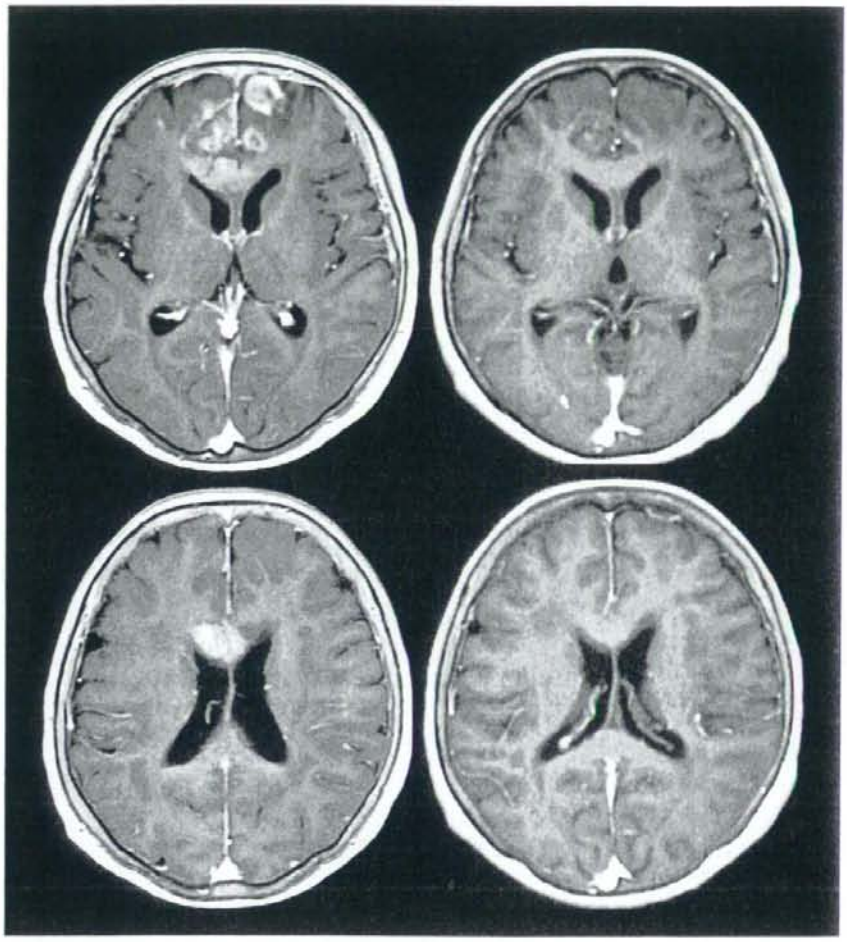
のこの血管透過性改善作用からsuper-steroidという別称がある。そしてこの作用を応用して放射線壊死に対して投与し改善したという報告もある[10]。いずれにせよbevacizumabはきわめて興味深い薬剤で，欧米を中心に作用機序や治療成績，その評価方法等について積極的な研究が進められている。今後の動きから目が離せない。

図2　bevacizumabとirinotecanによる再発GBMの治療効果

症例は64歳女性。初回再発。左上下は治療前，右上下は治療後3日目の造影MRI T1強調像である。



すでにグリオーマにおける新しい化学療法は分子標的薬の時代に突入している。今までの常識では測れないめざましい効果を予感させるとともに，経験のない副作用が現れる危険性もはらんでいる。また分子標的薬は次々と現れている故に，症例数の決して多くないグリオーマにおける有効性を検証するには国際協力が必須である。国際的な臨床試験に参加するためには，十分な意識の高まりが必要であり，臨床試験や薬剤に対する知識においても国際的に対等に討論に参加できる実力が要求される。さらにまた，分子標的薬はいずれもきわめて高価であり，医療経済上も見過ごせない問題になりつつある。変革の時代である。

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特 集 ◎ がん治療と神経障害

放射線照射による脳障害

Radiation-induced Brain Injury

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Abstract

Radiation-induced brain injury is a life-threatening or at least QOL-compromising pathological entity induced by therapeutic irradiation to malignant brain tumors. Although life-threatening late delayed radiation necrosis and radiation-induced leukoencephalopathy had been assumed to be major complications of radiation therapy to the brain classically, these complications seem to be less frequently seen in therapeutic irradiation to the brain recently because in many treatment protocols to brain tumors, irradiation field is now confined to tumors and their margins and adjuvant chemotherapy consisting of methotherexate etc. has been avoided as much as possible. Instead, less aggressive but still QOL-compromising encephalopathy has been recognized for the past 20 years. This encephalopathy occurs in senior adults several months after the extended field irradiation with even less amount of irradiation dose such as 40 Gy whole brain irradiation. This encephalopathy is characterized by cognitive impairment and brain atrophy which attenuates QOL of the patients. In this article, these radiation-induced brain injuries are reviewed clinically, etiologically and hisotopathologically based on reports in the literature.

Key words : radiation-induced brain injury, radiation encephalopathy, radiation necrosis, leukoencephalopathy, neural stem cells, dementia

はじめに

この20年の間に，悪性脳腫瘍に対する集学的治療の内容は飛躍的に充実した。手術を支援するさまざまな機器の発達により，脳機能を温存しつつ腫瘍を最大限に摘出する手術が可能となり，施設によってできることに差はあるものの，少なくともその方針の妥当性は広く認識されるようになった。化学療法では，テモゾロミドの出現により悪性グリオーマの治療成績もわずかではあるが改善が期待でき[1)]，大量メトトレキセート療法により脳原発悪性リンパ腫の治療成績は飛躍的に改善し[2)]，プラチナ製剤を主力とする化学療法により，悪性胚細胞腫の治療成績も一定の改善をみている[3)]。放射線治療の内容はというと，これまで行われてきた局所あるいは全脳に対する分割照射（conventional radiation therapy）が依然golden standardであるものの，ガンマナイフをはじめとする定位放射線治療の普及により治療の幅が大きく広がった。これら悪性脳腫瘍の集学的治療は，いずれも放射線治療なしでは考えられず，放射線治療は依然，悪性脳腫瘍治療の中心的役割を果たしていると言っても過言ではない。悪性脳腫瘍の治療成績を考えるとき，生存の期間（survival time）と質（quality of life: QOL）の両方を考えなければならない。悪性脳腫瘍治療後の5年生存率はこの20年間，腫瘍の種類によって差はあるが，おおむね不変ないし若干の改善をみるのみであるが，2年生存率あるいはprogression free survivalになると，どの腫瘍でも飛躍的に改善している。これは初期治療で局所コントロールが十分にできるようになったことと，初期治療後のQOLが向上したことに起因していると考えられる。初期治療後のQOLの向上には，上述の手術方法の改善と放射線の照射法の改善が大きく寄与していると

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考えられる。それほど遠くない昔，悪性グリオーマに対して全脳照射が広く行われていた時代もあった。

初期治療後，急速に認知障害が進行するため仮に寛解導入できたとしてもQOLは不良で，初期治療後，離床できないまま腫瘍再発をきたし死に至る症例も多々みられた。照射範囲が局所照射に変わってからQOLが格段に改善したという経緯がある。昨今，上述のconventional radiation therapy，定位放射線治療以外に，中性子捕捉療法，強度変調放射線治療，tomotherapy等々，さまざまな種類の放射線治療が単独のみならず組み合わせでも行われつつあり，このあたりで一度QOLに多大な影響を及ぼしうる，これら放射線治療による正常脳組織障害を再認識することは極めて重要であると考える。

本稿では，これまで報告されている正常脳組織に対する放射線障害をreviewし，標的となる組織，細胞，分子機序などを整理して解説する。

Ⅰ．放射線照射による脳障害の臨床分類と組織学的特徴

1．急性脳浮腫

照射直後から照射野を中心に起こってくる脳浮腫で，毛細血管の内皮細胞傷害により毛細管レベルでの血管透過性亢進によって起こってくると考えられている[4]。浮腫の程度は内皮細胞の傷害の程度に比例するため，線量依存性である。1回2 Gy程度の低線量では極めて稀で，1回10 Gyを超える高線量で起こってくるため，conventional irradiationの際にはあまり問題にならず，1回大量照射を行う定位放射線治療の際の，周辺浮腫による局所症状の悪化などが問題となる。通常，一過性で可逆的であり，組織学的な変化は残さず軽快すると考えられている。

2．晩発性放射線性壊死

一般に放射線の耐容線量というのが組織ごとに設定されているが，これはそれ以上照射すると組織の壊死を起こす可能性が出てくる線量として定義される。脳の場合には60 Gyに設定されている。すなわち，それ以上照射するとこの晩発性放射線性壊死が起こってくるわけである。実際には52.5 Gy以下ではまったく起こらず，55 Gyでは1.5%，60 Gyでは4%，65 Gyでは25%，67.5 Gyでは100%起こるという報告もある[5]。照射後1～5年後に照射野に一致して起こってくるとされる。腫瘍局所への60 Gyの30分割照射（2 Gy/fraction）では，周辺正常脳にこの壊死が起こることは稀で，姑息的照射を追加したり，他のmodalityの照射と重複した時に起こる危険性が高い。例えば，転移性脳腫瘍の全脳照射後の再発あるいは*de novo*の転移巣に対して定位放射線治療を行う場合など，その危険性が高くなる。臨床的には発生部位に応じた局所症状を呈し，腫瘍再発との鑑別が問題となる。画像上はCT，MRI上いずれも造影剤による増強を受け，腫瘍再発との鑑別は困難である。PET，SPECTにより鑑別がつく場合もあるが，偽陽性，偽陰性いずれを呈する場合もあり，確定診断には至らない[6]。確定診断はやはり病理組織診断となる。病理組織所見としては，中小動脈の血栓化，出血，フィブリンの漏出，血管拡張，ヒアリン線維化による血管内腔の狭窄，血管そのもののフィブリノイド壊死など，血管の変化が著明である。また，これら血管の閉塞による二次的な凝固壊死像，およびneurofibrillary tangleなどの神経変性疾患的変化等の脳実質の変化も著明である[7]。本病態における放射線障害の標的は血管内皮細胞と考えられている。

3．放射線性白質脳症

Priceら[8]あるいはRubinstein[9]らによって報告された放射線性脳障害である。小児の急性リンパ球性白血病の中枢神経浸潤を予防するために行われる全脳照射＋メトトレキセート髄注治療後数カ月から1年後に起こってくる病態で，精神運動機能の低下，軽度の意識障害から重症例では痙攣，進行性の意識障害，除脳硬直を起こし予後不良である。組織学的には，白質にび漫性に脱髄を認め，oligodendrocyteに胞体の大小不同，核の濃縮，崩壊を認める。重症例では，白質全体の脱髄に加えて，斑状の凝固壊死巣を伴うものもある[9]。放射線あるいは化学療法に対して，感受性の高い発育期の脳に対して放射線とメトトレキセートが相乗的に作用するためと考えられる。本病態における放射線障害の一義的な標的はoligodendrocyteと考えられている。

4．高齢者における認知機能低下を伴う放射線性脳症

これはAsaiらが最初に新たな病態として報告したもので，50歳以降の中高年者に対して全脳照射を含む広範囲放射線照射を施行した際に，50%以上の患者で照射後数カ月から1年の間に著明な脳萎縮を伴った進行性の認知機能の低下，意識障害の出現を認める。これらは髄液ドレナージ，シャントなどによって改善されないことにより，正常圧水頭症から区別される[10,11]。標的線量が30 Gy以上で50%以上の症例で発症する可能性があり，照射範囲の大小と患者の年齢が発症の重要な因子となっている。すなわち照射範囲が広く，高齢なほど本病態に対

して感受性が高いことが明らかになっている。組織学的には，神経細胞，血管組織に変化がなく，oligodendrocyteにはわずかにpiknoticな変化を認め，白質の髄鞘の膨化などごくわずかな変化を認めるのみで，晩発性放射線壊死あるいは放射線性白質脳症に比べて，全般に組織学的変化に乏しいのが特徴である。著明な脳萎縮，認知障害があるにもかかわらず組織学的変化に乏しいという，あい矛盾する事実をどう説明するかが長い間の課題であったが[12]，近年のneural stem cellあるいはprecursor cellの存在が証明されたことによってその説明が可能になったように思われる[13]。長らく脳内の神経系細胞の運命はわからないままでいたが，neural stem cellが確認されて以降，他臓器のstem cell同様，neural stem cellは死んでいく細胞に代わって新たな細胞を供給することによって，脳を構築する細胞数を一定の状態に保っていると考えられるようになっている。すなわちneuron，astrocyte，oligodendrocyteはすべて生まれては死んでいくことを繰り返しているわけである。Neural stem cellは他のstem cell同様，放射線照射に対して感受性が高く，それが照射によって細胞死を起こし脱落すると脳を再構築する種々の細胞の新生が低下する。このため，oligodendrocyteのみならずneuron，astrocyte等神経系細胞全体の絶対数が減少して脳萎縮および認知機能の低下をきたすもので，どれか特定の細胞だけが脱落したり形態変化があるわけではないので，組織学的検索をしても所見に乏しいのではないかというように考える。なぜ高齢者で感受性が高いかということについては，1つには高齢者ではかなりの数のneural stem cellがsenescence（老化死）により脱落して絶対数が減少しているうえに，さらに放射線照射によって細胞数が減少することによる可能性がある。また，もう1つの可能性としては，老化したneural stem cellが放射線によって容易に細胞死を起こしやすくなっているのかもしれない。しかし，いまだ明らかなことはわかっていない。

本病態で年齢とともに危険因子となっているのが，広範囲照射である。これは転移性脳腫瘍や悪性リンパ腫に対する全脳照射はもちろん，脳梁伝いに反対側まで伸展している神経膠芽腫などにおいて，広範囲に照射した症例で高率に起こってくる[11]。これらの照射はいずれも照射野に側脳室を広範囲に含んでおり，neural stem cellが脳室上衣下（いわゆるsubependymal zone）に存在することを考えると，広範囲照射で脳萎縮が起こりやすい理由も放射線照射の標的がneural stem cellであると考えると容易に理解できる。例えば片側の側脳室を照射範囲に含む照射後に，同側の側脳室の拡大と脳萎縮を認めることがしばしばあるが，これは脳室上衣下のneural stem cellが標的になっていることの傍証となる。本病態は30 Gyあるいは40 Gy程度のいわゆる脳の耐容線量をはるかに下回る「安全線量」で起こってくる。年齢，照射範囲以外に危険因子はわかっていないので，高齢者に対して放射線を照射する際には，特に1年以上の余命が期待できる場合には全脳照射は避けて，極力局所照射を組み合わせて脳室壁への被曝を回避するように努めるべきと考える。

II．放射線性脳障害の標的となる正常組織，細胞，分子

1．放射線照射の標的となる正常組織，正常細胞と組織変化

脳内の細胞にはサイトカイン，イオンあるいはneurotransmitterを介したクロストークが存在する[14-17]。したがって放射線照射により傷害を受ける細胞は放射線の影響のみならず，他の細胞からの何らかの影響も受けていることを念頭において考えなければならない。

1）脳組織とneuron, astrocyte, oligodendrocyte, microglia, neural stem cell

Neuronは長らく放射線に対して感受性はないと考えられてきた。ただこれはあくまでも細胞形態の面から照射後に変化が認められないということで，例えばoligodendrocyteのように明らかな形態変化を起こして細胞死に至って脱落してしまうことはないという意味で，決して放射線の影響を受けない丈夫な細胞であるという意味ではない。実際に*in vitro*では，neuronは臨床上被曝する量と等価の放射線量でp53依存性にアポトーシスを起こすことが知られている[18-20]。しかし，*in vivo*で脳に同量を照射してもneuronのアポトーシスは起こらない[21]。これは*in vivo*ではneuronは何かによって保護されているからと考えるのが妥当であろう。その放射線傷害からneuron保護の役割を果たしているのはastrocyteであることが明らかになってきた。*In vitro*でneuronとastrocyteを共に培養したり，あるいは，astrocyteの培養上清中でneuronを培養すると，放射線による細胞死が有意に起こりにくくなることが示されている[22]。これらはいずれもastrocyteから分泌される何らかの液性因子が，保護的に働いていることを示している。また，neuronでは*in vitro*で放射線照射後に遺伝子発現が変化することが示されている[23]。遺伝子発現の変化は当然，neuronの産生するneurotransmitterをはじめとする，さまざまな蛋白質発現に変化を及ぼし，脳全

体として認知機能の変化等に影響してくる可能性がある。このため，照射後の認知機能の低下や意識障害などに一部関与している可能性がある。

Astrocyte は脳内の細胞成分の大半（neuron：astrocyte＝1：9）を占める[24]。以前は neuron を構造的に支えるだけの細胞として考えられていたが，種々のサイトカインや生物学的活性物質を分泌することにより他の細胞の機能，生存を調節する役目を持った細胞であることがわかってきた[25]。Astrocyte は platelet-derived growth factor（PDGF）や insulin-like growth factor 1（IGF-1）を分泌して oligodendrocyte の生存を維持していることが知られている[15]。Astrocyte はこれらの growth factor に加えて，basic fibroblast growth factor，その他の未知の液性成分を分泌して，上記のように neuron の生存の維持に寄与していると考えられる。生理的な状態のみならず，病的な状態においても astrocyte が種々の細胞の生存に寄与していることがわかっていて，例えば放射線照射など活性酸素による傷害から neuron をはじめ，oligodendrocyte，endothelial cell を保護することが示されている[26-28]。Oligodendrocyte はこれまで放射線照射による細胞傷害の標的と考えられてきた。放射線照射後の最も明らかな病理組織変化が demyelination であるからである。最終分化した oligodendrocyte が標的である可能性は否定できないが，むしろその前駆細胞である O-2A 細胞など増殖能を有する細胞が，標的となっていると考えるほうが自然である。O-2A 細胞に放射線を照射すると分裂能が失われること，あるいは線量によって細胞死が起こることが示されている[29-33]。O-2A 細胞の分裂停止あるいは消失により oligodendrocyte の turnover が停止し，demyelination が引き起こされるわけである。

Microglia は分裂能，貪食能を有していて脳の局所における免疫反応に関わっているのではないかと考えられているが，詳しいところはまだ不明な点が多い。ただ，脳に虚血や放射線照射などの侵襲が加わったときには，加水分解酵素や活性酸素を分泌して傷害を助長する働きがある[34-36]。

Neural stem cell は，他の組織の stem cell 同様，放射線による傷害の標的細胞と考えられる。Neural stem cell は分裂能および neuron，astrocyte，oligodendrocyte に分化しうる能力を持つ。Neural stem cell は脳室上衣下（subependymal zone）に存在するので，広範囲照射では側脳室の大半が照射野内に含まれ，ほとんどの neural stem cell が被曝することになる。脳内の neural stem cell は 15 Gy の 1 回全脳照射でほぼ全部の細胞が死滅し，2 Gy の 1 回全脳照射では約 20%死滅することが示されている[37]。臨床的に施行する全脳照射 2 Gy/回×20 回（40 Gy）を施行した際のデータはないが，相当数が死滅することは想像に難くない。この細胞が脱落したときにはそこから分化する三系列の細胞の turnover がすべて停止することになり，脳萎縮が進行することになる。

2）血管組織と endothelial cell

Endothelial cell は分裂能を有し，放射線照射に対して極めて感受性が高い。毛細血管の endothelial cell の傷害により血管壁の肥厚，血管拡張，endothelial cell の核の巨大化などの形態変化を生じる[38-41]。これらの変化により，二次的な白質の虚血性壊死を生じたものがいわゆる晩発性放射線性壊死と考えられている。

また，毛細血管レベルではなくもっと大きな内頸動脈レベルでも，放射線照射による血管の狭窄が起こり，側副血行路としてのもやもや血管の増生をみることが報告されている[42,43]。これは主として小児のトルコ鞍近傍の病変に対して放射線照射した際に，照射野に内頸動脈が含まれる場合に 3.5%の患者で照射後 4 カ月ないし 240 カ月後に発症してくる[42,43]。照射線量は 22 Gy から 120 Gy の間で起こりえ，線量依存性に発症頻度が高くなる。また，neurofibromatosis type 1 の患者での発症の可能性が，そうでない患者の 3 倍高くなることが明らかになっている[42,43]。閉塞血管は組織学的には，内膜の肥厚と中膜の壊死が主体である。

2．放射線照射の標的となる分子

放射線照射の一次的標的は細胞，組織内の水分子である。水分子が放射線により分解して種々の活性酸素種（reactive oxygen species: ROS）を生じるが，これが DNA 損傷や細胞の膜のリン脂質の過酸化，酵素の失活を惹起し，細胞に対して傷害性に働く。例えば ROS によって DNA 損傷が生じた場合，修復機序が活性化するが，それによる修復が不能な場合には p53 が活性化して細胞死が誘導される。逆にこれら細胞死を防ぐ機構も知られている。脳に放射線を照射すると，ROS により NFkB が活性化する。NFkB は転写因子で tumor necrotizing factor（TNF），nerve growth factor（NGF），insulin-like growth factor I（IGF-I）の発現を増加させる。これら 3 つのサイトカインは，いずれも neuron に対して保護的に働くことがわかっている[44-46]。Basic fibroblast growth factor（bFGF）の発現も上昇するが，これも neuron に対して，酸化的ストレスから保護的に働くことがわかっている[26-28]。これらの防御機構はある

ものの，照射線量が多くなるとROSも増加し防御機構の限界を超えてしまうことになる。

III. 新しい放射線治療と放射線障害軽減への展望

以上，悪性脳腫瘍に対する放射線治療の副作用としての放射線性脳障害についてその病態，成因等について解説してきたが，現行の放射線治療はこれらのことを踏まえて，可能な限り正常脳の障害を最小限にとどめる努力がなされている。定位放射線治療をはじめ，強度変調放射線治療，tomotherapyなど新しい放射線治療装置はすべて腫瘍部分に高い線量を照射して，周囲の正常脳組織へは極力照射線量を減らすための工夫がなされている。転移性脳腫瘍に対して，定位放射線治療は有効かつ周辺脳への影響の少ない治療法として確立している。筆者は高齢者の単発あるいは数個程度の転移巣であれば，全脳照射は避けて，手術および定位放射線治療を用いて治療する方針をとっている。不規則な形状をした悪性グリオーマに対しては，今後強度変調放射線治療，tomotherapyなど，従来の照射法に比して腫瘍辺縁部で急峻な線量低下を実現できる照射法が普及するものと思われ，治療成績の向上と同時に副作用の軽減を期待したい。

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〈お知らせ〉

第27回 The Mt. Fuji Workshop on CVD

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演題募集

1）主　題：ディベート「脳血管内治療VS外科的治療／内科的治療」

2）細　目：上記の主題について下記の細目で演題を募集いたします。

カテゴリー

I．ランチョンセミナー
「脊髄血管性病変に対する脳血管内治療」　ルーズベルト病院　新見康成先生
「脊髄血管性病変に対する外科的治療」（演者未定）

II．イブニングセミナー
「脳血管障害に対する抗血小板療法（仮題）」（演者未定）

III．一般演題（口演・ポスター）
脳血管障害に関する演題はすべて応募対象とします。

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CLINICAL STUDY - PATIENT STUDY

Immunohistochemical profiles of brain metastases from breast cancer

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Abstract The aim of present study is to explore the immunohistochemical profiles of brain metastases from breast cancer. We retrospectively performed immunohistochemical staining for estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor type 2 (HER2/neu), and cytokeratin (CK) 5/6 in 29 patients with resected tumor specimens of brain metastases. Immunohistochemical staining for ER, PgR and HER2/neu was performed in 24 patients with primary tumors. The positive frequency of immunohistochemical profiles of ER, PgR, HER2/neu, and CK5/6, in the brain metastases were 13.8%, 6.9%, 37.9%, and 24.1%, respectively. The immunohistochemical profiles including ER, PgR, and HER2/neu of the primary tumor and the brain metastasis differed in seven patients (29.2%, N = 7/24). Interestingly, the biological characteristics of brain metastasis sometimes changed which were represented by immunohistochemical staining. Therefore, the changes in the biological features of breast cancer should be taken into account when developing treatment strategies, including new molecular-targeted drugs, for brain metastases.

Keywords Immunohistochemical staining · Discordance · Hormone receptor · HER2/neu · Breast cancer · Brain metastasis

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Introduction

Brain metastasis is the most common type of malignancy found in the brain and is responsible for a substantial fraction of the total morbidity and mortality of metastatic breast cancer patients. Brain metastasis is generally a late feature in the history of metastatic breast cancer. The incidence of symptomatic brain metastases in metastatic breast cancer ranges from 10% to 16% and has been reported to be even higher in human epidermal growth factor receptor type 2 (HER2/neu)-positive tumors [1]. Current systemic therapy, including hormone therapy, chemotherapy and molecular-targeted drug therapy, is not effective for the treatment of brain metastasis, and the development of treatment strategies for brain metastasis has become a critical issue.

A recent study reported that patients with HER2/neu-positive metastatic breast cancer had a high incidence of subsequent brain metastasis than HER2/neu-negative patients [2–4]. The human epidermal growth factor

receptor family is involved in cell proliferation, differentiation, and survival. HER2/neu amplification is widely known to indicate an aggressive tumor behavior and poor clinical outcome in breast cancer patients. HER2/neu over expression occurs in approximately 20–30% of breast cancer patients [5]. Trastuzumab, a monoclonal antibody against HER2/neu, has been shown to be significantly effective in both adjuvant and metastatic settings [6, 7].

One interpretation of these results was that HER2/neu-positive breast cancer may have a biological affinity toward the development of brain metastasis or trastuzumab-containing therapy prolongs survival until the eventual development of brain metastasis. On the other hand, evidence indicated trastuzumab cannot enter cerebrospinal fluid [8]. Blood brain barrier creates a "sanctuary" for cancer cells where antitumor agents cannot penetrate in high enough concentrations to have any substantial effect. However, whether prolonged patient survival enables cancer cells that have become resistant to trastuzumab to metastasize to the brain remains uncertain. Furthermore, whether brain metastases continue to overexpress HER2/neu and to be sensitive to trastuzumab therapy also remains unknown.

On the other hand, triple negative breast carcinomas (TNBCs) are a group of primary breast tumors with aggressive clinical behavior that account for 10–15% of all breast cancers [9]. Most TNBCs possess a basal phenotype and show varying degrees of basal cytokeratin (CK) and myoepithelial marker expression. Histologically, such cancers are poorly differentiated, and most fall into the basal subgroup of breast cancers, characterized by staining for basal markers (i.e., CK5/6) [10]. A previous study, in which primary tumors were immunohistochemically analyzed, reported that patients with estrogen receptor (ER)-negative and progesterone receptor (PgR)-negative tumors either with or without HER2/neu over-expression had a high risk of brain metastasis in a case-controlled study. A multivariate analysis of a database containing 10,782 patients in another study also reported that the independent risk factors for central nervous system metastasis were ER negativity, a young age, and a histology of invasive ductal carcinoma [11].

We therefore investigated the immunohistochemical profiles, including ER, PgR, HER2/neu, and CK5/6 of brain metastases in breast cancer patients. In addition, we investigated the changes in the immunohistochemical profiles of the primary tumors and the brain metastases.

Patients and methods

Patients

Two hundred fifty-two patients with breast cancer received trastuzumab-based chemotherapy between January 1999 and January 2006 at the National Cancer Center Hospital (NCCH), in Japan. (48 patients in neo-adjuvant setting and 204 patients in metastatic or recurrent setting) Of these, 74 patients (36.3%) developed brain metastases. Twenty-nine patients with brain metastasis were retrospectively identified based on records in the hospital's surgical database. All clinical information was collected from patient chart. Trastuzumab had been initially administered at an intravenous loading dose of 4 mg/kg, followed by weekly infusions of trastuzumab (2 mg/kg) in combination with chemotherapy. The present study was approved by the Institutional Review Board of the National Cancer Center.

Tissue samples and microscopic and immunohistochemical analysis

Hematoxylin–eosin stained specimens were reviewed by two pathologists (K.T. and K.S.) and were confirmed to contain an adequate amount of cancer tissue available for use in the present study. All tumor specimens from brain metastasis resections were available for immunohistochemical analysis. Tumor specimens from primary breast cancers were available for immunohistochemical analysis of ER, PgR, and HER2/neu in 24 of the 29 patients.

The pathological and immunohistochemical examinations were conducted by the same pathologists (K.T. and K.S.), who were blinded to the clinical statuses of the patients. Formalin-fixed, paraffin-embedded tissue samples were sectioned (4-μm thick) and mounted on charged slides. Immunohistochemical staining for ER (clone 1D5; DAKO, Carpinteria, CA), PgR (clone PgR636; Dako), and CK5/6 (clone D5/16B4; Dako) were performed using the streptavidin-biotin method and were considered positive if 10% or more of the nuclei in the invasive component of the tumor were stained [12, 13]. The HER2/neu status, as assessed using Herceptest (Dako), was scored on a scale of 0 to 3+, according to the Dako scoring system. HER2/neu-positive was defined by HER2/neu 3+ or HER2/neu 2+ and fluorescence in situ hybridization-positive. Negative controls, in which the primary antibody was omitted, were also included in each run.

Statistical analysis

The comparisons were made between two groups using a Chi-square test, a Fisher exact test. All the statistical analyses were performed using SPSS 12.0J (SPSS Inc., Chicago, IL, USA), and the significance level for the results was set at 0.05 (two-sided).

Results

The present study included 29 patients with brain metastasis, and the median age at the time of the diagnosis of

Table 1 Patient characteristics

Characteristics of brain metastasis	Prior history of trastuzumab		Total
	(+)	(−)	
Median size (in mm) of brain metastasis (range)	32 (30–40)	36 (20–60)	35 (20–60)
Number of brain metastases			
1	6	18	24
2	2	2	4
3	0	1	1
Side (right/left/bilateral)	1/6/1	13/8/0	14/14/1
Site			
Frontal lobe	0	1	1
Parietal lobe	0	2	2
Temporal lobe	1	5	6
Occipital lobe	1	2*	3*
Cerebellum	6	10*	16*

* One patient had brain metastases in both the cerebellum and occipital lobe

brain metastasis was 53 years old (range, 39–78 years). The median time to brain metastasis from the time of breast cancer diagnosis was 2.9 years (range, 0–23.1 years).

In this study, eight patients had a prior history of receiving chemotherapy containing trastuzumab, seven of these patients had received trastuzumab-containing chemotherapy in a metastatic setting, and one had received trastuzumab-containing neo-adjuvant chemotherapy, five patients had a prior history of receiving hormone therapy, seven patients had a prior history of receiving chemotherapy, two patients had a prior history of receiving both hormone therapy and chemotherapy, and seven patients had received no systemic therapy prior to the brain tumor resection.

The patient characteristics are shown in Table 1. After the brain tumor resection, most patients (59%) received whole brain radiotherapy, eight patients received local brain radiotherapy, one patient received γ-knife radiotherapy, and three patients did not receive additional brain radiotherapy. After the completion of the local treatment for metastatic brain tumor, 14 patients received systemic chemotherapy, including three patients who received combination chemotherapy including trastuzumab, one patient who received trastuzumab therapy alone, five patients who received hormone therapy, and three patients who received intrathecal chemotherapy because of the rapid progression of meningeal carcinomatosis. Six patients received supportive care alone. The median overall survival time was 14.7 months.

The positive frequency of immunohistochemical profiles of ER, PgR, and HER2/neu, in 24 primary tumors were 12.5% (N = 3/24), 8.3% (N = 2/24), 37.5% (N = 9/24), respectively. The positive frequency of immunohistochemical profiles of ER, PgR, HER2/neu, and CK5/6, in 29 brain metastases were 13.8% (N = 4/29), 6.9% (N = 2/29), 37.9% (N = 11/29), and 24.1% (N = 5/29), respectively (Table 2). Among patients with both hormone-negative and HER2/neu-negative statuses, basal type (CK5/6 positive) breast cancer was observed in 42.9% (N = 6/14). The staining results for ER, PgR, HER2/neu and CK5/6 in the brain metastases were not statistically correlated with a prior history of receiving trastuzumab-containing chemotherapy (Table 2).

Table 2 Relationship between prior history of receiving trastuzumab and immunohistochemical profiles in specimens obtained from brain metastasis (Chi-square test and Fisher exact test)

Variables	Total (%) n = 29	Prior history of trastuzumab		*P*-value
		(+)	(−)	
ER				0.99
Negative	25	7	18	
Positive	4	1	3	
PgR				0.99
Negative	27	8	19	
Positive	2	0	2	
HER2/neu				0.402
Negative	18	2	16	
Positive	11	6	5	
CK5/6				0.99
Negative	22	6	16	
Positive	7	2	5	
Basal type*				0.647
No	23	7	16	
Yes	6	1	5	

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor; HER2/neu, human epidermal receptor type 2; NA, not applicable

* Basal type in present study defined as ER negative, PgR negative, HER2/neu negative, and CK5/6 positive brain metastasis

The frequencies of immunohistochemical change in ER, PgR, and HER2/neu were 12.5% (N = 3/24), 4.2% (N = 1/24), and 12.5% (N = 3/24), respectively (Table 3). The immunohistochemical profiles for ER, PgR, and HER2/neu differed between the primary tumors and the brain metastases in seven patients (29.2%; N = 7/24, see Table 4). With regard to the systemic treatment options, the treatment options for six patients (25%) were changed based on the immunohistochemical profiles of their brain metastases. Among the patients who had been previously treated with trastuzumab, 25% of the patients (N = 2/8) had changed to a HER2/neu-negative status.

Discussion

The present study demonstrated immunohistochemical characteristics for ER, PgR, HER2 receptor, and CK5/6 of

Table 3 Changes in immunohistochemical profiles in estrogen receptor, progesterone receptor or HER2 receptors between primary tumors and brain metastases (n = 24)

Primary tumor	Brain metastasis	N
ER (−)	ER (−)	19
	ER (+)	*2*[*]
ER (+)	ER (−)	*2*
	ER (+)	1
PgR (−)	PgR (−)	22
	PgR (+)	0
PgR (+)	PgR (−)	*1*
	PgR (+)	1
HER2/neu (−)	HER2/neu (−)	14
	HER2/neu (+)	*1*
HER2/neu (+)	HER2/neu (−)	*2*[*]
	HER2/neu (+)	7

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor; HER2/neu, human epidermal receptor type 2

Italic number indicated seven patients who had changes of immunohistochemical profiles

[*] One patient with an ER-negative, PgR-negative, HER2-positive primary tumor developed an ER-positive, PgR-negative, HER2/neu-negative brain metastasis

Table 4 Discordance cases of immunohistochemical profiles between primary tumor and brain metastasis (N = 7)

Case no.	ER		PgR		HER2/neu	
	Primary	Brain meta	Primary	Brain meta	Primary	Brain meta
1	+	−	−	−	−	−
2	−	−	+	−	−	−
3	+	−	+	−	−	−
4	−	+	−	−	+	+
5	−	−	−	−	−	+
6	−	−	−	−	+	−
7	−	+	−	−	+	−

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor; HER2/neu, human epidermal receptor type 2

brain metastases from breast cancer patients. Although present study had small number of patients and potential selection bias, it is remarkable that the biological characteristics of brain metastasis sometimes changed from the primary tumors.

ER, PgR and HER2/neu immunohistochemical profiles of the primary tumors differed from those of the brain metastases in 29.2% of the patients in the present study; in particular, the HER2/neu status of the two specimens differed in 12.5% of the patients. Generally, marked intratumoral heterogeneity is rare than the degree of heterogeneity (as determined using immunohistochemical analyses and FISH) between primary tumors and their metastases [14–16]. Some previous reports have compared the HER2/neu status of primary tumors and metastases, including axillary lymph node metastasis, local recurrence, distant metastasis, and autopsy findings, but only one study addressed brain metastases. These previous studies, excluding the study that examined brain metastases, described discordance rates of between 0% and 37.5% [14, 16–22]. In a study comparing primary tumors and axillary or metastatic lymph nodes, 0–2% of the patients had a discordance in their HER2/neu statuses [23, 24]. Similar to the results of these studies, discordance has been reported in <5% of primary tumors and their local recurrences [14, 16]. Meanwhile, the rate of discordance in studies examining distant metastasis at various sites was between 6% and 37.5% [17–21]. Among these studies, only two included patients with brain metastasis, but less than five patients were reported and they were not described in detail [17, 18].

Only one study examining patients with brain metastasis reported that 51.5% (N = 17/33) of the patients exhibited the over-expression of HER2/neu (as determined using immunohistochemical analyses) and 25.8% (N = 8/31) exhibited HER2/neu gene amplification. The concordance rate between the immunohistochemical analyses and FISH was 86% when compared with individual metastatic lesions (43 lesions). The concordant rates between the primary tumors and the brain metastases were 58% (N = 7/12) according to the immunohistochemical analyses and 100% (N = 10/10) according to FISH [25]. Although the frequency of discordance between the primary tumors and the brain metastases differed among the studies, probably because of differences in the measurement methods and the small sample sizes, it is important to realize that the brain metastases in some patients have different HER2/neu statuses from those of the primary tumors.

Hormone positivity in the brain metastases was relatively low in this cohort. Hormone receptor discordance in locoregional recurrences or lymph node metastasis may be more frequent than HER2/neu discordance. The rates of discordance have been reported to be 10–25% for ER and 21–44% for PgR between the primary tumor and locoregional recurrence or lymph node metastasis [14, 24, 26, 27]. The rate of discordance between the primary tumor and distant metastasis was similar to that of regional lymph node metastasis or recurrence, which were reported to be 28–42% for ER and 17% for PgR [26, 27]. The discordance rate for hormone receptors in the present study was slightly lower than those reported in previous studies, but whether the hormonal characteristics of brain metastasis differ from those of other distant metastases remains uncertain.

Although the overall frequency of CK5/6 positive brain metastases was low (24.1%, N = 7/29) in present

study, most of patients (71.3%, N = 5/7) were basal type subgroup in patients with CK5/6 positive brain metastases. A previous study revealed that primary tumors which were negative for ER but that expressed basal CK5/6 and overexpressed HER1 or HER2/neu were more likely to develop brain metastasis [28]. The changes in basal type markers between the primary tumors and the brain metastases are uncertain in the present study because CK5/6 staining was not performed in the primary tumors.

There is one report that suggests chemotherapy do not modify the HER2/neu status in metastatic lesions [16]. On the other hand, Regitnig et al. reported that HER2/neu amplification and overexpression may occur de novo in distant metastasis at a late stage of disease [18]. Currently, possible mechanisms of trastuzumab-resistance include the down-regulation of p27, the activation of insulin-like growth factor receptor (IGF-1R), the loss of PTEN, pAkt, interactions between HER family members, the masking of HER2/neu by membrane-associated glycoprotein mucin-4, angiogenesis, and the induction of antibody-dependent cellular toxicity by the immune system [29, 30]. These hypotheses remain controversial, and some studies assessing IGF-1R and p53 in clinical samples have reported negative results [12, 31]. Our study suggests that some patients with HER2/neu negative primary whose brain metastasis were HER2/neu positive responded to trastuzumab therapy after diagnosis of brain metastasis [21]. However, whether the biological changes in the breast cancer cells described in the present study and in previous studies influence the mechanism of resistance to trastuzumab therapy remains unknown.

The present results suggest that all distant metastases should not be assumed to be biologically equal to locoregional metastasis, and that a re-assessment of the immunohistochemical status of the brain metastasis, if possible, may be useful to optimize treatment. Further studies are warranted to address the reason of discordance in the immunohistochemical profiles of the primary tumors and the brain metastases.

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Image of the Month

Three Cases of Sub-scalp Tumor Presenting with Protrusion of the Head

Case 1 was an 81-year-old male who underwent right nephrectomy for renal cell carcinoma 5 years ago. When he fell down and hit his back of the head, he noticed an occipital lump (Fig. 1). The bulging was growing gradually within several months. He underwent an excisional biopsy and pathological findings showed a metastatic tumor from renal cell carcinoma. Irradiation was administered. Case 2 was a 58-year-old female who suffered head trauma 3 years ago. She noticed a protrusion on her parietal cranium while grooming her own hair (Fig. 2). She received a needle biopsy and the pathological diagnosis was meningioma. She underwent surgical resection of the tumor and cranioplastic surgery. Case 3 was an 81-year-old male who had tingling-numb on his forehead for 6 months. Because of the persistent dysesthesia accompanied by progressive swelling (Fig. 3), he consulted our hospital and was operated on for histological confirmation. Obtained tissues were composed of poorly differentiated carcinoma. He subsequently received irradiation on the frontal lesion.

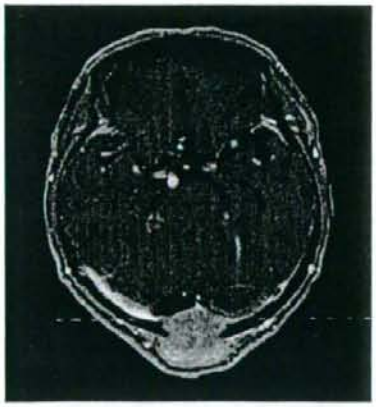

Figure 1.

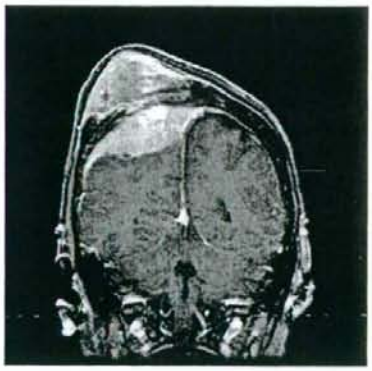

Figure 2.

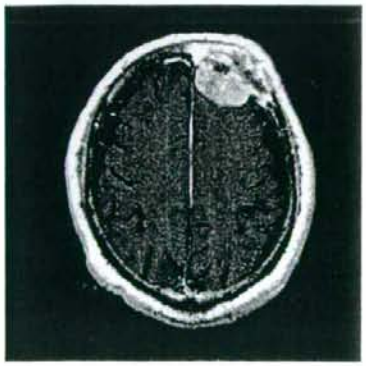

Figure 3.

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Clinical Trial Note

A Multicenter Phase I Trial of Interferon-β and Temozolomide Combination Therapy for High-grade Gliomas (INTEGRA Study)

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A multicenter phase I clinical trial, namely, Integrated Japanese Multicenter Clinical Trial: A Phase I Study of Interferon-β and Temozolomide for Glioma in Combination with Radiotherapy (INTEGRA Study), is being conducted for patients with high-grade glioma in order to evaluate the safety, feasibility and preliminary clinical effectiveness of the combination of interferon-β and temozolomide. The primary endpoint is incidence of adverse events. The secondary endpoints are progression-free survival time and overall survival time. In addition, objective tumor response will be evaluated in a subpopulation of patients with the measurable disease. The reduction rate of tumor will be calculated according to Response Evaluation Criteria In Solid Tumors for measurable tumors as determined by magnetic resonance imaging. Subsequently, the overall response will be evaluated based on the results of measurable and non-measurable tumors. Ten newly diagnosed and 10 recurrent patients will be enrolled in this study.

Key words: chemo-phase I-II-III – clinical trials – CNS

INTRODUCTION

Gliomas account for ~40% of all brain tumors and are thus the most common primary tumors of the central nervous system. Primary brain tumors are classified according to their cell type and histological grade into categories defined by the World Health Organization (WHO) (1). High-grade (WHO grades III and IV) gliomas, which include anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), anaplastic oligoastrocytoma (AOA) and glioblastoma multiforme (GBM), are often resistant to treatment; GBM, the most common glioma in adults, kills patients within a median time span of a year after diagnosis despite treatment with aggressive surgical resection, nitrosourea-based chemotherapy and radiotherapy (2–4). A number of studies by large cooperative groups have shown the benefits of radiation therapy in doses up to 60 Gy after surgery for improving overall survival and time to progression (5). In Japan, nitrosourea agents such as 1-(4-amino-2-methyl-5-pyridiminyl)methyl-3-(2-chloroethyl)-3-nitrosourea and methyl-6-[3-(2-chloroethyl)-3-nitrosoureido]-6-deoxy-alpha-D-glucopyranoside have been used to treat malignant gliomas for a long time; however, this treatment offered few clinical benefits. Temozolomide (TMZ), an oral alkylating agent, has been demonstrated to possess antitumor activity against malignant gliomas, with minimal additional toxicity; furthermore, in a previous study of concomitant radiation therapy and chemotherapy with TMZ followed by adjuvant TMZ, survival duration substantially improved (6). In 2006, TMZ

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was certified as the treatment agent for malignant gliomas by the National Ministry of Health and Welfare of Japan, and a combination of radiotherapy and chemotherapy with TMZ is now used as the first-line therapy. However, its clinical outcomes depend on the *O*-(6)-methylguanine-DNA methyltransferase (MGMT) status, and MGMT modification is one of the key factors to obtain greater clinical benefits in the future.

Interferon-β (IFN-β) exhibits pleiotropic biological effects and has been widely used either alone or in combination with other antitumor agents in the treatment of malignant gliomas and melanomas (7). In the treatment of malignant gliomas, IFN-β can act as a drug sensitizer, enhancing toxicity against various neoplasms when administered in combination with nitrosourea. IFN-β and nitrosourea combination therapy has been particularly used for the treatment of gliomas in Japan (8). Previously, we demonstrated that IFN-β markedly enhanced chemosensitivity to TMZ in an *in vitro* study of human glioma cells (9); this finding suggested that one of the major mechanisms by which IFN-β enhances chemosensitivity is the downregulation of MGMT transcription via *p53* induction. This effect was also observed in an experimental animal model (10). These two studies suggested that chemotherapy with IFN-β and TMZ plus radiation might further improve the clinical outcome in malignant gliomas when compared with TMZ plus radiation therapy. Here, in order to evaluate the safety, feasibility and preliminary clinical effectiveness of the combination of IFN-β and TMZ, we are conducting a clinical study, namely, Integrated Japanese Multicenter Clinical Trial: A Phase I Study of Interferon-β and Temozolomide for Glioma in Combination with Radiotherapy (INTEGRA study). This study involves eight medical institutions, covering the entire regional population of Japan.

PROTOCOL DIGEST OF THE STUDY

Purpose

The main aim of this study is to evaluate the safety, feasibility and preliminary clinical effectiveness of IFN-β and TMZ for the treatment of malignant gliomas.

Study Setting and Protocol Review

This is a multicenter clinical trial involving eight neurosurgical institutions: Yamagata, Saitama Medical, Nippon Medical, Nagoya, Osaka, Kyoto, and Hiroshima Universities and Kitano Hospital. The protocol has been reviewed and approved by institutional review boards of each of these institutions.

Registration and Monitoring

Participating investigators are instructed to send an eligibility criteria report to the Data Center at Nagoya University, which is a third party different from the study director. Ten newly diagnosed and 10 recurrent patients are registered for a period of 6 months from December 2007. Data, including those of magnetic resonance imaging (MRI), blood tests, and pathology, will be collected at the data center. The quality of data will be checked and verified at the data center. If required, the data center would provide feedback to the institutions. The data center will send high-quality data to the study director. Committees of safety and efficacy (Dr Kazuo Tabuchi, Koyanagi Memorial Hospital, Saga), radiotherapy (Dr Shinji Naganawa, Department of Radiology, Nagoya University School of Medicine), pathological review (Dr Youichi Nagasato, Department of Pathology, Gunma University School of Medicine) and statistics (Dr Kunihiko Hayashi, Gunma University School of Health Science) will send their reports to the head office.

Endpoints

The primary endpoint is incidence of adverse events. The secondary endpoints are progression-free survival time and overall survival time. In addition, objective tumor response will be evaluated in a subpopulation of patients with measurable disease. The reduction rate of tumor will be calculated according to Response Evaluation Criteria In Solid Tumors for measurable tumors as determined by MRI. Non-measurable tumors are classified into four grades: complete remission, partial response, progression and not evaluable. Subsequently, the overall response will be evaluated based on the results of measurable and non-measurable tumors.

Eligibility Criteria

The eligibility criteria are as follows:

(i) Histologically confirmed diagnosis of newly diagnosed or recurrent high-grade glioma (AA, AO, AOA or GBM). More than 50% volume of tumor is located in the supratentorial region.
(ii) No tumor recognized in the optic nerve, olfactory nerve and pituitary gland on pretreatment MRI.
(iii) No dissemination detected by MRI. Age between 18 and 75 years at the time of registration.
(iv) Performance status is 0–2, 3 only due to neurological deficits.
(v) Sufficient organ function before chemotherapy according to the following laboratory data: WBC $\geq$ 3000/mm^3 or neutrophils $\geq$1500/mm^3, platelets $\geq$100 000/mm^3, hemoglobin $\geq$8.0 g/dl, bilirubin $\leq$1.5 mg/dl, serum glutamic oxaloacetic transaminase $\leq$ 100 IU, serum glutamic pyruvic transaminase $\leq$ 100 IU, creatinine $\leq$1.5 mg/dl, creatinine clearance $\geq$50 ml/min and electrocardiogram showing no serious arrhythmia and no serious ischemic heart disease.
(vi) No prior chemoradiotherapy for newly diagnosed patients.

(vii) The interval from the end of prior anti-tumor therapy (e.g. chemotherapy, radiotherapy, immunotherapy) must be at least 4 weeks for recurrent patients, regardless of the regimen.
(viii) Written informed consent.

Exclusion Criteria

The exclusion criteria are as follows:

(i) synchronous double cancer or metachronous double cancer in last 5 years; carcinoma *in situ* accepted;
(ii) meningitis or pneumonia;
(iii) pregnant, possibly pregnant, or nursing women;
(iv) mental disorder;
(v) uncontrolled diabetes mellitus (DM) or under treatment with insulin for DM;
(vi) myocardial infarction in last 3 months;
(vii) history of pulmonary fibrosis or interstitial pneumonia.

Treatment Methods

For newly diagnosed patients:

Radiotherapy	60 Gy/30 fr, 2 Gy ×5 days/week;
IFN-β	3 MIU/body, administered intravenously on alternate days during radiotherapy;
TMZ	75 mg/(m^2 day), daily from the first day to the last day of radiotherapy.

After completing this induction period, all patients will have 4 weeks of washout period, and they will be then shifted to adjuvant period.

IFN-β	3 MIU/body, administered on the first day morning every 4 weeks;
TMZ	150 mg/(m^2 day) (days 1–5: first cycle); 200 mg/(m^2 day) (days 1–5: second to sixth cycle).

In the absence of hematologic toxicity, the dose is increased to 200 mg/(m^2 day), beginning with the second cycle to the sixth cycle.

This cycle is repeated six times every 28 days in the absence of tumor progression, serious adverse events such as grade 4 hematological toxicity, refusal of therapy and deviation from the protocol.

For recurrent patients:

IFN-β	3 MIU/body, administered the first day morning every 4 weeks (day 1);
TMZ	150 mg/(m^2 day) (days 1–5: first cycle); 200 mg/(m^2 day) (days 1–5: second to sixth cycle).

In the absence of hematologic toxicity, the dose is increased to 200 mg/(m^2 day), beginning with the second cycle to the sixth cycle.

This cycle is repeated six times every 28 days.

This regimen has been considered to be the most promising based on previous clinical studies (8,11–14). Thus, dose-limiting toxicity was not evaluated in this study.

Follow-up and Statistical Methods

Disease progression and occurrence of new disease will be examined by MRI performed at baseline and at least after every 4–5 weeks during treatment. Blood tests and symptom checks will be carried out before treatment and at least after every 2 weeks during treatment. Follow-up will continue for 3 months from the end of treatment. In cases wherein therapy is discontinued due to toxicity, clinicians would follow-up patients until they recover from toxicity. In addition, overall survival, progression-free survival and treatment success curves are constructed as time-to-event plots by the Kaplan–Meier method.

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Conflict of interest statement

None declared.

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