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特集3

グリオーマに対する手術療法と放射線療法の進歩

グリオーマ手術時の覚醒下手術

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SUMMARY

覚醒下手術（awake surgery）とは文字どおり患者が覚醒状態のまま脳神経外科的手術を行うことである．近年の神経放射線診断学の進歩により，脳の機能局在は術前にある程度は予測可能になってきているが，脳腫瘍やてんかんなど病的な脳において，実際にどこまでの病変摘出が可能であるかを決定できるまでには至っていない．そのため，術中に機能マッピングを施行して摘出範囲の決定を行う必要がある．脳機能マッピングには，術中に患者を覚醒させて種々のタスク（数唱や物品呼称など）を施行する．より安全で適切な手術のため，日本Awake Surgery研究会（会長：嘉山孝正）にて現在ガイドラインを作成中である．機能マップを作成後も覚醒状態を保つことで摘出中に神経症状の出現・悪化がないかというモニタリングの役割も果たすことができる．手術による腫瘍摘出率が予後因子と考えられる悪性脳腫瘍の手術において，覚醒下手術は術後の後遺症を最小限にとどめ最大限の摘出を可能とする極めて重要な手術手技である．

KEY WORDS
覚醒下手術
脳機能マッピング
モニタリング
高次脳機能
脳腫瘍

覚醒下手術の歴史

覚醒下手術の歴史は以外に古く，1900年代前半より行われ，歴史的にはPenfieldらのてんかん患者に対する覚醒下手術中に行った大脳機能マッピングに関する研究が有名である．近年では，Ojemannらが言語野について117例のやはりてんかん患者の大脳皮質電気刺激を行い，その詳細な検討から言語関連領域は脳表にモザイク状に存在していると報告している[1]．このように，従来考えられていた脳機能局在がすべての人に必ずしもあてはまらないことから，脳神経外科手術に際しては個々人の脳機能局在を決定，手術を行う必要性が認識されるに至った．さらに，アメリカでは1989年，日本では1995年から使用可能となった静脈麻酔剤プロポフォール（propofol）により，術中の良好な覚醒が速やかにかつ安全に得られるようになり，awake surgeryによる術中脳機能マッピングは近年注目を浴び，また日本国内でも普及してきている．

脳腫瘍（特にグリオーマ）手術における意義

グリオーマ治療における手術摘出率が予後因子であるか否かについては，これまで種々の報告がなされcontroversialであったが，近年MRIを用いた残存腫瘍の正確な把握と予後の検討がなされ，グリオーマにお

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いてもやはり摘出率の向上が予後を改善に寄与することが広く認められるようになってきている[2]．しかしながら摘出率の向上だけを優先させ，術後 morbidity をまねくような手術は避けねばならない．近年 MRI をはじめとする神経放射線診断学の進歩はめざましく，例えば functional MRI による個人の脳機能マッピングも精力的に行われており，重要な知見が多く得られている．しかし，この所見だけをもとに腫瘍摘出範囲を決定できるほどの信頼性はまだ無く，現時点で確実に脳機能局在を決定し，安全な治療を行うためにはやはり脳表電気刺激による脳機能マッピングが不可欠であると思われる．特に高次脳機能である言語機能は，言語機能が全身麻酔下に評価不能であることから，硬膜下電極挿入術を行い言語機能マッピングを行った後に，第二期手術として腫瘍摘出を行うか，あるいは覚醒下手術を行う必要がある．硬膜下電極挿入では，様々な言語タスクを繰り返し検討し詳細な言語機能マッピングを行えるという利点があるが，腫瘍摘出前に侵襲度の決して低くない電極挿入術を行う必要があること，脳浮腫を伴う患者の硬膜下に電極を入れること自体の危険性もあることから，我々はこれまで言語機能マッピングは覚醒下手術を第一選択としてきた．また，言語野がシルビウス裂に接して存在することに注目し，シルビウス裂内の言語機能の有無について検討し言語機能を認めない部位より病変にアプローチし（**図1**），言語野直下に存在する病変を morbidity なく治療することが可能であった[3]．このような評価は硬膜下電極ではなし得ず，言語野をはじめとする eloquent area 近傍病変の手術における awake surgery の重要性，有用性は今後もますます高まるものと考えられる．また，摘出中も覚醒状態を維持することで，機能モニタリングとして用いることができること，皮質下刺激による皮質下マッピングも可能であり，最近は弓状束をはじめとする皮質下の言語機能のネットワークの同定・研究も試みられている[4]．

Awake surgery の実際

図2，3 に awake surgery の流れと手術風景を示す．体位は側臥位とし，頭部は円座や馬蹄形のヘッドレストの上に半固定か，もしくは3点固定にて固定する．半固定の方が患者の苦痛は少なく長時間覚醒状態を保つ上で有利と考えられる．対して3点固定を用いることで顕微鏡操作中に突然術野が動くという危険を回避

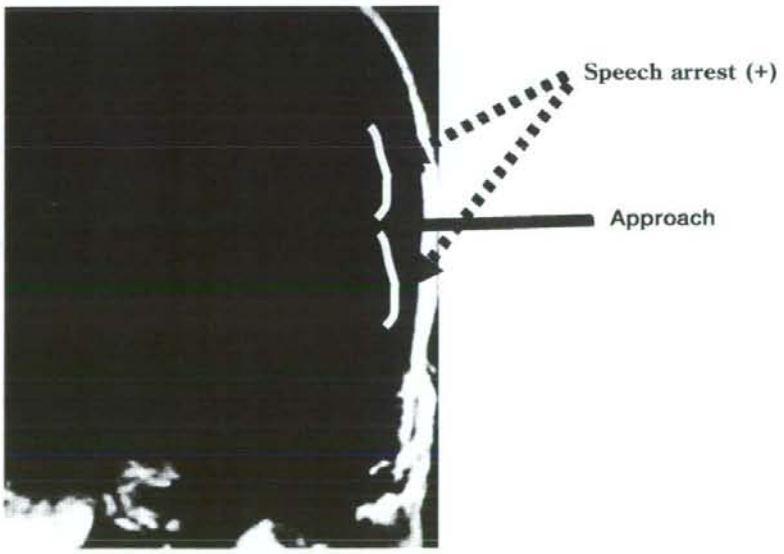

図1　言語野近傍腫瘍に対する経シルビウス裂アプローチ

術　前：神経放射線学的検査（MRI, fMRI, DTI, MEG, SAS, venography など）
　　高次脳機能検査，タスク練習

術前日：手術室にてシミュレーション，機器レイアウト確認

当　日：
1．体位確認
2．麻酔開始
3．ライン確保
4．局所麻酔・頭位固定
5．咽頭マスク挿入
6．モニタリング電極挿入
7．ナビゲーションレジストレーション
8．ドレーピング
9．開頭
10．SEP（中心溝同定）
11．覚醒
12．皮質脳波モニタリング
13．皮質刺激，マッピング
14．腫瘍摘出，適時モニタリング
15．麻酔再開，再挿管*
16．閉頭

＊覚醒を維持するのが難しいと判断した場合，摘出開始前に麻酔再開，再挿管

図2　手術の流れ

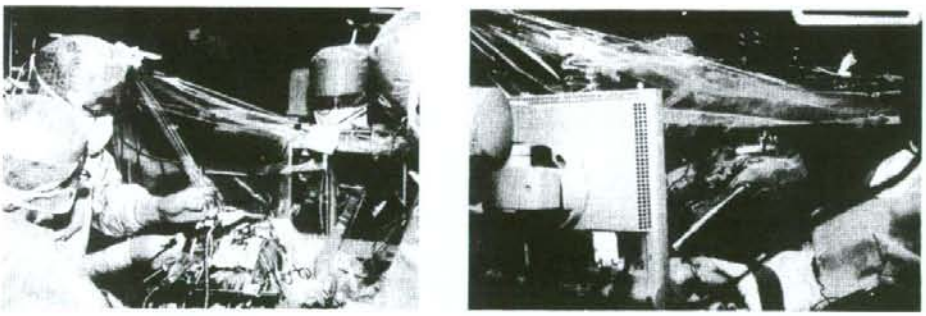

図3　手術風景

することができる．どちらの方法を用いるかは，患者・術者・その他の種々の因子から総合的に決定する必要がある．挿管は行わず，propofol による静脈麻酔で自発呼吸を保ちながら，頭皮に十分な局所麻酔を行って皮切，開頭を行う．硬膜を開き脳表を露出してから，propofol 投与を中断するとおよそ15分で患者は覚醒する．患者が会話可能となったところで皮質脳波を記録しながら双極電気刺激装置を用いて，電気刺激を行う．患者が頭部を動かさずに見ることのできる位置に設置したモニタ等を見せながら言語タスク（物品の呼称，数唱，読字，簡単な質疑応答など）を与えつつ，言語機能が存在すると予測される大脳皮質を電気

刺激し，一過性に神経機能を抑制する．物品呼称の停止や遅れ，失名詞（anomia）などを生じる部位を詳細に調べ，最終的に言語機能地図を作成する．これにより，個々の患者の言語中枢が特定され，切除しても言語障害の出現しない安全な部分と言語機能に影響を与えるcriticalな部分が同定でき，新たな後遺症を来すことなく最大限の腫瘍摘出が可能となる．現在，日本Awake Surgery研究会にて，高次脳機能や麻酔なども含めた覚醒下手術に関するガイドラインを作成中であり，2009年には正式な発表を予定している．

症例提示（図4, 5）

症例は31歳の右利きの女性である．MRI上，解剖学的なBroca area近傍に腫瘍性病変を認めた．覚醒下手術による脳機能マッピングで，腫瘍は舌，口唇の運動野直下に存在していることが明らかとなり，脳機能マッピングの結果言語機能障害が生じなかったシルビウス裂内側面よりアプローチし，言語障害を来すことなく腫瘍を全摘出し得た．

今後の課題と展望

現時点での覚醒下手術の第一の問題点は，体位，頭位，開頭範囲が制限される点である．特に側頭葉切除の際に，中頭蓋底まで開頭することはしばしば困難である．補足運動野（supplementary motor area: SMA）は，優位半球においては運動機能のみならず言語機能にも関与する部位である．補足運動野は運動野の前方に接して帯状回の上方の大脳半球内側面に存在し，言語表出も含めた運動の企画及び開始の役割を担っている．切除することにより対側の片麻痺と，優位半球の場合には一過性の無言症が生ずる．通常これらの障害は1ヵ月程でほぼ消失するが，現時点で覚醒下手術，術中マッピング，モニタリングでは予測が困難であり，この領域の手術には注意が必要である．
また覚醒下手術による頭頂葉の機能診断についても，いくつかの報告が認められる[9]．

近年，急速に普及したfunctional MRIは，非侵襲的に神経活動を評価できる大変魅力的な手法であるが，

図4　術中脳機能マッピングと手術アプローチ
術中の脳機能マッピングで病変が舌の運動や直下に存在しており，スライド左下に示す如くシルビウス裂を開放後，内側の言語機能を認めない部分から病変にアプローチし腫瘍を全摘出した．

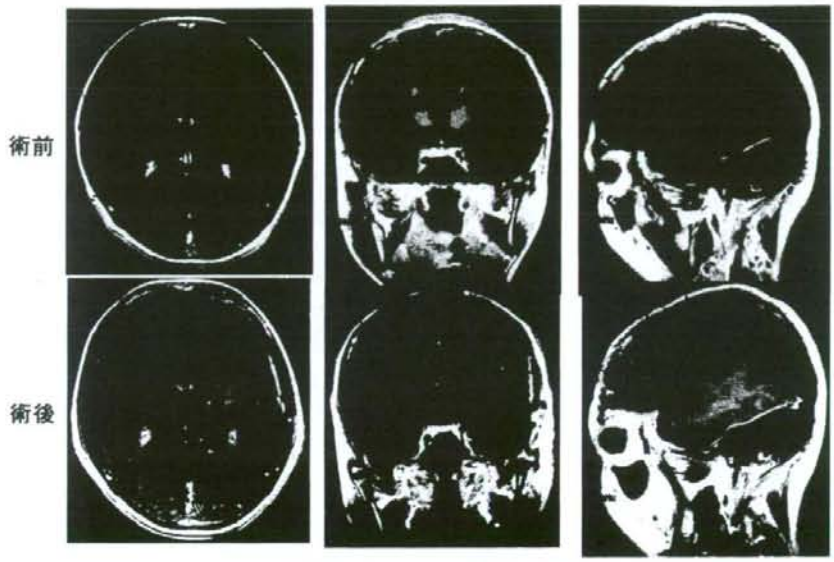

図5　左前頭葉グリオーマ　術前・術後MRI

現時点では未だfunctional MRIで得られた結果を術中マッピングで検証している段階であり，functional MRIのデータに基づいて脳内病変の切除計画を決定するには至っていない．またfunctional MRIで得られる言語関連領域は，awake surgeryでのマッピングで得られるような言語機能に必須の領域よりも広いことが予想される．悪性神経膠腫のように手術摘出率，KPSが予後因子である場合には，言語必須領域の損傷はさけなければならないが，言語関連領域の損傷を恐れるあまり摘出率が低下するということがないようにしなければならないと考えている．

覚醒下手術は，個々人の脳機能局在を知り「確率の医療から個別の医療へ」と安全に最大限の治療成果を得るために今後益々役立っていくとともに，脳科学の発展にも大きく貢献するものと考えられる．

おわりに

近年様々な分野でテーラーメイド治療の重要性が強調されているが，個々人の大脳機能局在を知り，それに応じた腫瘍摘出術を行う覚醒下手術という方法はこれからますますその必要性が増すものと考えられる．

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No Shinkei Geka, 37(3): 277 - 282, 2009

症例 ◆ Case Report

自然退縮を呈した鞍上部・松果体部 germinoma の 1 例*

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Spontaneous Regression of Primary Intracranial Germinoma: A Case Report

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Key words:
germinoma,
spontaneous regression

Here we report a case in which an intracranial germinoma displayed spontaneous regression. An 11-year-old boy presented with polyuria and headache. Computed tomography (CT) and magnetic resonance imaging (MRI) revealed tumors in the suprasellar and pineal regions, and obstructive hydrocephalus. As repeat MRI demonstrated shrinkage of these tumors, resection was deferred. The patient was discharged and followed up with serial MRI. The tumor continued to regress for three weeks; however, the patient was readmitted due to tumor regrowth. We performed endoscopic biopsy, and histopathologic diagnosis was germinoma. The patient underwent three courses of combined chemotherapy and radiotherapy, and complete response was achieved. Although the precise cause of the transient regression is unknown, intracranial germinoma may occasionally undergo spontaneous regression.

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I. はじめに

悪性腫瘍において自然退縮を呈する頻度は極めて稀であり，6 ～ 10 万人に 1 人と報告されている[1]．今回われわれは，自然退縮を認めた鞍上部・松果体 germinoma の稀な 1 例を経験したので文献的考察を加え報告する．

II. 症　例

〈患　者〉 13 歳　男性

主　訴　多飲，多尿，頭痛

既往歴　生下時より発達，成長に異常なし

家族歴　特記事項なし

現病歴　2006 年 6 月頃より飲水，排尿回数が増加し，頭痛も出現したため近医を受診した．頭蓋内精査にて鞍上部および松果体に腫瘍性病変を認めたため，当科へ紹介され，入院となった．

入院時所見　神経学的に異常所見は認めなかった．

血液・内分泌学的検査　内分泌学的には下垂体前葉ホルモンの低下は認めなかった．一日尿量は 4,000 ml，尿比重 1.002，antidiuretic hormone (ADH) は 0.2 pg/ml 以下であり，中枢性尿崩症を呈していると考えられた．

血中 AFP 3.2 ng/ml，HCG-β 0.1 ng/ml 未満と腫

*(2008. 6. 5 受稿，2008. 11. 5 受理)

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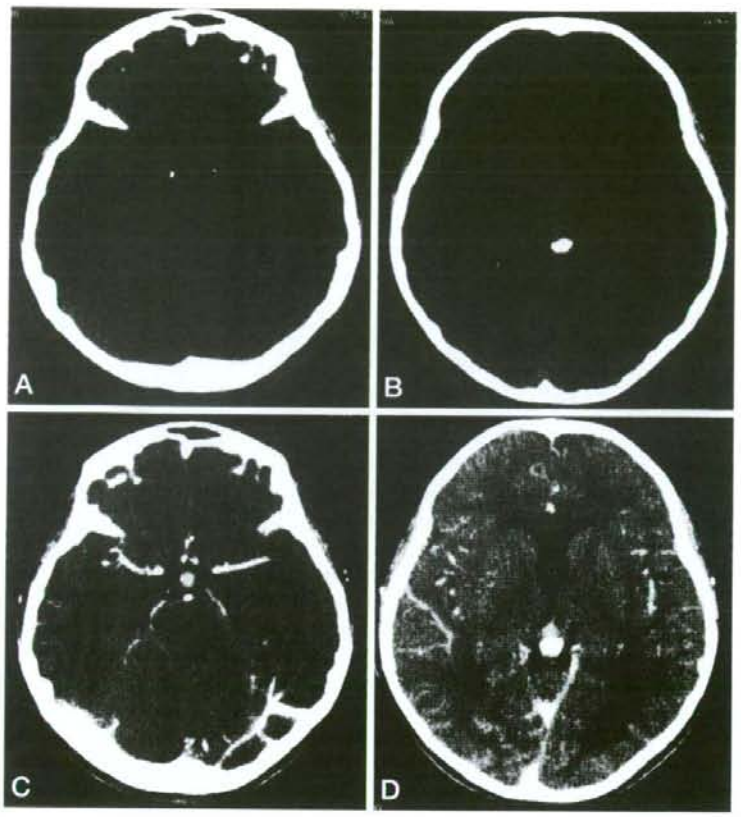

Fig. 1 Plain (**A**, **B**) and enhanced (**C**, **D**) CT findings: Plain CT demonstrates isodense masses in the suprasellar and pineal regions, and enhanced CT shows homogenous enhancement of these masses.

瘍マーカーの上昇は認めなかった．また髄液細胞診では異常細胞は認めなかった．

神経放射線学的所見　前医で2006年6月13日に施行された頭部CTでは鞍上部に8×8 mm大，松果体部に20×20 mm大の腫瘍性病変を認め，松果体部の病変は10 mm大の石灰化を伴っていた．造影CTでは鞍上部，松果体部の病変はいずれも均一に造影された（**Fig. 1**）．6月14日に施行された頭部MRIでこの病変は，T1強調画像，T2強調画像ともにiso intensity，Gd-DTPAでは充実性部分は均一増強された（**Fig. 2**）．

入院後経過　6月14日に前医にて施行されたMRIでは松果体部病変は22×15 mm，鞍上部病変は13×8 mmであったが，当科にて施行したMRIでは18×15 mm（6/26），10×8 mm（7/3），10×7 mm（7/14）と病変の縮小を認めた．同様に鞍上部病変は10×8 mm（6/26），10×8 mm（7/3），10×7 mm（7/14）と病変の縮小を認めた（**Fig. 3**）．この時点で腫瘍性病変は退縮傾向を示していたため，外来にて2週間ごとのMRIフォロー予定とし一時退院となった．

退院後8月1日のMRIで松果体部・鞍上部の病変ともに再増大を認め入院となった（**Fig. 3**）．

手　術　2006年8月30日，経側脳室経由に神経内視鏡下に松果体部腫瘍の生検術を施行した．腫瘍は鞍上部，松果体部ともに色調は灰白色であり，可視範囲内では脳室内への播種を疑わせる病変は認められなかった．

病理学的所見　クロマチンに富む大型の円形核と淡い高酸性細胞質を持つ細胞が増殖し，細胞は胎盤性アルカリホスファターゼ陽性であった．間質にリンパ球の浸潤が認められ，two cell patternを呈していた（**Fig. 4**）．以上よりpure germinomaと診断した．

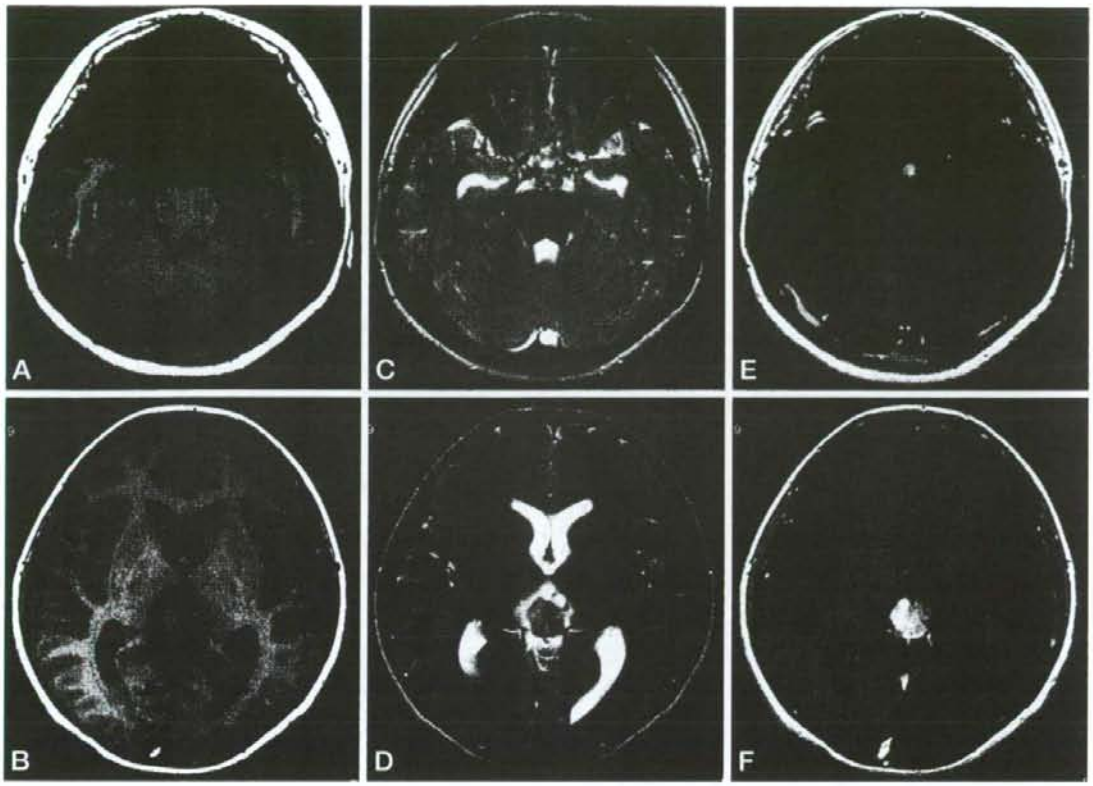

Fig. 2 Preoperative MRI findings: Suprasellar and pineal region tumors were isointense on T1-weighted images (**A**, **B**) and T2-weighted images (**C**, **D**). Gadolinium-enhanced MRI demonstrated homogeneous enhancement (**E**, **F**).

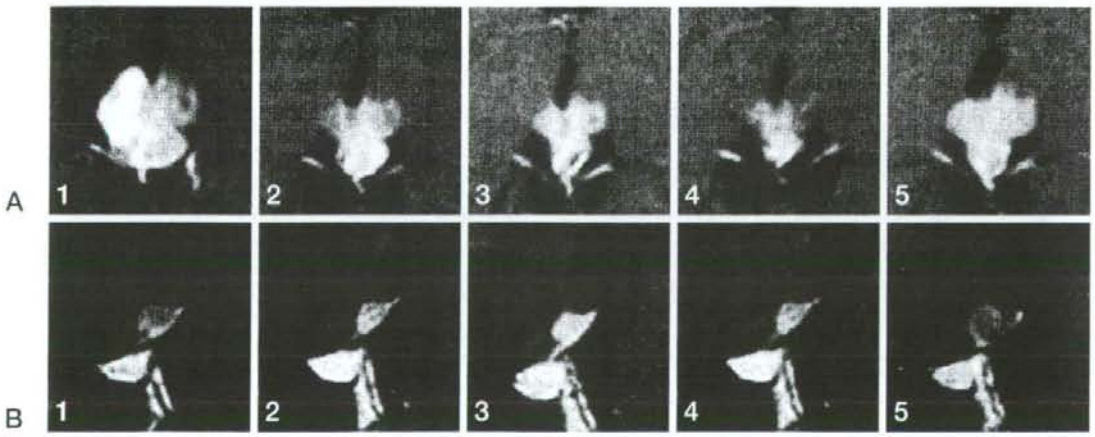

Fig. 3 Serial gadolinium-enhanced MR images obtained on June 13 (**1**), June 26 (**2**), July 3 (**3**), July 14 (**4**), and August 1 (**5**). The pineal (**A**: axial) and suprasellar tumors (**B**: sagittal) continued to decrease in size until July 14 and subsequently began to enlarge.

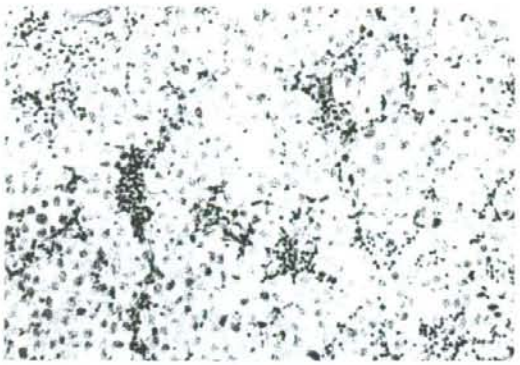

Fig. 4 Histology of biopsy specimen showing polygonal cells with large nuclei and minimal lymphocytic infiltration (hematoxylin & eosin stain, ×200).

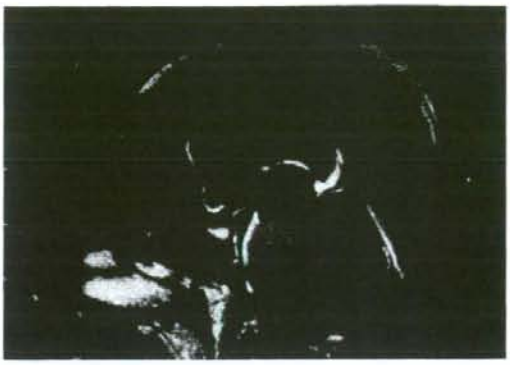

Fig. 5 Gadolinium-enhanced MRI after radio-chemotherapy. Complete response was achieved.

治療経過 術後新たな神経脱落症状は認めなかった．Germinoma に対して ICE 3 コース（IFOM 1,300 mg，VP-16 85 mg，CDDP 25 mg×5 日間）および拡大局所照射 24 Gy を施行した．初期治療終了時，鞍上部および松果体部の腫瘍ともに完全に消失した．初期治療終了後 1 年 9 カ月経過したが，腫瘍の再発は認めていない（**Fig. 5**）．

III. 考　察

自然退縮を来す頭蓋内腫瘍は稀であり，悪性リンパ腫[12,13]，neurofibromatosis に関連した神経膠腫[2,8,14]，腎癌からの転移性脳腫瘍[11]等の報告がある．頭蓋内 germ cell tumor の自然退縮例はわれわれが渉猟した限りでは本症例を含め 4 例の報告を認めるのみであった[5,6,10]（**Table**）．

一般に悪性腫瘍が自然退縮を引き起こす要因としては，免疫状態の変化，ホルモン状態の変化，手術侵襲，感染，発熱などの種々の誘因が推測されている．

ステロイドの投与は免疫状態を変化させるため，結果として腫瘍の縮小を引き起こすと考えられている．頭蓋内リンパ腫はステロイドにより退縮を来す腫瘍であるが，頭蓋内 germ cell tumor に対するステロイドの影響は不明である．今回渉猟し得た 4 例においてステロイドの投与を受けている症例は 2 例のみであった．また，Matsutani ら[9]の 153 例の頭蓋内 germ cell tumor の報告でも多くの症例でステロイド投与を受けているが，自然退縮を来した症例は認めてはいない．

手術侵襲も腫瘍を退縮させる誘因の 1 つと考えられている．Everson ら[4]の報告では，自然退縮を呈した悪性腫瘍 176 例中 71 例（約 40%）で何らかの手術が行われていた．自然退縮を来した germ cell tumor の 4 例中 3 例で手術が行われていた．2 例は合併した水頭症に対する脳室―腹腔シャント術であり，1 例は松果体部と第 4 脳室に腫瘍を認めた症例で，第 4 脳室腫瘍摘出術が行われている．しかしながら，本症例では手術は施行しておらず，これまでの症例と異なり手術による影響はないと考えられた．

また，頭蓋内 germ cell tumor は放射線への感受性が高い腫瘍であり，術前の放射線被曝により退縮を来した可能性も示唆される．これまでに 10 Gy の照射で消失した症例も報告されているが[1]，被曝量は施設により差異はあるものの CT ではおおよそ 0.02 ～ 0.04 Gy，頭部単純 X 線ではおおよそ 0.001 Gy であり，このような極少量の放射線が germ cell tumor の退縮に影響を引き起こすかは不明である．

今回のわれわれの症例では腫瘍退縮前にステロイド投与や手術は施行しておらず，また CT 検査も 1 回しか行われていない．ヒトの自然退縮した腫瘍において組織学的検討を行っている報告は少

Table Summary of cases demonstrating spontaneous regression of intracranial germinoma.

Author	Age/ Sex	Symptoms	Lesion	Pathology	Size	Operation	Steroid	An involution period of tumor
Ide, et al. [6]	21/M	polydipsia, polyuria	neurohypophysis	germinoma	large	VP shunt	+	2 months
Fujimaki, et al. [5]	39/M	consciousness disturbance	pineal, IV ventricle	germinoma	large	tumor removal	+	not observed
Murai, et al. [10]	17/M	headache	pineal	pure germinoma	30 mm	VP shunt	none	not observed
Present case	13/M	polyuria, headache	neurohypophysis pineal	pure germinoma	13 mm 22 mm	none	none	3 weeks

なく，Kitanaka ら[7]は自然退縮した neuroblastoma の組織においてネクローシスともアポトーシスとも形態的に異なる細胞死（autophagy）が生じていることを報告している．現在のところ，腫瘍の自然退縮においてどのようなメカニズムでどのような細胞死が生じているかについては不明であり，今後のさらなる研究が期待される．

自然退縮後の経過であるが，4 例中 2 例で腫瘍退縮後に経過観察を行っているが，縮小期間は Ide ら[6]の報告では 2 カ月，本症例では 3 週間と比較的早期に再増大を来し治療を行っている．症例数は少ないものの，germ cell tumor は自然退縮を呈したとしても消失には至らず再増大を来しており，経過観察する際には厳重なフォローが必要であると考えられた．また生検が可能な大きさであるならば，早期に病理診断を確定させることも重要ではないかと考えられた．

IV. 結　語

自然退縮を呈した鞍上部，松果体部 germinoma の稀な 1 例を報告した．自然退縮を来す頭蓋内病変として germ cell tumor の可能性を念頭におく必要がある．また，退縮後も比較的早期に再増大を来すため厳重なフォローが必要であると考えられた．

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書評

姫井　昭男●著

『精神科の薬がわかる本』

書評者
長嶺　敬彦（清和会吉南病院内科部長）

このたび医学書院から姫井昭男先生が書かれた『精神科の薬がわかる本』が出版された．これは精神科で使われている全領域の薬の使い方，作用，副作用，禁忌，患者さんへの説明の仕方がざっと理解できるお薦めの本である．解説されているのは「抗うつ薬」「睡眠薬」「抗精神病薬」「抗てんかん薬」「老年期に使う薬」「気分安定薬」「抗躁薬」「抗不安薬」「抗酒薬」「悪性疾候群の治療薬」「発達障害をもつ人への薬物療法」と，ざっと11領域にわたる．コンパクトながら，精神科の薬が“ざっと”理解できる．このような本を望んでいた人は多いのではないだろうか．

向精神薬に関する本は，一般的に言って非常に難解である．脳のさまざまな受容体や神経回路がひと通り頭に入っていることを前提に書かれているからである．もっと簡便で，それでいて臨床の場で使える向精神薬の薬理の本はないものかと思っていたところ，姫井先生のこの本を見つけた．タイトル通り，向精神薬が「わかる」本である．

向精神薬の薬理は複雑で，簡単な模式図で示すことが困難である．その上，抗うつ薬，睡眠薬，抗精神病薬，抗てんかん薬，認知症の薬，気分安定薬と，種類も多い．それらを網羅し，その作用と臨床的意義を簡便に記述することは不可能に近い．しかしこの本を一読すると，難解な向精神薬の薬理が身近に感じられる．それはこの本が，理論の押し売りをしていないこと，それから表面的で辞書的な記述を避け，現実的な記述を用いているためと思われる．ここに著者の，「薬理は難しいからと毛嫌いするのではなく，臨床現場での向精神薬の振る舞いを知ってほしい」という真摯な願いが感じられる．

大多数の精神薬理の本は，薬理学的理論を重視するあまり，向精神薬の一面をデフォルメしてしまう危険性がある．それは向精神薬を分厚く化粧し，見せかけの美人に仕立てるようなものである．だから読んでいても実感が湧かない．私は，優れた向精神薬は過剰に化粧をしなくても美しいと思っている．だから向精神薬は，多くの患者さんに福音をもたらしているのである．著者もおそらく同じ意見ではなかろうか．

向精神薬はけっして「魔法の薬」ではない．言い換えれば，非のうちどころがない「絶世の美女」ではない．向精神薬の欠点を理解し，それでもなおかつ素顔の向精神薬は美しいと感じるからこそ，著者は難解な薬理がわかりやすく書けるのだと思う．

この本を紹介するのに「臨床での向精神薬の振る舞いを，著者の膨大な薬理学的知識を背景に解説した本である」と簡単に記すこともできる．しかしそれではこの本の本当のよさは伝わらない．この本で著者は，「素顔の向精神薬の美しさ」を表現しようとしたのではなかろうか．私にはそう思えるのである．

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ORIGINAL ARTICLE

A combination of IFN-β and temozolomide in human glioma xenograft models: implication of p53-mediated MGMT downregulation

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Abstract
Purpose Methylation of the O^6-methyguanine-DNA methyltransferase (MGMT) gene promoter in gliomas has been reported to be a useful predictor of the responsiveness to temozolomide (TMZ). In our previous experiments, we observed that IFN-β sensitized TMZ-resistant glioma cells with the unmethylated MGMT promoter and that the mechanism of action was possibly due to attenuation of MGMT expression via induction of TP53. In this study, (1) we explored the synergistic effect of IFN-β and TMZ in the animal model, and (2) clarified the role of IFN-β induced TP53 in the human MGMT promoter.
Methods (1) Nude mice with either subcutaneous T98 (TMZ-resistant) or U251SP (TMZ-sensitive) tumor were treated with IFN-β/TMZ for 5 consecutive days. (2) The MGMT promoter activity was assayed by a luciferase reporter system in Saos2 (p53-null) cells transduced with a p53-adenoviral vector, and T98 glioma cells treated with IFN-β.
Results (1) A combination of IFN-β/TMZ had significant synergistic antitumor activity on the growth of both T98 and U251SP tumors. (2) MGMT promoter activity was suppressed by either adenovirally transduced p53 or IFN-β.
Conclusions It would be appealing to consider a prospective clinical trial in which genetic markers are used for personalized drug selection, eliciting other forms of treatment or inhibition of MGMT for those with MGMT expression. In this context, IFN-β inactivates MGMT via p53 gene induction and enhances the therapeutic efficacy to TMZ.

Keywords Glioma · Temozolomide · IFN-beta · MGMT · Methylation · P53

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Introduction

High-grade (WHO grades III and IV) gliomas consisting of anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), anaplastic oligoastrocytoma (AOA), and glioblastoma multiforme (GBM) often resist treatment; GBM, the most common glioma in adults, kills patients within a median time span of a year after diagnosis, even when aggressive surgical resection, chemotherapy, and radiotherapy are carried out. This dismal outcome has not been improved substantially over the last three decades. Temozolomide (TMZ) is a well-tolerated orally bioactive alkylating agent used in glioma patients. It has been adopted as the first-line treatment in patients with high-grade gliomas with the expectation that it might improve poor prognosis [2, 4, 11, 25–27]. TMZ induces the O^6 methyl-guanine (MG) lesion. Subsequently, during DNA synthesis, thymine is mispaired to the O^6 MG by DNA polymerase, where aberrant mismatch repair (MMR) processing of O^6-MG:T mispair generates DNA single-strand gaps and/or double-strand breaks, which leads to cell killing [5, 20]. A cellular DNA repair protein—the O^6-methylguanine-DNA methyltransferase (MGMT) protein—repairs the alkylation at the O^6 position of guanine [15, 21]. A number of studies have suggested that *MGMT* deficiency of brain tumors is closely related to the sensitivity to alkylating agents [1, 13, 16]. Furthermore, since MGMT protein loss may be a result

of promoter hypermethylation, methylation of the MGMT promoter in gliomas has been reported to be a useful predictor of their responsiveness to alkylating agents [4, 12]. Thus, MGMT promoter methylation may enable the selection of patients that are most likely to benefit from TMZ treatment.

For patients with gliomas in which the promoter is not methylated, alternative treatments involving a different mechanism of action or methods that inhibit *MGMT* should be developed. One such inhibitor is interferon-β (IFN-β), and it is being investigated for this purpose [18]. IFN-β, one of the type I IFNs, in its role as a drug sensitizer, has been widely used in combination with other antitumor agents such as nitrosoureas. In our previous experiments, we found that IFN-β sensitized TMZ-resistant glioma cells containing an unmethylated MGMT promoter [18]. With regard to the mechanism of action, the sensitizing effect of IFN-β was possibly due to the attenuation of MGMT expression via induction of TP53, which binds to the MGMT promoter, as determined by the ChIP assay. In this study, we further explore the synergistic effect of IFN-β and TMZ on nude mice with subcutaneously transplanted human gliomas. Here, we report that a combination of IFN-β and TMZ decreased tumor growth to a very significant extent, and complete regression was observed in 20% of the treated animals.

The mechanism of MGMT inactivation caused by IFN-β-induced p53 upregulation in glioma cells is not completely understood. The possibility that MGMT may be regulated by p53 has received much attention. Briefly, studies in murine cells have indicated that MGMT can be induced by ionizing radiation in a wild-type *p53* gene-dependent manner [6, 22], and p53 may upregulate the basal expression of MGMT [19]. On the contrary, the findings in human cells have been inconsistent with p53 being a negative regulator of MGMT expression [7, 9]. A clear explanation for the specific role of IFN-β inducible p53 in *MGMT* gene expression in human glioma cells is critical. In this context, we have examined the role of TP53 in the human MGMT promoter in a luciferase reporter plasmid. We have demonstrated that MGMT promoter activity in a reporter gene system was suppressed by adenoviral vector-mediated wild-type p53 expression in Saos-2, a p53-null cell line, or by IFN-β mediated p53 overexpression in a glioma cell line, i.e., T98.

Materials and methods

Cell lines and reagents

We used two human glioma cell lines (T98 and U251SP), which were determined to be TMZ-resistant and TMZ-sensitive, respectively, in a previous study [18]. These cell lines were obtained from the American Tissue Culture Collection. Human IFN-β (Toray Co., Ltd., Kamakura, Japan) and TMZ (the Schering-Plough Research Institute, Kenilworth, NJ, USA) were resolved in phosphate-buffered saline (PBS) and dimethyl sulfoxide, respectively.

Animal experiments

Balb/c nude mice (female and 5–6 weeks old) bearing T98 or U251SP tumors were randomized to separate them into six (T98) or four (U251SP) groups (five to six animals per group) and treated when the subcutaneous tumors had reached a volume between 200 and 400 mm^3. IFN-β(2×10^5 IU/animal) was administered i.p. 6 h before an i.p. injection of TMZ (50 or 100 mg/kg). Control mice, or mice receiving IFN-β or TMZ alone, also received the corresponding vehicle. Treatments were repeated at 24-h intervals for a total of five doses. Tumor length (a) and width (b) were measured in situ with digital calipers at 7-day intervals, and tumor volumes were calculated according to the following formula: $V\ (mm^3) = a \times b^2/2$. The statistical significance between treated and control tumors was evaluated by a one-tailed Mann–Whitney test.

MGMT expression in tumors after treatment with IFN-β

Mice bearing T98 tumors of volume 200–400 mm^3 were randomized; divided into groups of three; and treated as described with PBS, TMZ (50 mg/kg) alone, and a combination of TMZ (50 mg/kg) and IFN-β (2×10^5 IU/animal). Treatments were repeated at 24-h intervals for a total of five doses. Seven days after the initial treatment, total RNA was immediately isolated from the removed tumor samples by using a standard Trizol preparation protocol (Invitrogen, Carlsbad, CA, USA). To investigate MGMT mRNA expression, semiquantitative RT-PCR was performed using the Superscript First-Strand Synthesis System for RT-PCR (Invitrogen), as previously described [18]. β-Actin-specific PCR products from the same RNA samples were amplified and used as internal controls.

Construction of a reporter plasmid

The 955-bp DNA fragment containing the human MGMT promoter was amplified from T98 genomic DNA by PCR amplification using the forward primer with the *Mlu*I site, 5′-c*gacgcg*tatcctgctccctctgaaggctc-3′ and the reverse primer with the *Bgl*II site, 5′-ga*agatct*ggacctgagaaaagcaagagag-3′.

The fragment was then subcloned into the pGL3-luciferase enhancer vector (Promega, Madison, WI, USA) through the *Mlu*I and *Bgl*II sites to generate the luciferase expression plasmid under the human MGMT promoter, i.e., p-952/+3 MGMT LUC. The corresponding construct was amplified with the primer sets RV primer3 (5′-ctagcaaaataggctgtccc-3′) and GL primer2 (5′-ctttatgtttttggcgtcttcc-3′) and sequenced using the BigDye Terminator Sequencing Kit (Applied Biosystems) in order to confirm the vector construct.

Assay for transiently expressed reporter gene

In the preliminary experiment, Saos2 (p53-null) osteosarcoma cells were plated in a 35-mm dish at a density of 2×10^5 cells/well. At 24 h, they were infected with the recombinant wild-type p53 or lacZ adenoviral vector under the control of the cytomegalovirus promoter (a gift from Dr. Hamada, Hokkaido, Japan) at multiplicity of infection (MOI) = 1. The MOI was necessary to infect 100% of the cells, and TP53 was successfully transduced in the cells, as determined by western blotting analysis. Cell lysis and western blotting were carried out as described previously [18]. Antibodies against the following proteins were purchased: p53 (DO-1: Santa Cruz Biotechnology, Santa Cruz, CA, USA), and β-actin (AC-15: Sigma–Aldrich, St. Louis, MO, USA). In this study, Saos2 and T98 glioma cells were plated in a 35-mm dish at a density of 2×10^5 cells/well, respectively. At 24 h, the Saos2 cells were infected with the p53 adenoviral vector at MOI = 1, and the T98 cells were treated with 1,000 IU/ml of IFN-β. After an additional 24 h, Saos2 and T98 cells were transfected with the p-952/+3 MGMT LUC plasmid (1 μg) along with the β-galactosidase expression plasmid (0.5 μg) as the internal control; transfection was carried out by using FuGENE6 (Roche, Indianapolis, IN, USA) and Lipofectamine (Invitrogen), respectively. The cells were harvested 48 h after plasmid cotransfection. Luciferase and β-galactosidase activity were measured using kits from Promega and the protocols recommended by the manufacturer. Luciferase activity was normalized based on the β-galactosidase activity to correct for variations in tranfection efficiency. The assay was performed in triplicates.

Results

A combination of TMZ and IFN-β reduced the growth of the human glioma xenografts

As shown in Fig. 1, IFN-β alone did not decrease the growth of the T98 tumor significantly (Fig. 1, filled diamond). TMZ (100 mg/kg) alone suppressed T98 tumor growth (filled square, $P \leq 0.05$) but increased the associated body weight loss (Fig. 2); it produced toxicity-related deaths in two of seven animals. In contrast, a combination of IFN-β and 50 mg/kg TMZ decreased T98 tumor growth to a very significant extent (triangle, $P \leq 0.05$), and complete regression was observed in one of five animals (20%). A combination of IFN-β and 100 mg/kg TMZ reached more statistical significance (filled triangle, $P \leq 0.01$), however increased the body-weight loss. Semiquantitative RT-PCR demonstrated that MGMT expression decreased substantially in the T98 tumor 7 days after the combined treatment with TMZ and IFN-β (Fig. 3).

Similarly, for the U251SP (TMZ-sensitive) tumor, while a TMZ dose of 50 mg/kg alone reduced tumor growth, IFN-β preadministration marginally enhanced tumor growth delay ($P \leq 0.05$) (Fig. 4).

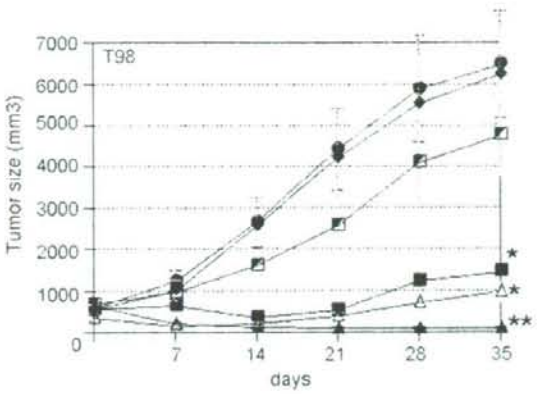

Fig. 1 Growth inhibition of T98 xenografts treated with IFN-$\beta \pm$ TMZ. BALB/c nude mice (female, 5–6 weeks old) bearing T98 were separated into six treatment groups; *filled circle* vehicle; *filled diamond* IFN-β (2×10^5 IU); *half filled square* TMZ (50 mg/kg); *filled square* TMZ (100 mg/kg); *triangle* IFN-β (2×10^5 IU) +TMZ (50 mg/kg); *filled triangle* IFN-β (2×10^5 IU) +TMZ (100 mg/kg). IFN-β was administered i.p. 6 h before an i.p. injection of TMZ. Control mice, or mice receiving IFN-β or TMZ alone, also received the corresponding vehicle. Treatments were repeated at 24-h intervals for a total of five doses. Tumor length (**a**) and width (**b**) were measured in situ with digital calipers at 7-day intervals, and tumor volumes were calculated according to the following formula: V (mm^3) = $a \times b^2/2$. Points represent mean values ± SE. The statistical significance between treated and control tumors was evaluated by a one-tailed Mann–Whitney test. *$P < 0.05$ compared with vehicle; **$P < 0.01$ compared with vehicle

IFN-β and TP53 expression inhibit MGMT promoter function

Saos-2 cells were used in this study because they are p53-null and therefore contain no endogenous p53 protein, wild type, or mutant, which might influence the transfection

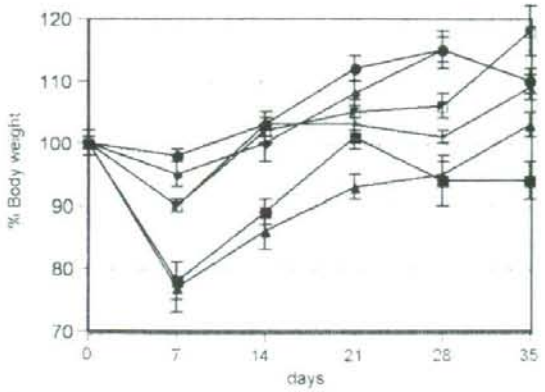

Fig. 2 Body weight of mice receiving IFN-β ± TMZ. The mean body weight of six treatment groups; *filled circle* vehicle; *filled diamond* IFN-β (2 × 105 IU); *half filled square* TMZ (50 mg/kg); *filled square* TMZ (100 mg/kg); *triangle* IFN-β (2 × 10^5 IU) +TMZ (50 mg/kg); *filled triangle* IFN-β (2 × 10^5 IU) +TMZ (100 mg/kg), was measured and expressed as a percentage of the day 0 untreated weight. The mice receiving TMZ (100 mg/kg) (*filled square* and *filled triangle*) had the associated body weight loss; toxicity-related deaths were found in four of 14 animals. Points represent mean values ± SE

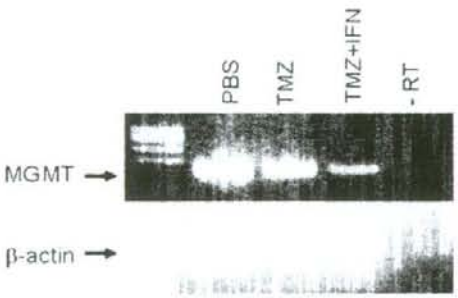

Fig. 3 Semi-quantitative RT-PCR for MGMT expression in T98 tumor. Mice bearing T98 tumors were treated with either PBS, TMZ (50 mg/kg) alone, or a combination of TMZ (50 mg/kg) and IFN-β (2 × 10^5 IU/animal). Treatments were repeated at 24-h intervals for a total of five doses. To investigate MGMT mRNA expression, 7 days after the initial treatment, semiquantitative RT-PCR was performed. β-Actin-specific PCR products from the same RNA samples were amplified and used as internal controls

result. To demonstrate that overexpression of wild-type p53 could affect MGMT promoter function, Saos-2 cells were transduced with a wild-type p53 adenovirus at MOI = 1. Overexpression of p53 was obtained 24 h after adenovirus-mediated transduction of p53 gene into Saos-2 cells (Fig. 5a, lower panel). Then, cells were transiently transfected with p-952/+3 MGMT LUC together with a control plasmid pSVβ-gal. Luciferase activity was normalized based on β-galactosidase activity to correct different transfection efficiencies. In the presence of wild-type p53, the

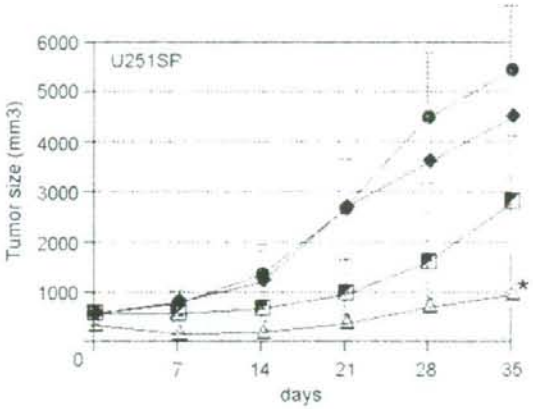

Fig. 4 Growth inhibition of U251SP xenografts treated with IFN-β ± TMZ. BALB/c nude mice (female, 5–6 weeks old) bearing U251SP were separated into four treatment groups; *filled circle* vehicle; *filled diamond* IFN-β (2 × 105 IU); *half filled square* TMZ (50 mg/kg); *triangle* IFN-β (2 × 10^5 IU) +TMZ (50 mg/kg). IFN-β was administered i.p. 6 h before an i.p. injection of TMZ. Control mice, or mice receiving IFN-β or TMZ alone, also received the corresponding vehicle. Treatments were repeated at 24-h intervals for a total of five doses. Points represent mean values ± SE. The statistical significance between treated and control tumors was evaluated by a one-tailed Mann–Whitney test. *$P < 0.05$ compared with vehicle

MGMT promoter produced only 5% of the luciferase activity generated in the absence of p53 expression (Fig. 5a, upper panel).

IFN-β upregulated p53 expression in T98 glioma cells (Fig. 5b, lower panel). Previously, we have demonstrated that knockdown of p53 by siRNA increased MGMT expression in T98 cells, and the ChIP assay revealed that the MGMT promoter coprecipitated with p53 [18]. We analyzed whether the p53 induction caused by IFN-β curtailed the MGMT promoter function in T98 cells. T98 cells were treated with IFN-β and after 24 h, the cells were transfected with p-952/+3 MGMT LUC. Luciferase activity in T98 cells transfected with p-952/+3 MGMT LUC was dramatically decreased in the presence of IFN-β (Fig. 5b, upper panel).

Discussion

Previous studies have demonstrated that epigenetic silencing of the *MGMT* DNA repair gene by promoter methylation compromised DNA repair, and this was shown to be associated with longer survival in glioblastoma patients who received TMZ chemotherapy [4, 12]. The methylation status of the MGMT promoter may have prognostic value. Additionally, it may be a clinically relevant predictor of the beneficial effects of TMZ chemotherapy. Thus, MGMT

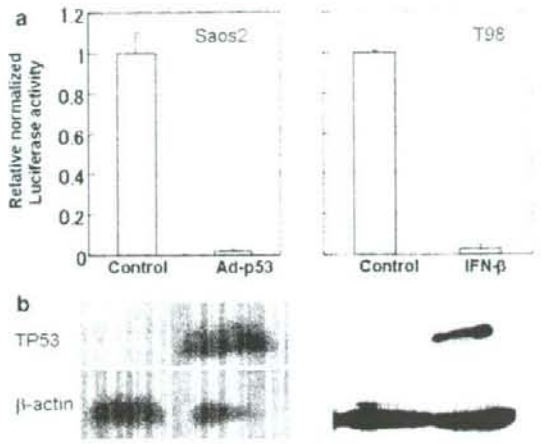

Fig. 5 Relative MGMT promoter luciferase activity. Saos2 (**a**) and T98 glioma (**b**) cells were plated in a 35-mm dish at a density of 2×10^5 cells/well, respectively. At 24 h, the Saos2 cells were infected with the p53 adenoviral vector at MOI = 1, and the T98 cells were treated with 1,000 IU/ml of IFN-β. Overexpression of TP53 was confirmed by immunoblotting, and β-actin was also immunoblotted as a loading control (*lower panels*). After an additional 24 h, Saos2 and T98 cells were transfected with the MGMT promoter-luciferase construct (1 μg) along with the β-galactosidase expression plasmid (0.5 μg) as the internal control. The cells were harvested 48 h after plasmid co-transfection. Luciferase activity was normalized based on the β-galactosidase activity to correct for variations in tranfection efficiency. The assay was performed in triplicates

promoter methylation may enable the selection of patients that are most likely to benefit from TMZ treatment.

Molecular studies have shown that specific tumor characteristics such as the presence of a silenced *MGMT* gene may allow the treatment of individual patients to be tailored. The role of MGMT in the resistance to alkylating chemotherapy has been shown; therefore, in several studies, an MGMT-depleting agent was added in order to increase efficacy. For this purpose, both drugs with intrinsic cytotoxic activity as well as those that deplete MGMT were used. O^6-Benzylguanine (O^6-BG) was developed based on its restricted mechanism of action [3]. O^6-BG reacts with MGMT by covalent transfer of the benzyl group to the active site cysteine and thereby causes irreversible inactivation of the enzyme. The first results of this strategy showed that adding O^6-BG to carmustine did not induce regression in carmustine-resistant gliomas, although a similar trial in TMZ-pretreated patients showed some evidence that addition of O^6-BG to TMZ was beneficial. At therapeutic levels, O^6-BG alone is not toxic, and one might expect that it could be advantageous if MGMT depletion can be accomplished by a drug that also has antitumor activity; therefore, synergistic effects with the alkylating agent may occur. Type I IFNs, including IFN-α and IFN-β, a family of cytokines that elicits pleiotropic biological effects, are widely used either alone or in combination with other antitumor agents such as nitrosoureas in the treatment of malignant gliomas. Among the multiple functions of type I IFNs against human neoplasias, IFN-β, in particular, can act as a drug sensitizer that enhances the toxicity against a variety of neoplasias when administered in combination with nitrosoureas [10]. Consistent with the results of in vitro studies [18], the present study indicates that administration of IFN-β 6 h prior to TMZ for 5 consecutive days has a significant synergistic antitumor activity on the growth of both T98 (TMZ-resistant) and U251SP (TMZ-sensitive) tumors in a xenograft model. Intensive TMZ treatment (100 mg/kg) alone suppressed the ever-resistant T98 tumor growth but increased the associated body weight loss and produced toxicity-related deaths in two of the seven animals. In contrast, a combination of IFN-β and a 50 mg/kg dose of TMZ decreased T98 tumor growth to a very significant extent, and complete regression was observed in one of five animals (20%). Additionally, while TMZ (50 mg/kg) alone suppressed TMZ-sensitive U251SP tumor growth, the two drugs in combination enhanced antitumor activity significantly. This study suggests that prior administration of IFN-β can lead to an increase in the therapeutic index of TMZ. RT-PCR analysis revealed a decrease in MGMT expression in a tumor xenograft treated with an IFN-β/TMZ combination; this observation was consistent with the results of a previous study [18].

With regard to the mechanism of action, we have speculated that IFN-β suppresses MGMT transcription via TP53 induction. Previous studies using murine cells [6, 19, 22] and human cells [7, 9] in which different experimental approaches were used have yielded confusing results on the possibility that MGMT may be regulated by p53. Our study sought to clarify the direct and specific effects of p53 on *MGMT* gene expression in human glioma cells. Our observations clearly show that p53 downregulates the transcription of the *MGMT* gene, and may have clinical relevance. Our studies agree with the initial observations of Harris et al. and Srivenugopal et al. who showed suppression of *MGMT* gene expression in IMR human fibroblasts after infection with an adenoviral p53 construct [9] and in p53-null H1299 human lung cancer cells engineered to express p53 in a tetracycline-regulated system [24]. It appears that modest to high level induction of p53 expression by either infection with an adenoviral construct, regulated gene expression, or treatment with a drug such as IFN-β (this study) is capable of inhibiting MGMT expression. The MGMT gene promoter lacks the TATA and CAAT boxes and has 10 Sp1 transcription factor binding sites [8]. There is growing evidence that TATA-less promoters are subject to p53 repression [14], and the *MGMT* gene may belong to this category. Alternatively, a variety of mechanisms

involving protein–protein interactions between p53 and the TATA-binding protein [23], p300/CBP transcriptional coactivator/histone deacetylation machinery [17], and Sp1 transcription factor have been proposed to explain p53-mediated transcriptional repression. It is possible that p53 could operate through one or more of these mechanisms to curtail the transcription of the *MGMT* gene. Since MGMT expression occurs in approximately 70% of gliomas, the present study highlights the important role of p53 in *MGMT* gene expression.

In summary, it would be attractive to inhibit MGMT in cases with MGMT expression. In this context, IFN-β inactivates MGMT via *p53* gene induction and enhances the therapeutic efficacy to TMZ. Clinical examination of an IFN-β/TMZ combination may therefore be warranted.

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Identification of a human leukocyte antigen-A24–restricted T-cell epitope derived from interleukin-13 receptor α2 chain, a glioma-associated antigen

Laboratory investigation

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Object. The human leukocyte antigen-A24 (HLA-A24) allele is highly expressed in Asians. This allele is expressed in 60% of the Japanese population and in a significant number of people of other ethnicities. The interleukin-13 type α2 receptor (IL-13Rα2) has been shown to be a glioma-specific antigen, and is abundantly expressed in a majority of high-grade astrocytomas. In this study, the authors first investigated the suitability of IL-13Rα2 as a target antigen of malignant glioma cells, and then identified a potential HLA-A24–restricted peptide derived from IL-13Rα2.

Methods. The expression of IL-13Rα2 in glioma tissues was examined by reverse transcription–polymerase chain reaction analysis. To identify the desired epitope, the authors selected 5 candidate peptides from IL-13Rα2 that were predicted to bind to HLA-A24. The lytic activity of cytotoxic T lymphocytes (CTLs) induced by peptide-pulsed dendritic cells was analyzed against various glioma cell lines and freshly isolated human glioma cells.

Results. In a series of glioma tissues obtained in 29 patients, the authors found that > 50% of high-grade gliomas expressed IL-13Rα2. Of the 5 peptides tested, P174 (WYEGLDHAL) was found to be the most useful for the induction of HLA-A24–restricted and IL-13Rα2–specific CTLs. A CTL line induced by P174 also showed antigen-specific cytotoxicity to surgically removed glioma cells depending on their level of expression of IL-13Rα2 and HLA-A24.

Conclusions. Interleukin-13Rα2 is a glioma-specific antigen, and the immunogenic peptide P174 may contribute to a peptide-based immunotherapy against malignant glioma cells expressing HLA-A24.
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Key Words • glioma • human leukocyte antigen-A24 • interleukin-13 receptor α2 chain • T-cell epitope

Malignant gliomas remain untreatable and lethal despite the extensive application of surgical excision and adjuvant radio- and/or chemotherapy. Therefore, various immunotherapy approaches are being explored and appear promising as new therapeutic methods.[21,24] Immunotherapy for tumors in humans has been proposed based on the finding that $CD8^+$ CTLs are capable of effective recognition and destruction of tumor cells.[20] Consequently, much attention has focused on the identification and characterization of glioma-associated antigens that elicit strong and highly glioma-specific immune reactions.

Recently, IL-13Rα2 has been shown to be abundantly expressed in a majority of high-grade astrocytomas.[5–7] Interleukin-13 is an immunoregulatory cytokine that shares a variety of functions with IL-4 through an IL-13/IL-4 receptor complex, which commonly contributes to normal physiological functions;[25,26] IL-13Rα1 is a component of this receptor complex.[14] However, IL-13Rα2 does not interact with IL-4.[16] Because IL-13Rα2 is expressed in tumor cells but not in most adult somatic tissues—with the exception of the testis—it may be considered a kind of cancer/testis antigen.[4] Mintz et al.[15] reported that mice treated with IL-13–based cytotoxins showed significant regression in IL-13Rα2–expressing tumors without any obvious complications. Interleukin-13Rα2 is therefore a promising target for glioma-specific immunotherapy.

Abbreviations used in this paper: BIMAS = BioInformatics and Molecular Analysis Section; CTL = cytotoxic T lymphocyte; DC = dendritic cell; FBS = fetal bovine serum; HLA = human leukocyte antigen; IL = interleukin; IL-13Rα2 = IL-13 type α2 receptor; MHC = major histocompatibility complex; PCR = polymerase chain reaction; rh = recombinant human; rhGM-CSF = rh granulocyte–macrophage colony-stimulating factor; RT-PCR = reverse transcription–PCR.