including neoplasms originating from the optic pathways and pituitary gland; absence of subarachnoid dissemination; and eligibility for aggressive resection of the lesion. The main exclusion criterion was a history of photosensitivity or porphyria.

Study Design

This prospective clinical trial was developed and carried out in 2 neurosurgical centers with well-established neurooncology programs, namely Tokyo Women's Medical University and Tokyo Medical University. An open-label, investigator-initiated clinical study was conducted in accordance with the Declaration of Helsinki. The research protocol was approved by the Pharmaceuticals and Medical Devices Agency of Japan as well as by the ethics committees and institutional review boards of both participating universities. A special review board was formed for central radiology assessment, evaluation of data related to treatment efficacy and safety, and handling of the enrolled cases and overall data management. Additionally, a pathology board was created for central review of the permanent formalin-fixed tissue specimens to determine the histopathological tumor type and grade. The 3-year study period was scheduled from March 21, 2009, to February 28, 2012. The clinical trial information for this study can be found at https://dbcentre3.jmacct.med.or.jp/jmactr/App/JMACTRS06/JMACTRS06.aspx?seqno=862.

Patients who were considered eligible for enrollment into study received a single intravenous injection of talaporfin sodium (Laserphyrin, Meiji Seika Pharma Co., Ltd.) in a dose of 40 mg/m^2 on an inpatient basis 1 day prior to undergoing the elective craniotomy. The next day, surgery was done, the neoplasm was resected, and irradiation of the resection cavity with a 664-nm semiconductor laser beam (Panasonic Healthcare Co., Ltd.), with a diameter of 1.5 cm, radiation power density of 150 mW/cm^2, and radiation energy density of 27 J/cm^2, was performed. Particular emphasis was put on irradiation of the areas at risk for recurrence, such as the genu of the corpus callosum.[9] If tumor resection was incomplete and the residual lesion was macroscopically identified, additional irradiation by the laser was applied at 1 to 3 sites with avoidance of overlap of the irradiation areas. In all cases laser irradiation was done 22–27 hours after administration of talaporfin sodium.

Postoperative Treatment and Follow-Up

Postoperatively all patients with newly diagnosed gliomas underwent fractionated radiotherapy (total dose 60 Gy) with concomitant and adjuvant chemotherapy using ACNU (in cases of WHO Grade III tumors) or temozolomide[18] (in cases of GBM). Patients with recurrent neoplasms were treated according to the preference of their doctors, taking into consideration the details of the primary management.

Adverse effects of treatment were graded according to the Common Terminology Criteria for Adverse Events version 3.0.[3] Follow-up examinations were performed every 2–3 months and included physical and neurological assessments with evaluation of PS score, blood and urine tests, and contrast-enhanced MRI. Tumor progression was defined as a 25% or greater increase in the volume of the contrast-enhanced lesion or the appearance of

new brain lesions. At the time of recurrence the salvage treatment was applied according to the preference of the individual doctors and usually included a combination of re-resection, second-line chemotherapy, and/or vaccine therapy.

End Point Evaluation

The primary end point of the study was overall survival (OS) rate at 12 months after PDT. Secondary end points were progression-free survival (PFS) and local PFS rates at 6 months after PDT. The OS, PFS, and local PFS were all estimated from the date of surgery. Additionally, in cases with a maximal diameter of the residual neoplasm of 16 mm or more, the overall tumor response to treatment was evaluated. All brain MRI data before surgery and during follow-up were assessed by review board members. Safety end points included rates of adverse events, side effects, and results of skin photosensitivity testing.

Data Analysis

Analysis of the treatment efficacy was done in all patients who underwent PDT based on administration of talaporfin sodium and intraoperative laser irradiation of the residual neoplasm and/or resection cavity if the diagnosis of primary malignant parenchymal brain tumor was confirmed by the pathology review board after investigation of the permanent formalin-fixed tissue sections (study cohort). Separate analysis of the treatment efficacy was also done in the subgroup of patients with newly diagnosed GBMs. Survival was assessed using the Kaplan-Meier method. Analysis of the treatment safety was done in all patients initially enrolled into the study who received talaporfin sodium.

Results

Patient Characteristics

Detailed characteristics of patients enrolled in the study are presented in Table 1. In all, 27 patients initially received talaporfin sodium. However, 3 patients were deemed ineligible for study participation during surgery and did not receive irradiation with the laser based on the results of the intraoperative histopathological investigation of the resected tissue on the frozen sections, which revealed lymphoma, low-grade glioma, and cavernoma (1 case each). Additionally, 2 patients were excluded from the study later on because the pathology review board did not confirm the diagnosis of a primary malignant parenchymal brain tumor based on the postoperative examination of the permanent formalin-fixed tissue sections. Therefore, the study cohort included 22 patients with a male/female ratio of 1:1 and a median age of 50.5 years (range 24–69 years). The frontal lobe was affected most frequently (59.1% of cases). In 72.7% of patients the tumor was located within or close to eloquent brain areas. Total, subtotal (> 90% of the lesion volume), and partial resections of the neoplasm were performed in 36.4%, 50%, and 13.6% of cases, respectively. No significant differences in clinical characteristics were observed between the entire group of initially enrolled patients (n = 27) and the study cohort (n = 22). Thirteen (59.1%) of 22 patients included in the study cohort had newly diagnosed GBMs and corresponded to recursive partitioning analysis Classes III (4 cases), IV (5 cases), and V (4 cases).[6]

Treatment Efficacy

Among all 22 patients included in the study cohort, 1 death occurred within 12 months after surgery. This patient died 3.4 months after resection and PDT of a newly diagnosed gliosarcoma due to local progression of the tumor. Therefore, the 12-month OS rate was 95.5%. Two tumors demonstrated progression despite treatment within 6 months after surgery, and both recurrences were local. Therefore, the 6-month PFS and local PFS rates were 91%. The maximum length of follow-up was 38.6 months. The median OS was 27.9 months (95% CI lower, 24.8 months; upper, not estimated), the median PFS was 20 months (95% CI lower, 10.3 months; upper, not estimated), and the median local PFS was 22.5 months (95% CI lower, 17.2 months; upper, not estimated).

Among 13 patients with newly diagnosed GBM, the 12-month OS, 6-month PFS, and 6-month local PFS rates after surgical removal of the tumor and PDT were all 100% (Fig. 1). In this subgroup the maximum length of follow-up was 32.0 months. The median OS was 24.8 months (95% CI 18.5–32.0 months), the median PFS was 12.0 months (95% CI 10.3–24.2 months), and the median local PFS was 20.0 months (95% CI 16.2–32.0 months).

In only 1 patient was it possible to evaluate the overall tumor response to treatment. In this case, a newly diagnosed GBM showed complete response 4 months after surgery and PDT.

Treatment Safety

Among all 27 patients who received talaporfin sodium the day before surgery, serious adverse events were noted postoperatively in 6 patients (22.2%). These included aphasia (2 cases) and hemiplegia, hemiparesis, unilateral blindness, visual field defect, homonymous hemianopia, postoperative pyrexia, and infection (1 case each). The overall frequency and distribution of postoperative adverse events were within the range of our usual neurosurgical practice in cases of primary malignant parenchymal brain tumors, and their causal relationships with administration of talaporfin sodium and/or intraoperative laser irradiation were very unlikely. None of these adverse events resulted in the death of a patient.

The laboratory test results in all patients were abnormal, most frequently with an increase in γ-glutamyltransferase (59.3%), alanine aminotransferase (48.1%), aspartate aminotransferase (37.0%), blood alkaline phosphatase (25.9%), and blood lactate dehydrogenase (22.2%). In 18 (66.7%) of 27 patients such abnormalities could be considered as side effects after administration of talaporfin sodium. Postoperative adverse events by system organs, particularly abnormal liver function, were relatively frequent but never exceeded Grade 3 toxicity (Table 2). Only 2 patients (7.4%) had skin disorders, which could be con-

TABLE 1: Characteristics of patients enrolled into study

Demographics & Clinical Characteristics	Value*		
	Initially Enrolled Patients (n = 27)	Study Cohort	
		Total (n = 22)	Newly Diagnosed GBM (n = 13)
age in yrs			
mean ± SD	47.1 ± 13.5	48.1 ± 13.5	46.0 ± 14.1
median (range)	50.0 (24–69)	50.5 (24–69)	49.0 (24–69)
sex			
male	13 (48.1)	11 (50.0)	6 (46.2)
female	14 (51.9)	11 (50.0)	7 (53.8)
histopathological type of tumor†			
GBM	13 (48.1)	13 (59.1)	13 (100.0)
gliosarcoma	1 (3.7)	1 (4.5)	0 (0)
anaplastic astrocytoma	3 (11.1)	3 (13.6)	0 (0)
anaplastic oligoastrocytoma	2 (7.4)	2 (9.1)	0 (0)
anaplastic oligodendroglioma	2 (7.4)	2 (9.1)	0 (0)
pilocytic astrocytoma w/ anaplastic features	1 (3.7)	1 (4.5)	0 (0)
oligodendroglioma	2 (7.4)	0 (0)	0 (0)
central review not performed‡	3 (11.1)	0 (0)	0 (0)
WHO grade†			
IV	14 (51.9)	14 (63.6)	13 (100.0)
III	8 (29.6)	8 (36.4)	0 (0)
II	2 (7.4)	0 (0)	0 (0)
central review not performed‡	3 (11.1)	0 (0)	0 (0)
tumor status			
newly diagnosed	26 (96.3)	21 (95.5)	13 (100.0)
recurrent	1 (3.7)	1 (4.5)	0 (0)
tumor location			
frontal lobe	16 (59.3)	13 (59.1)	7 (53.8)
temporal lobe	5 (18.5)	3 (13.6)	2 (15.4)
parietal lobe	4 (14.8)	4 (18.2)	3 (23.1)
occipital lobe	2 (7.4)	2 (9.1)	1 (7.7)
tumor side			
rt	13 (48.1)	12 (54.5)	8 (61.5)
lt	14 (51.9)	10 (45.5)	5 (38.5)
tumor functional grade			
located in eloquent area	13 (48.1)	12 (54.5)	7 (53.8)
adjacent to eloquent area	6 (22.2)	4 (18.2)	2 (15.4)
located in noneloquent area	8 (29.6)	6 (27.3)	4 (30.8)
PS before treatment§			
0	14 (51.9)	10 (45.5)	3 (23.1)
1	10 (37.0)	9 (40.9)	8 (61.5)
2	0 (0)	0 (0)	0 (0)
3	3 (11.1)	3 (13.6)	2 (15.4)
extent of tumor resection			
total	9 (33.3)	8 (36.4)	5 (38.5)
subtotal (>90% of lesion vol)	13 (48.1)	11 (50.0)	8 (61.5)
partial	5 (18.5)	3 (13.6)	0 (0)

* Unless otherwise stated, values represent cases (%).
† According to central review based on WHO criteria.
‡ These patients did not receive laser irradiation during surgery due to results of the intraoperative histopathological investigation of the resected tissue on the frozen sections and exclusion of the diagnosis of primary malignant parenchymal brain tumor.
§ According to the Eastern Cooperative Oncology Group Performance Status Scale.

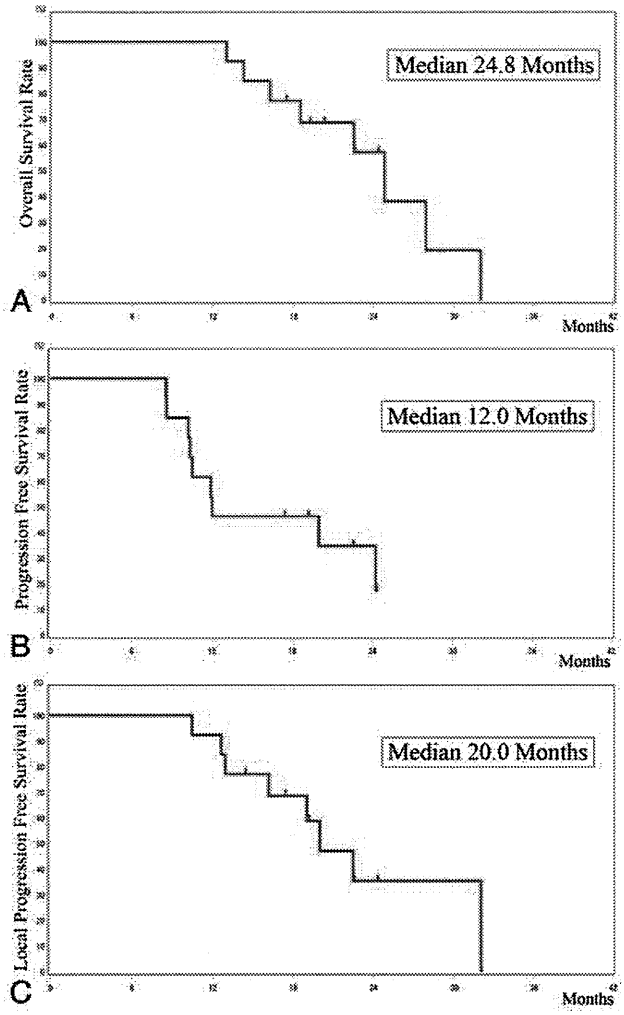

FIG. 1. Kaplan-Meier curves for OS (A), PFS (B), and local PFS (C) in the subgroup of patients with newly diagnosed GBM included in the study cohort. Censored observations are marked.

sidered as side effects after administration of talaporfin sodium. It included rash (2 cases), blister (1 case), and erythema (1 case).

Photosensitivity test results were relatively mild and most patients had a score of 1 (barely perceptible erythema) or 2 (distinct erythema); no patient had a score of 3 (marked erythema or edema). These reactions completely disappeared within 4, 8, and 15 days after administration of talaporfin sodium in 55.6%, 77.8%, and 100% of patients, respectively (Table 3).

Discussion

Management of primary malignant parenchymal brain tumors represents a significant challenge. According to the latest edition of the Japan Brain Tumor Registry, 1-, 2-, and 3-year survival rates of patients with high-grade gliomas constitute 64%, 37%, and 28%, respectively.[5] The poor survival rates are mainly due to an inability to perform complete removal of the neoplasm due to its infiltrative growth into functionally important neuronal structures, as well as the limited effectiveness of the postoperative radiotherapy and chemotherapy. Therefore, finding additional effective and safe treatment options in such cases is required.

As a highly selective treatment with minimal injury to the adjacent normal structures, PDT has demonstrated promising potential for management of the various cancers and nonneoplastic disorders, such as age-related macular degeneration, local infection, dermatological diseases, arteriosclerosis, and rheumatoid arthritis.[7] However, despite a large amount of basic and clinical research conducted during several decades and directed on testing of the various photosensitizers, light sources, irradiation types, and treatment regimens, PDT still was not approved to be used as a standard treatment for malignant brain tumors.[2,10] During the last decade there was considerable interest in the use of 5-aminolevulinic acid (5-ALA) in the surgical management of gliomas. Nevertheless, while its application for photodynamic diagnosis and fluorescence-guided resection was associated with a significant impact on effectiveness of tissue sampling, tumor resection rates, and clinical outcomes,[4,17] the attempts to use this photosensitizer for PDT were not so impressive.[2] These unimpressive results might be particularly caused by insufficient incorporation of the drug in the neoplastic cells, especially in necrotic regions and at the periphery of the neoplasm.[2]

In the present study PDT was based on administration of the relatively novel second-generation photosensitizer talaporfin sodium. This water-soluble compound is derived from plant chlorophyll. In the living body it binds to albumin and does not pass the blood-brain barrier. In neoplastic cells it is primarily distributed in the lysosomes.[14] Compared with conventional photosensitizers, talaporfin sodium is activated by light with longer wavelengths; therefore, its light absorption is not affected by hemoglobin and penetrates deeper.[13] Additionally, talaporfin sodium more selectively accumulates in glioma tissue, is rapidly eliminated from the normal tissues, and is less likely to cause adverse reactions.[14] It was demonstrated that PDT based on administration of talaporfin sodium with subsequent irradiation using a 664-nm laser led to necrosis and apoptosis of cultured human glioblastoma cells[13] and experimental tumors[14] in a dose- and time-dependent fashion. The adverse effects on the peritumoral brain were limited to mild temporary edema, and no damage to neurons or the myelin sheath was observed.[14] A pilot clinical study on 14 adult patients with unresectable malignant gliomas showed a median PFS of 23 months in newly diagnosed neoplasms.[1] In concordance, in our present prospective investigation, which included 21 patients with newly diagnosed high-grade gliomas treated according to strict research protocol, the median local PFS constituted 22.5 months.

The most impressive results of our study were obtained in patients with a newly diagnosed GBM. In this subgroup, the 12-month OS and 6-month PFS rates were 100%, and the median OS and median PFS were 24.8 and 12.0 months, respectively. These rates compare favorably

TABLE 2: Frequency of adverse events and side effects by grade*

System Organ Class†	No. of Patients (%)					
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total (n = 27)
adverse events						
investigations	3 (11.1)	12 (44.4)	10 (37.0)	2 (7.4)	0 (0.0)	27 (100.0)
gastrointestinal disorders	5 (18.5)	16 (59.3)	0 (0.0)	0 (0.0)	0 (0.0)	21 (77.8)
general disorders & administration site conditions	15 (55.6)	6 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	21 (77.8)
nervous system disorders	1 (3.7)	17 (63.0)	2 (7.4)	0 (0.0)	0 (0.0)	20 (74.1)
skin & subcutaneous tissue disorders	10 (37.0)	8 (29.6)	0 (0.0)	0 (0.0)	0 (0.0)	18 (66.7)
injury, poisoning, & procedural complications	9 (33.3)	6 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	15 (55.6)
eye disorders	7 (25.9)	1 (3.7)	1 (3.7)	0 (0.0)	0 (0.0)	9 (33.3)
infections & infestations	1 (3.7)	3 (11.1)	2 (7.4)	0 (0.0)	0 (0.0)	6 (22.2)
renal & urinary disorders	3 (11.1)	2 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)	5 (18.5)
psychiatric disorders	4 (14.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (14.8)
respiratory, thoracic, & mediastinal disorders	4 (14.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (14.8)
vascular disorders	0 (0.0)	0 (0.0)	4 (14.8)	0 (0.0)	0 (0.0)	4 (14.8)
musculoskeletal & connective tissue disorders	1 (3.7)	2 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)	3 (11.1)
blood & lymphatic system disorders	1 (3.7)	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.4)
metabolism & nutrition disorders	0 (0.0)	0 (0.0)	2 (7.4)	0 (0.0)	0 (0.0)	2 (7.4)
cardiac disorders	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.7)
ear & labyrinth disorders	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.7)
side effects						
investigations	7 (25.9)	6 (22.2)	5 (18.5)	0 (0.0)	0 (0.0)	18 (66.7)
skin & subcutaneous tissue disorders	1 (3.7)	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.4)

* According to the Cancer Therapy Evaluation Program.[3]
† According to the Medical Dictionary for Regulatory Activities version 14.1 (http://www.meddra.org).

with contemporary results obtained in such tumors with standard treatment. In a global Phase III randomized controlled study on radiotherapy with concomitant and adjuvant temozolomide for GBM, Stupp et al.[18] demonstrated a 12-month OS rate of 61%, a 6-month PFS rate of 54%, a median OS of 14.6 months, and a median PFS of 6.9 months. In the series by Stummer et al.[17] on fluorescence-guided resection of malignant gliomas with the use of 5-ALA, the 6-month PFS rate was 41% and the median PFS period was 5.1 months. Moreover, in our patients with a newly diagnosed GBM, the median local PFS was nearly two times longer than the median PFS (20.0 vs 12.0 months). It can therefore be speculated that prolonged survival was caused by improved local tumor growth control due to intraoperative PDT. It should be emphasized that in the present series all patients with newly diagnosed GBM underwent either total or subtotal resection. Aggressive removal of the tumor may be an important prerequisite for clinical effectiveness of intraoperative PDT, since the penetration depth of a laser is approximately 2.5–5 mm; therefore, the corresponding effective distance for irradiation is limited to 0.75–1.5 cm.[1,2] The limitations of the efficacy of PDT in bulky target tissues and recurrent tumors have been demonstrated.[1] It is also possible that metabolically active infiltrating tumor cells in the periphery of the GBM may be more sensitive to PDT because of incorporation of a greater amount of photosensitizer. It was reported that the tissue concentration of a photosensitizer directly correlates with the grade of malignancy of the neoplasm.[2]

In the present study PDT showed a high level of safe-

TABLE 3: Skin photosensitivity test results in 27 patients*

No. of Days†	No. of Patients (%)	Cumulative No. of Patients (%)
3	4 (14.8)	4 (14.8)
4	11 (40.7)	15 (55.6)
8	6 (22.2)	21 (77.8)
10	1 (3.7)	22 (81.5)
13	2 (7.4)	24 (88.9)
14	1 (3.7)	25 (92.6)
15	2 (7.4)	27 (100)

* For the skin photosensitivity test, between 11 a.m. and 2 p.m., the back of the individual's hand was exposed to direct sunlight for 5 minutes, and the occurrence of any photosensitivity reaction, such as erythema, was assessed. In cases in which photosensitivity reactions were detected, the subject was kept shielded from light until the reaction disappeared, and the skin photosensitivity test was subsequently repeated.
† From administration of talaporfin sodium to disappearance of reaction.

ty. While laboratory investigations have frequently revealed abnormalities likely attributable to the administration of talaporfin sodium, only 2 patients (7.4%) had definite symptoms on the skin, which did not exceed Grade 2 toxicity. In no case did we encounter brain edema or cerebral infarction, which may complicate PDT.[1,2] Therefore, the risk of clinically significant side effects caused by the administration of talaporfin sodium and intraoperative irradiation of the residual tumor and peritumoral brain with a 664-nm laser 22–27 hours thereafter may be considered low. Moreover, according to photosensitivity test results, any reactions completely disappeared in all patients within 15 days after administration of the drug.

The main limitations of the present study are related to its design. A nonrandomized noncontrolled prospective investigation was performed in just 2 neurosurgical centers with well-established neurooncology programs and enrolled a limited number of highly selected cases with rather heterogeneous histopathological diagnoses of malignant parenchymal brain tumors. It is evident that to prove clinical efficacy of the intraoperative PDT with talaporfin sodium and a semiconductor laser, further carefully designed Phase III studies should be performed in a sufficiently large number of patients with possible initial stratification according to tumor resection rate. Testing of the proposed treatment method is also planned in cases of low-grade gliomas and in incompletely resected benign extraaxial neoplasms, such as pituitary adenomas and meningiomas. Since appropriate use of equipment for PDT requires specific skills, the dedicated training program for neurosurgeons is currently under organization. Finally, advanced experimental investigations directed at further understanding the basic mechanisms of the therapeutic effectiveness of intraoperative PDT are also required, and additional studies to search for the most optimal treatment regimens should be continued as well.

Conclusions

The results of the present study demonstrate that novel PDT based on administration of talaporfin sodium and subsequent irradiation with a 664-nm semiconductor laser may provide an additional benefit to the combined management of primary malignant parenchymal brain tumors through possible improvement of their local growth control, which, in turn, may lead to prolongation of the patient's survival. The therapy seems sufficiently safe with a minimal risk of serious side effects. Therefore, application of the intraoperative PDT along with aggressive resection, radiotherapy, and chemotherapy may be of clinical significance, particularly in patients with newly diagnosed GBM.

Disclosure

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CASE REPORT

Administration of gefitinib via nasogastric tube effectively improved the performance status of a patient with lung adenocarcinoma-derived meningeal carcinomatosis

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Abstract Meningeal carcinomatosis (MC) is a refractory disease with a dismal prognosis, and no therapeutic strategy has been established to date. Herein we report a case of lung adenocarcinoma-derived MC in which the patient's performance status was dramatically improved by administration of gefitinib suspension via a nasogastric tube. The patient was a 71-year-old woman who was originally admitted to our hospital for a progressive headache and subsequently presented with severe consciousness disturbance. Cerebrospinal fluid examination and systemic imaging studies revealed MC that was derived from lung adenocarcinoma. Moreover, epidermal growth factor receptor (*EGFR*) mutations were detected in the tumor cells. Since the patient suffered from hydrocephalus, a ventriculoperitoneal shunt was placed. Nevertheless, her consciousness disturbance persisted. Subsequently, gefitinib suspension was prepared and administered via nasogastric tube, which dramatically improved her consciousness level and enabled her to tolerate oral intake. She died 14 months after the disease onset. The observations in this case report suggest that gefitinib might be a therapeutic option for patients with MC derived from cancers harboring *EGFR* mutations even though the patient exhibited severe consciousness disturbance.

Keywords Meningeal carcinomatosis · Lung adenocarcinoma · Gefitinib

Introduction

Meningeal carcinomatosis (MC) is a refractory disease with a dismal prognosis that occurs in 5–10 % of cancer patients [1]. No therapeutic strategy has been established to date; the median survival time is 4–6 weeks if the disease is left untreated [1]. On the other hand, the recent development of novel chemotherapies has markedly improved the outcome of advanced cancer patients. The advent of molecular-targeted drugs is the most prominent among them, and gefitinib is a representative drug for lung cancer. Gefitinib has been shown to prolong the progression-free survival of patients with lung cancer harboring epidermal growth factor receptor (*EGFR*) mutations compared with standard chemotherapy [2, 3]. However, gefitinib is supplied in a tablet form and therefore needs to be administered orally. For this reason, patients with brain metastasis and/or MC sometimes have difficulty tolerating standard gefitinib treatment because they frequently exhibit consciousness disturbance and/or swallowing difficulty. Herein we report a case of lung adenocarcinoma-derived MC in which the patient's performance status (PS) was dramatically improved by administration of gefitinib suspension via a nasogastric (NG) tube even though the patient exhibited severe consciousness disturbance.

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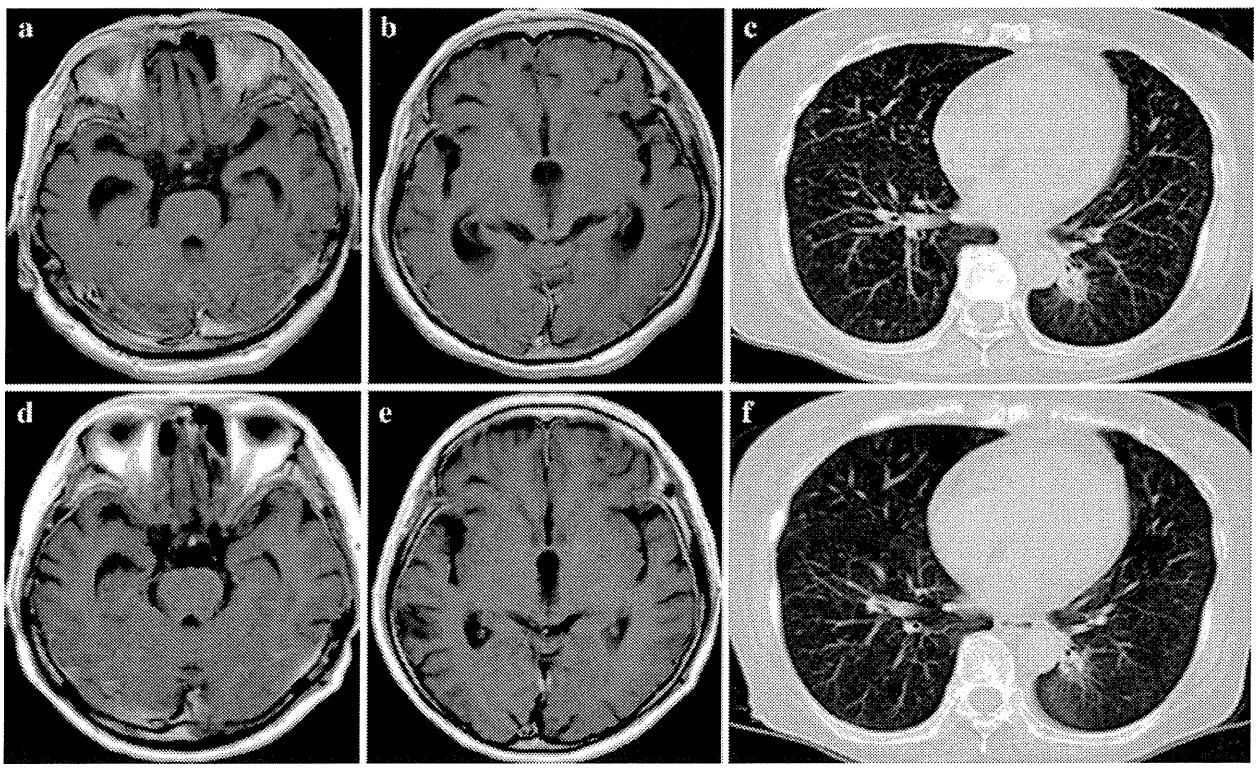

Fig. 1 Imaging studies pre- and post-gefitinib treatment. **a**, **b** T1-weighted gadolinium-enhanced MRI of the head reveals multiple small enhanced lesions. **c** Chest CT shows a mass-like lesion in the left lung S6 with diffuse granular shadows. **d**, **e** T1-weighted gadolinium-enhanced MRI reveals complete disappearance of the enhanced lesions. **f** Chest CT shows a decrease in size of the primary lesion

Case report

The patient was a 71-year-old woman who suffered from a progressive headache that had lasted several weeks. She suddenly presented with consciousness disturbance and was emergently admitted to our hospital. At the time of admission, her consciousness level was lethargic. Her past medical history was unremarkable. Magnetic resonance images (MRI) of the head revealed multiple small enhanced lesions and hydrocephalus (Fig. 1a, b). Cerebrospinal fluid (CSF) examination showed a cell count of 18/3 mm^3, protein 44 mg/dl, glucose 42 mg/dl, and carcinoembryonic antigen (CEA) 54.4 ng/ml (serum CEA 13.5 ng/ml). Adenocarcinoma cells were detected in the CSF. At the same time, computed tomography (CT) revealed a mass-like lesion in the left lung S6 segment along with diffuse granular shadows (Fig. 1c). These findings led us to diagnose MC that was derived from lung adenocarcinoma (cT1N0M1). *EGFR* mutations were also detected in exon 19 in the tumor cells, which was considered an appropriate target of gefitinib treatment.

Figure 2 shows the clinical course of the patient after the admission. The patient's consciousness level needed to recover for her to receive standard gefitinib treatment orally. Therefore we decided to place a ventriculoperitoneal (VP) shunt to treat the hydrocephalus. Nevertheless, the VP shunt failed to improve her consciousness level. Then, we sought to administer gefitinib suspension to the patient via an NG tube. Gefitinib tablets were finely crushed and suspended in 50 ml of sterile water (Fig. 3), and the patient received 250 mg/day gefitinib via an NG tube. On day 10 after the initiation of gefitinib treatment, her consciousness level improved dramatically, and she was able to tolerate oral intake on the following day. The imaging findings concurrently improved on the follow-up MRIs and CT (Fig. 1d–f). CSF cytology turned out to be negative on day 28. At the same time, CEA levels in the CSF also decreased to 11.3 ng/ml (serum CEA 11.8 ng/ml). The patient recovered with no neurological deficits and no adverse reactions. Gefitinib treatment was continued orally, and the patient was transferred for rehabilitation on day 82. She died 14 months after the disease onset without the cause of death identified.

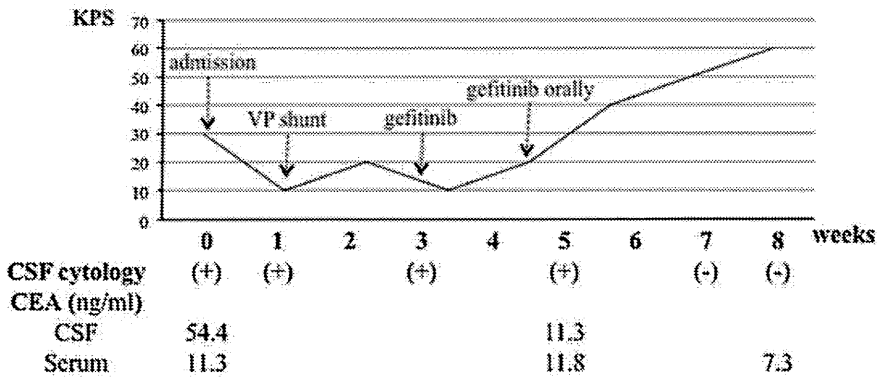

Fig. 2 Clinical course of the MC patient. *KPS* Karnofsky performance status, *CSF* cerebrospinal fluid, *CEA* carcinoembryonic antigen

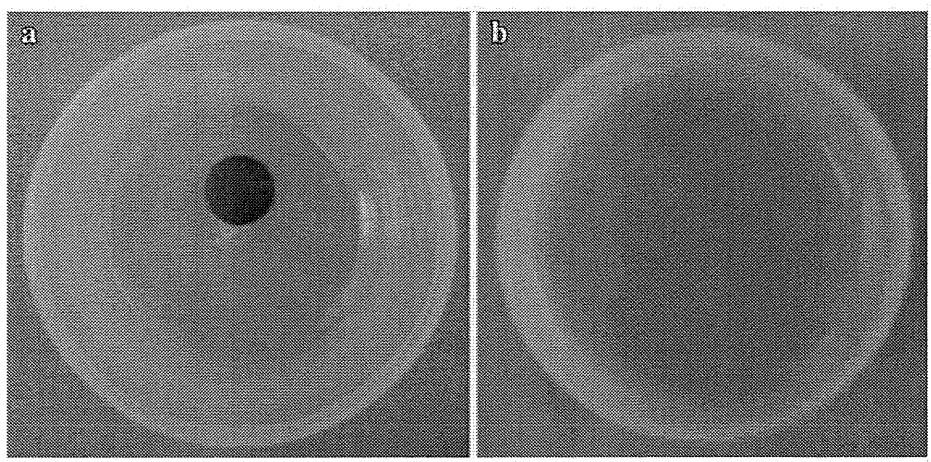

Fig. 3 Gefitinib processing for trans-NG tube administration. **a** Original gefitinib tablet. **b** Suspension of gefitinib in sterile water

Discussion

First-line gefitinib has been shown to improve the outcome of poor PS patients with *EGFR* mutation-positive lung cancers [4]. Therefore, examination of EGFR mutation as a biomarker is recommended in this patient population. However, since gefitinib is supplied in a tablet form and usually administered orally, standard gefitinib treatment is sometimes difficult for those with brain metastasis and/or MC because they frequently exhibit consciousness disturbance and/or swallowing difficulties. To treat these patients harboring *EGFR* mutations, gefitinib can be used in suspension by partially breaking the film coating and adding water [5]. Of note, the tablet film coating is not intended to enable sustained release or provide an enteric coating. Furthermore, administration of gefitinib suspension is comparable to administration of tablets in terms of bioavailability and safety [5]. On the basis of these findings, we postulated that the gefitinib suspension could provide the same therapeutic effect in this patient as the gefitinib tablets. Indeed, this therapeutic strategy successfully improved the patient's PS even though she had exhibited severe consciousness disturbance.

Although gefitinib is a small molecule inhibitor, intrathecal transfer rate is generally very low [6]. Particularly in MC patients, the concentration of gefitinib in the CSF has been reported as less than 1 % of the serum concentration [7, 8]. Nevertheless, the administration of gefitinib suspension improved the patient's PS in this case. We speculate several reasons for this. One is that even a low concentration of gefitinib would be effective against *EGFR* mutation-positive MC. Another reason is that the MC would destroy the blood–brain barrier (BBB) in situ and accelerate the drug transfer to each lesion. Indeed, whole-brain irradiation has been shown to enhance the intrathecal delivery of gefitinib by disruption of the BBB [9].

Erlotinib has been shown to induce higher bioactivities in plasma than gefitinib at similar or even lower doses of administration [10]. In addition, intrathecal gefitinib/erlotinib concentration can be elevated in a dose-escalating manner [7]. These findings suggest that erlotinib can be an alternative option for patients with MC or brain metastases if the primary cancer cells harbor *EGFR* gene mutations. We are currently in the process of determining the therapeutic efficacy of erlotinib for those with brain metastases harboring *EGFR* mutations.

A remaining issue is drug resistance exhibited by cancers. In the case of gefitinib/erlotinib, this typically occurs 8–12 months from the initiation of treatment. Over 50 % of resistance is caused by a mutation in the ATP binding pocket of the EGFR kinase domain involving substitution of a small polar threonine residue with a large nonpolar methionine residue (T790M) [11, 12]. In this regard, commencing treatment with a number of different therapeutic agents with differing modes of action is proposed to overcome the development of T790M and other resistance-conferring mutations [13].

In conclusion, we have reported the case of lung adenocarcinoma-derived MC in which the patient's PS was dramatically improved by the administration of gefitinib via an NG tube. The observations in this case report suggest that gefitinib/erlotinib might be therapeutic options for patients with MC derived from cancers harboring *EGFR* mutations even for the patients exhibiting severe consciousness disturbance.

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Conflict of interest The authors declare that we have no conflict of interest.

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Special Theme Topic:
Treatment of Malignant Brain Tumor

Mechanisms of Tumor Development and Anti-angiogenic Therapy in Glioblastoma Multiforme

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Abstract

Despite advances in surgical and medical therapy, glioblastoma multiforme (GBM) remains a fatal disease. There has been no significant increase in survival for patients with this disease over the last 20 years. Tumor vasculature formation and glioma cell invasion along the white matter tracts both play a pivotal role in glioma development. Angiogenesis and invasion are the major factors believed to be responsible for treatment resistance in tumors, and a better understanding of the glioma invasion and angiogenesis mechanisms will lead to the development of potential new treatments. In this review, we focus on the molecular characteristics of angiogenesis and invasion in human malignant glioma. We discuss bevacizumab and cilengitide, which are used to inhibit angiogenesis in GBM.

Key words: cilengitide, bevacizumab, angiogenesis, invasion

Introduction

Despite advances in surgical and medical therapy, glioblastoma multiforme (GBM) remains a fatal disease. The pathophysiological processes of angiogenesis and tumor cell invasion play pivotal roles in glioma development and growth, beginning in the earliest phase of tumor growth.[4] The main reasons for the resistance of treatment in these tumors were the formation of abnormal dysfunctional tumor vasculature and glioma cell invasion along white matter tracts. Recent insight into the glioma angiogenesis and invasion mechanisms have provided renewed hope for developing novel strategies aimed at reducing morbidity due to this fatal disease. However, glioma angiogenesis and invasion are challenging to investigate in experimental settings because most of the animal models fail to mimic the unique angiogenesis and invasiveness of human glioma cells.

In this article, we review histopathological studies that focus on invasion and angiogenesis of human malignant gliomas. We also focus on the molecular aspects of glioma angiogenesis and invasion and the key mediators of these processes. In addition, we consider several animal glioma models that are available for studying invasion and angiogenesis, including our novel animal models. Finally, we discuss bevacizumab (a recombinant humanized monoclonal antibody targeting vascular endothelial growth factor [VEGF]) and cilengitide (an inhibitor of αvβ3 and αvβ5 integrins).

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Histopathological Analysis of Angiogenesis and Invasion

GBM is known to have blood vessels of increased diameter with high permeability, thickened basement membranes, and highly proliferative endothelial cells.[41] The histopathological hallmark of GBM is the presence of microvascular proliferation with the formation of glomerular capillary loops in a garland-like formation.[54] One of the malignancy evaluation criteria is increased neoplastic proliferation of glial cells running parallel to endothelial vascular proliferation.[40] Vascular density in GBM is markedly higher than that in glioma of a lower histological grade.[53] An increase in vascularization

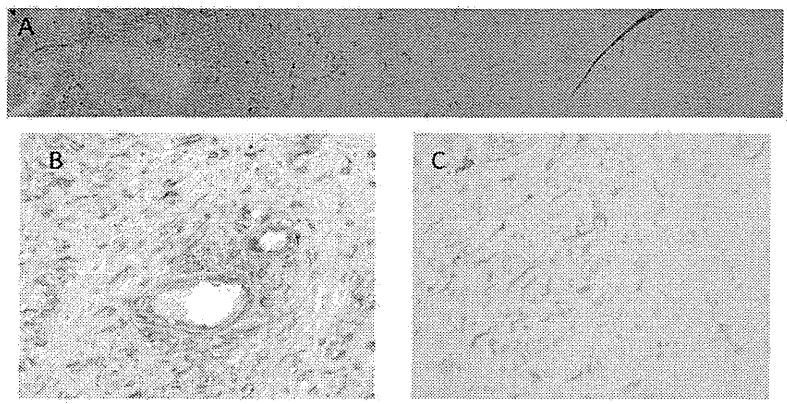

Fig. 1 Microtubule-associated protein (MAP) 2e and von Willebrand factor (vWF) immunohistochemical staining of human GBM samples. A: MAP2e, a splice variant of MAP2, was a candidate glioma-specific antigen. Tumor cells diffusely infiltrated from the tumor center to normal brain tissue; there is no border between them. B: At the tumor border, MAP2e-positive tumor cells clustered around dilated vessels. C: Single MAP2e-positive tumor cell infiltration into normal brain parenchyma that are independent of vasculature were also seen. MAP2e: diaminobenzidine (DAB), vWf: DAB-Ni, *Counterstain*: hematoxylin.

significantly worsens the disease's prognosis.[40]

Histopathological studies have given some insights into tumor invasion. We showed previously that there are at least two invasive and angiogenic glioma phenotypes. Clusters of glioma cells were seen around newly developed vessels in the normal parenchyma adjacent to the tumor margins. Single cell infiltrations were also seen in normal brain parenchyma independent of the vasculature (Fig. 1). These different invasive and angiogenic phenotypes are either angiogenesis-dependent or angiogenesis-independent. GBM consists of a mixture of subclones with both angiogenesis-dependent and angiogenesis-independent invasion phenotypes present in various proportions.[27,46,49]

Molecular Biology of Angiogenesisin GBM

Angiogenesis is one of the key events in GBM development, and the histological diagnosis of GBM was led by the presence of microvascular proliferation.[65] Among all solid tumors, GBM has been reported to be the most angiogenic because it displays the highest degree of endothelial cell hyperplasia and vascular proliferation.[9] The peritumoral edema resulting from a defective blood brain barrier (BBB) in the newly formed tumor vasculature is a pathological feature of GBM.[17,67] Vascular homeostasis is maintained by a balance between pro-angiogenic and anti-angiogenic stimuli.[29] Angiogenesis is activated in developing GBM when the pro-angiogenic stimuli outweigh the anti-angiogenic stimuli. Tissue hypoxia is the most potent activator of angiogenic mechanisms in brain tumors. The hypoxia-inducible factor (HIF) -1/VEGF-A pathway is one of the well-studied pathways. The HIF-1/VEGF-A pathway leads to endothelial cell proliferation and migration.[30] HIF-1 activates deoxyribonucleic acid (DNA) promoter regions, which are known as hypoxia response elements (HREs). HREs induce transcription of > 100 genes that help the cell to adapt to low O_2 conditions.[8,62] VEGF is an example of a gene that is regulated by an HIF-1 through an HRE. VEGF regulates brain edema surrounding brain tumors and blood vessel formation; specifically, VEGF-A is known to be upregulated in GBM.[24] VEGF-A regulates endothelial cell survival, permeability, proliferation, and migration primarily via the VEGF-receptor 2 (VEGFR2).[13] VEGF promotes endothelial proliferation by activation of the mitogen-activated protein kinase (MAPK) pathway.[65] VEGF also enhances vascular permeability through the MAPK signaling cascade. VEGF rearranges adherin/catenin complexes and loosens adhering junctions between endothelial cells.[19,31] VEGF stimulates endothelial production of urokinase-type plasminogen activator (uPA).[45] uPA induces the conversion of plasminogen to plasmin and the breakdown of extracellular matrix (ECM) components, and leads to ECM remodeling.[65] Immature, highly permeable blood vessels with subsequent poor maintenance of BBB and parenchymal edema are produced as a result of VEGF signaling in tumors.[28,29]

Angiogenesis is the formation of new blood vessels by rerouting or remodeling of existing vessels. It is