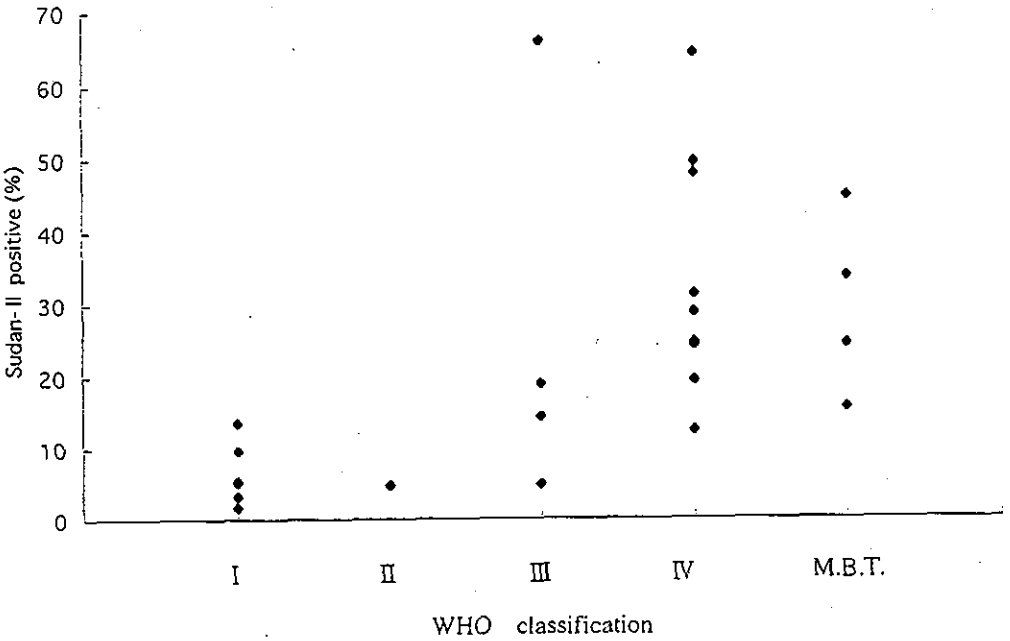

Fig. 2. The comparision of Sudan-II stain positive cells and classified WHO classification is shown.

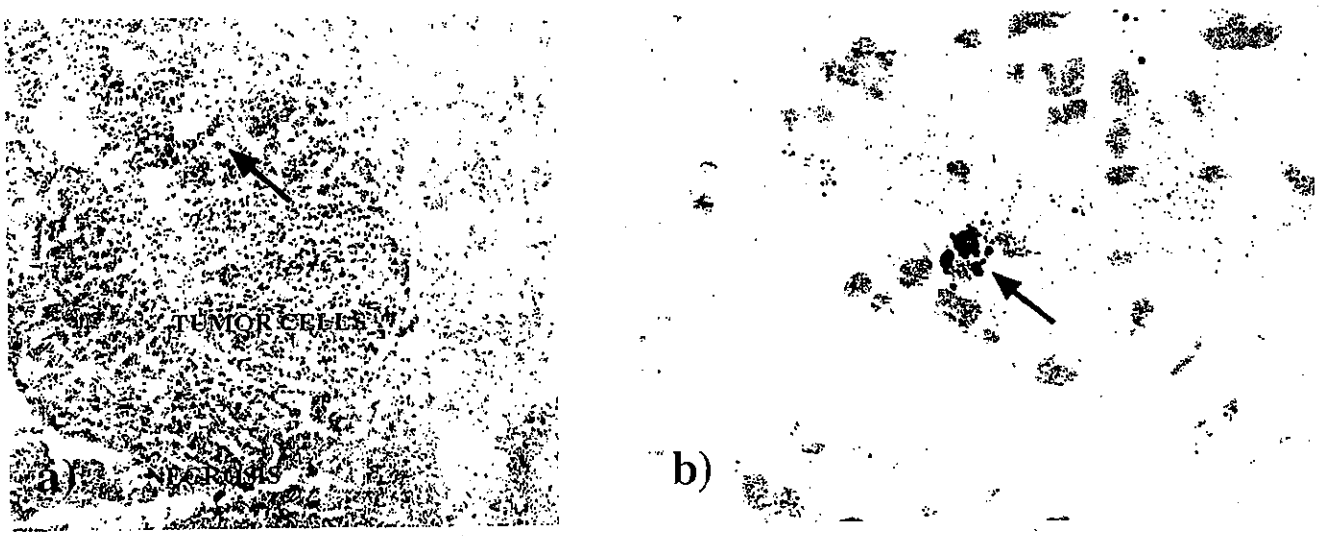

Fig. 3. a) The droplet (indicated by arrows) is shown in the necrotic tissue. (Sudan-II stain×80)
b) The lipid droplet is visible in the tumor cell (indicated by arrows). (Sudan-II stain×400)

The proliferative rate of cells close to a vessel, which are well nourished, is higher than those of tumor cells close to a necrotic area[19]. Some tumors do not become necrotic but instead form a watery cyst. ^{1}H-MRS is a means by which the growth stage and rate of tumors, along with the biological grades of the tumors can be precisely determined.

From the pathological examination at the early stage of tumor growth, it can be said that there is an increase of Cho, and a decrease of Cr and NAA. With further growth of the tumor, a low nutrient and hypoxic state occurs and Lac appears. Moreover, with growth of the tumor, Lip from lipid droplets, micronecrosis, and necrosis appears (**Fig. 7 a-c**). When the setting of voxel is completely focused on a necrotic portion, Cho, Cr, and NAA will decrease or be eliminated, and the signals will consist of only Lac and Lip, and the findings of a cyst and/or infarction will become similar (**Fig.7 c-e**).

As if supporting the relation between the bio-

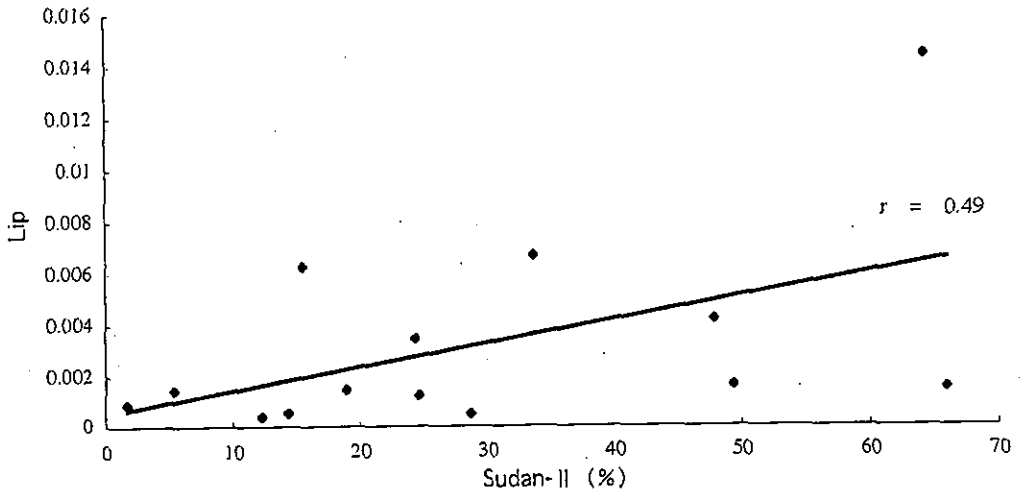

Fig. 4. The relation between lipid resonances (Lip) in ^{1}H-MRS and Sudan-II stain positive cells rate (%) is shown. The correlation coefficient is 0.49.

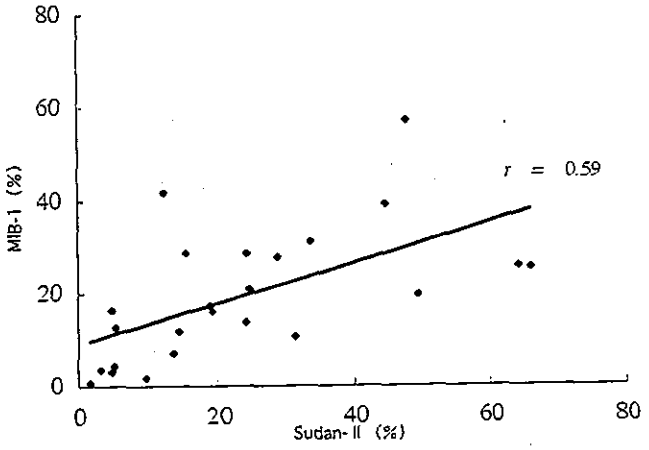

Fig. 5. The correlation between MIB-1 index (MIB-1) and Sudan-II positive cells rate (Sudan-II) is shown. The correlation coefficient is 0.59.

logical grade of malignancy and the appearance of Lip, when the cases were surveyed according to the groups classified by histological diagnosis, more than half of the cases of malignant glioma showed Lip. This concept stemmed from the fact that the glioblastoma cases with Lip (Nos. 8, 17 and 21) showed a high positive cell rate of Sudan-II staining and MIB-1 index. It is indicated that the biological activity and the proliferating capacity of tumors are higher at those areas, vascular proliferation lags behind, and many cells utilize lipid droplets as a source of energy by absorbing them.

Ganglioneuroblastoma showed a similar trend. Contrarily, there was a case (No. 20) of an anaplastic astrocytoma that showed a high Cho/Cr ratio, about three times as high as the normal value, and was suspected of having a high tumor activity from an MIB-1 index of 16.3%, but did not show Lip and only a low positive cell rate of Sudan-II staining of 4.9%. This indicated that the early stage of tumor growth when the proliferating capacity of the tumor was high, but it was not with an inadequate blood supply.

In metastatic brain tumor and malignant lymphoma, Lip was observed in a higher incidence. These are malignant tumors with high activity as indicated by the MIB-1 index. Commonly, metastatic brain tumors growth rapidly and cause central necrosis earlier, and lipid droplets also appear in malignant lymphomas. However, one case without Lip was encountered in a metastatic brain tumor. Case No. 1 was a case in which a tumor was detected at a relatively early stage, because pathological necrosis was observed in spite of a solid tumor shown on the MRI[20][21]. This was probably due to the voxel setting problem of the ^{1}H-MRS and a problem of accuracy. It was detected by the NAA because it is in the normal brain tissue.

There was no correlation between Sudan-II staining and the MIB-1 index for a meningioma. But there were 2 cases with no necrosis and no positive Sudan-II staining despite the appearance of Lip. There are the possibility that there were lipid producing cells and benign microcyst which were degenerated in this tumors. Moreover it remains difficult to identify several subtypes of meningioma with ^{1}H-MRS[22]. Conversely, in cases with necrosis

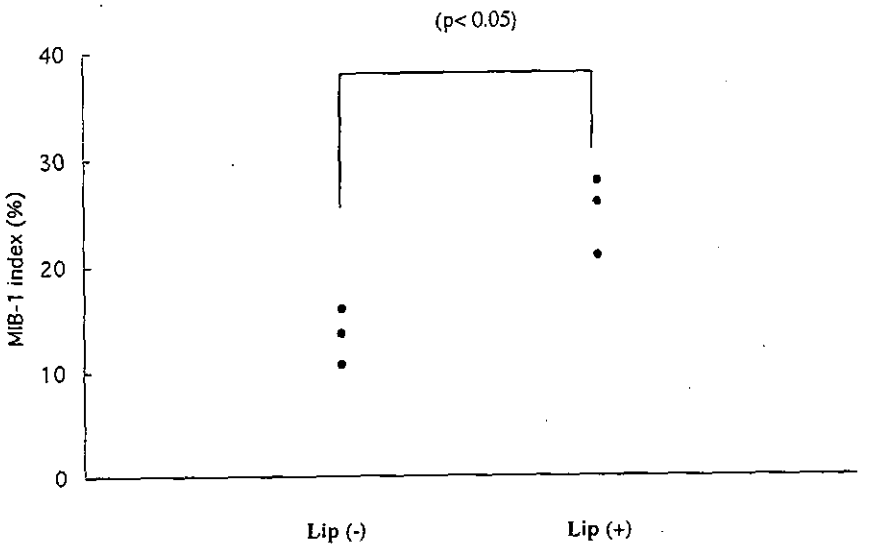

Fig. 6. The comparison between Lip resonances and MIB-1 index in Glioblastoma is shown.

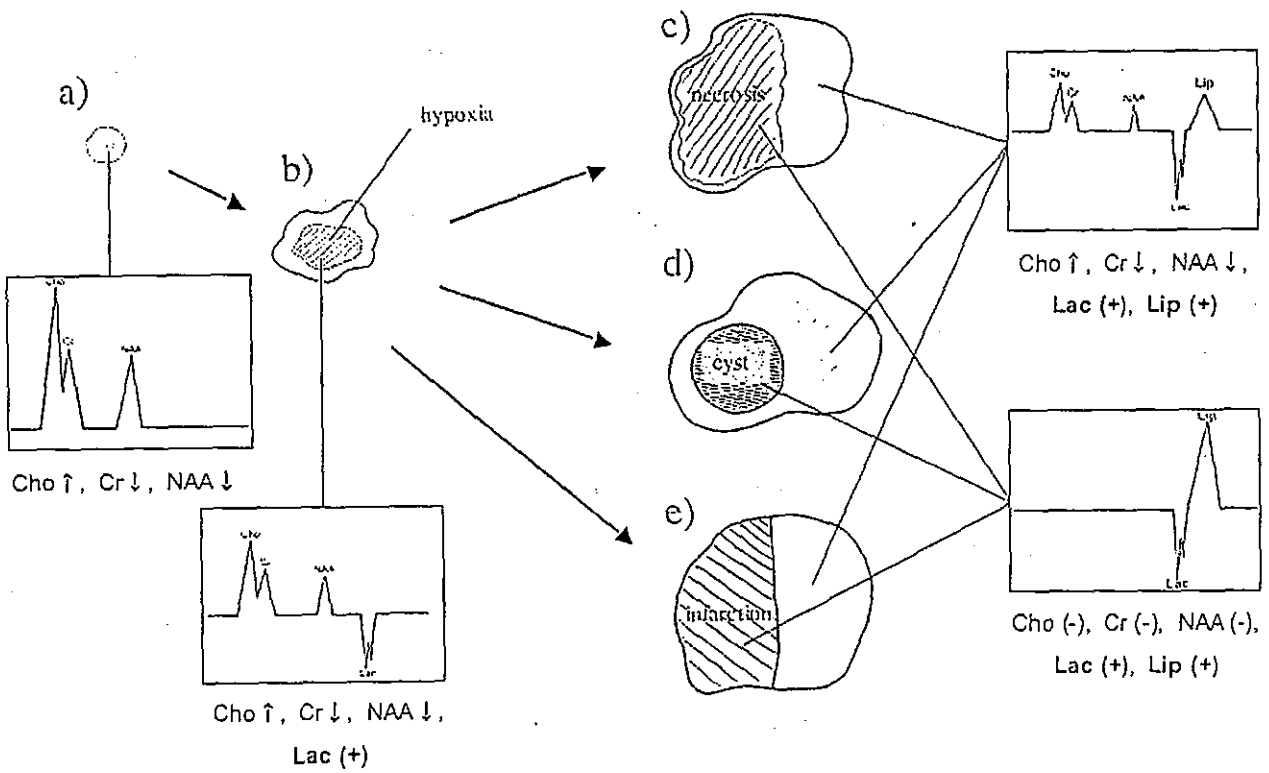

Fig. 7. The relation between process of tumor growth and ^{1}H-MRS.
The early stage of tumor growth, increase of Cho, decrease of Cr and NAA can be observed (a). When hypoxia is occurred, appearance of Lac can be observed (b). Growth proceeded further, appearance of Lip can be observed (c). Only Lac and Lip can be observed in necrotic parts, cystic parts and infarction parts (c, d, e).

without the appearance of Lip, a characteristic pattern showing only an increase in Cho was observed.

There were no cases with Lip appearance in pituitary adenoma and acoustic neurinoma. They are benign tumors according to the WHO classification, and their MIB-1 index are also low, therefore the result was as expected.

In this study, there were 5 cases (3 malignant gliomas, 1 metastatic brain tumor, and 1 meningioma) none of which showed Lip, despite the necrosis in H&E staining or a high positive cell-rate as observed with Sudan-II staining. The reason for this was that this study placed emphasis on the diagnosis, so the tests were carried out with the normal setting of TE=136 ms. A means to enhance the accuracy of Lip detection is to shorten TE to 30 ms[23)24)]. If this measure was adopted, identification might have been possible.

In ^{1}H-MRS, though there are problems such as a change of pattern, due to settings and precision, it is a useful test for differential diagnosis and evaluation of grading malignancy from the state of metabolism. Furthermore, the developmental stage and the growth rate of a tumor can be determined by its biological behavior.

Conclusion

^{1}H-MRS, though there are problems such as change of pattern due to settings and precision, is a useful test for differential diagnosis and evaluation of grading malignancy from the state of metabolism. Furthermore, in this study, the developmental stage and the growth rate of a tumor can be determined by its biological behavior. The potential effectiveness for the prognoses and determination of therapeutic effects were indicated, proving that Lip resonances in ^{1}H-MRS are important in brain tumors.

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悪性脳腫瘍における在宅療養の問題点

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坪田 由紀子[2)]、佐藤 千秋[2)]、福田 羽衣[2)]、斉藤 裕子[3)]、佐々木 千帆[3)]、

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【はじめに】

これまで悪性脳腫瘍において、患者のQOLを考慮した治療方針を立てると同時に、積極的に在宅療養支援を計画し、患者および家族の負担を軽減し安心して在宅で療養できるようにしてきた。しかし、他の悪性腫瘍患者と異なり意識障害や身体的障害を伴う脳腫瘍においては、数々の問題があると考える。

まず患者や家族においては、脳腫瘍やその随伴症状、その後の病状に関して、また介護や看護、経済的な不安がある。在宅における療養では開業医の協力は不可欠であるが、緊急時の対応の問題と、何よりも往診医が少ない現状がある。また悪性脳腫瘍の臨床的多様性から、告知に関しては議論も多い[1)]。さらに脳腫瘍の治療においても、緩和治療から緩和ケアへの過程において在宅療養は大切であり、様々な社会的・経済的側面からの支援が必要であるが、主治医や受持医の社会的資源の認識不足もあるのが現状と思われる。

そこで、最近の在宅療養の症例を通じて、提供できる社会的資源を挙げ、その問題点を検討したので報告する。

【症 例】

症例①

7歳の男児。小脳髄芽腫の再発症例である。初期治療後2年半後に再発し、脊髄への髄液播種から四肢麻痺、呼吸筋麻痺を来たしながらも、在宅における終末期療養に移行した。最終的には、臨床的脳死状態になりながらも3ヶ月間を在宅で過ごした。緊急時対応は本院ですべて受け入れ、家族の病状の受け入れが良好であった。開業医や訪問看護ステーションによる訪問看護は基本的に3回／週、ただし身体障害者手帳と重度障害者医療費助成制度の利用により自己負担なく、医療保険における訪問看護も回数制限なく行われた(終末期加算)。介護のマンパワーは両親のみであったが、必要な医療機器はすべて医療保険や社会的資源の利用で行えた。

症例②

30歳の女性。左視床から中脳に至るanaplastic astro-cytomaの症例で、術後からの右片麻痺のため身体障害者手帳を取得、在宅への移行もご家族兄弟が多かったことから良好に移行できた。また往診医との連携も良好であった。訪問看護ステーションによる訪問看護は基本的に3回／週、やはり重度障害者医療費助成制度の利用により自己負担なく、医療保険における訪問看護を回数も制限なく行われた(終末期加算)。介護のマンパワーは両親・兄弟が多く、必要な医療機器はすべて医療保険や社会的資源の利用で行えた。

症例③

30歳の女性。中脳のanaplastic astrocytomaであり、再発を来しながらも高気圧酸素療法併用化学療法にて寛解に至った症例である。著しい記銘力障害と認知障害を残し、通常の社会生活を営むことはできず、また運動麻痺がないため身障手帳の申請もできなかった。そのため、医療費助成は受けられず、介護のマンパワーもなく、在宅での療養は行えなかった。

【脳腫瘍患者における社会的資源】

実際に脳腫瘍患者が在宅で生活していく上で、家族の介護負担を軽減し、安心した在宅生活が過ごせるために、受けられる社会的資源について説明する。

①介護保険[2)]

まず介護保険は重要で知っておくべき制度である。この保険制度は社会保険方式がとらえ、給付と負担の関係が明確になっており、利用する側の自由な選択、契約により保健・医療・福祉サービスを総合的に受けられるものである。

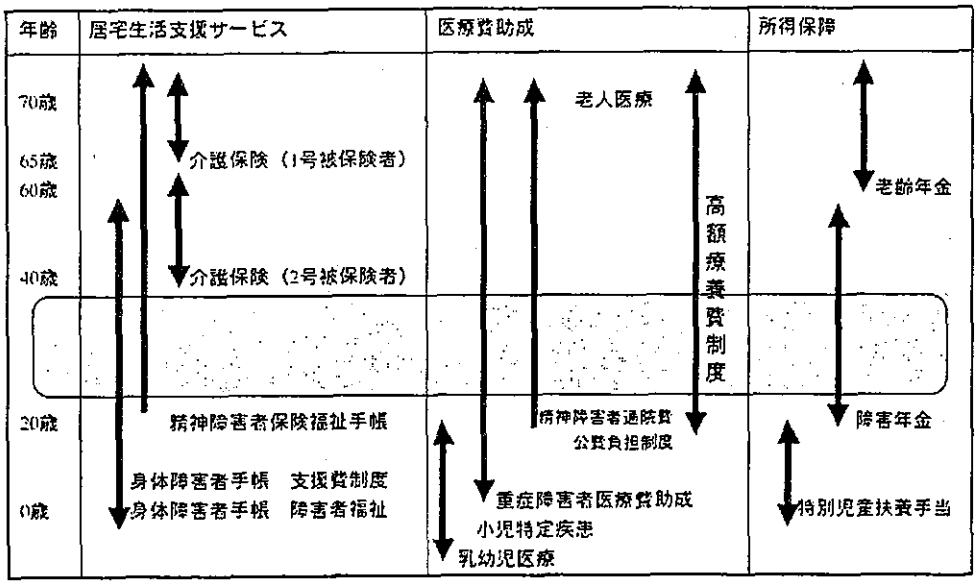
介護保険の仕組みとして、保険者は、市町村および特別区であり、それぞれが運営している。被保険者は、第1号被保険者が、市町村内に住所のある65歳以上の者であり、第2号被保険者は、市町村内に住所がある40~64歳の者が該当する。申請は、患者の生活する市町村役所窓口で行う。負担額は、第1・2号被保

特定疾病の種類

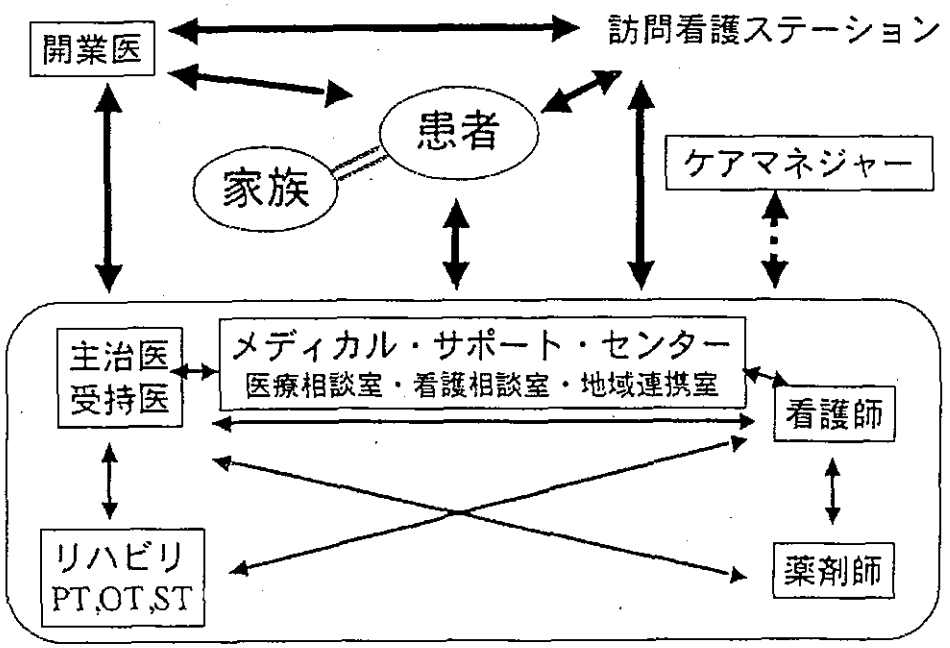
①筋萎縮性側索硬化症
②後縦靭帯骨化症
③骨折を伴う骨粗鬆症
④シャイ・ドレーガー症候群
⑤初老期における痴呆
⑥脊髄小脳変性症
⑦脊柱管狭窄症
⑧早老症
⑨糖尿病性神経障害、糖尿病性腎症および糖尿病性網膜症
⑩脳血管疾患
⑪パーキンソン病
⑫閉塞性動脈硬化症
⑬慢性関節リウマチ
⑭慢性閉塞性肺疾患
⑮両側の膝関節または股関節に著しい変形を伴う変形性関節症

⑤初老期における痴呆は、脳腫瘍による痴呆症状も該当する

脳腫瘍患者の療養支援



若年脳腫瘍における在宅療養支援の現状



険者においてはそれぞれの保険料と、サービス利用額の1割が自己負担となる。実際に保険給付を受けるには、要介護認定を受ける必要がある。なお、第2号被保険者への保険給付は、要介護状態が老化に伴う特定疾患が原因のものに限られる(表1)。

つまり脳腫瘍患者においては、器質性疾患に伴う初老期痴呆が該当すると、申請が可能となる。

居宅サービスは、利用額の1割が自己負担となる。訪問介護、訪問入浴介護、訪問看護、訪問リハビリテーション、居宅療養管理指導、福祉用具貸与だけでなく、要介護者のQOL向上や介護者の負担軽減のための通所介護、通所リハビリテーション、短期入所生活介護、短期入所療養介護などの12項目の居宅サービスがある。入浴・排泄のために直接肌に触れて使用する福祉用具を購入した際には居宅介護福祉用具購入費(限度額10万円)支給される。在宅での生活に支障がないように、手すりの取付け、段差を解消するなど住宅改修を行った場合には居宅介護住宅改修費(限度額20万円)が支給される。また介護サービス計画の作成やサービス提供機関等関係機関との連絡調整などの居宅介護支援においては利用者の負担は生じることはない。

訪問看護は、病状の急性増悪時や特別に医師が必要と指示した場合、2週間以内に限り訪問回数に制限がなくなるので、重要な点である(特別訪問看護指示書)。

②身体障害者福祉[3]

いわゆる身体障害者手帳(身障手帳)である。身障手帳を所持している人がサービスを利用することができる。ただし、まず介護保険が優先されるため、原則として介護保険対象者には給付されない。また、それぞれのサービスには対象となる障害の種類や等級が定められており、注意が必要である[4]。

身障手帳の取得により受けられるのは、訪問リハビリテーションや入浴援護事業などの在宅生活支援と、装具・座位保持装置・車イス・歩行器・歩行補助杖など20種類におよぶ補装具交付、特殊寝台や移動用リフト、吸引器など日常生活用具の給付や貸与、その他として交通費割引、税金免除、手当金支給などがある。

さらに支援費制度[5]とは、障害のある人が、その人らしく暮らせるように自分自身にあったサービスを自ら選択して、利用者の希望に添った質の高いサービスを提供することを目指して、平成15年度から導入された制度である。対象者は身体障害者手帳ないし療育手帳を取得している者である。ホームヘルプサービス、ショートステイ、デイサービス、グループホームなどの在宅サービスを利用し、利用者は利用したサービスに対して、所得に応じて自己負担があるが、市町村がかかった費用から自己負担分を差し引いた額を事業者に支払うものである。

③重度心身障害者医療費助成制度

身体障害者手帳取得者が、医療を受ける際医療費の自己負担分が助成される制度である。対象者は身体障害者手帳1・2級取得者であり、都道府県によっては3・4級取得者も対象のことがある。また場合により所得制限を設けていることもあり、注意を要する。申請は居住地における担当窓口で行ない、申請書および所得証明書が必要となる。給付内容は障害の種類に関わらず、全診療科における保険適応の医療費となり、保険適応外は給付されない。

④精神障害者保健福祉手帳[6]

精神障害者福祉にはこの手帳と通院費公費負担制度とがある。

精神障害者保険福祉手帳は、精神障害者に対して各種の援助を受けやすくし、社会復帰を促がし自立させ、さらに社会参加の促進を図ることを目的としている。対象となる精神疾患は、分裂病や躁鬱病だけでなく、器質的精神疾患も含まれる。手帳交付の対象となるのは、6ヶ月以上の精神障害の状態にあり、日常生活や社会生活に制約があるもので、その患者の状態により、障害の程度によって1級～3級に等級が決められている。1級は他者の援助がなければほとんど自分自身のこともできない状態であり、2級は必ずしも他者の助けは必要ないが日常生活は困難な状態、3級は日常生活や社会生活に制限を受けるか、制限を設けることを必要とする状態である。

申請は初診から6ヶ月を経過した時点で、必要申請書類を保健所に提出する。必要書類は保健所に用意されており、申請書と診断書であり、診断書は障害年金証明書でもよい。有効期限は2年で更新時にも同様の手続きが必要となる。

手帳の交付により優遇される処置は、各種税金の減免・控除、交通費割引、各種施設の入場料等減免・割引であるが、自治体により差があるため、予め説明しておく必要性がある。また平成14年度から施行された精神障害者へのホームヘルプ事業では、この手帳取得が条件となっている。ただし、このホームヘルプ事業は患者に触れるケアは実施できないので注意すべき点である。

⑤精神障害者通院費公費負担制度

精神保健福祉法によって規定されている病名で、当該疾患の通院にかかる医療費が一定の割合で公費負担される。割合は加入している保険により異なる。申請は保健所で行う。

⑥小児特定疾患[7]

小児において該当疾患では、入院または通院など医療費の公費負担は全額受けることが可能となる。対象者は該当疾患により入院または通院する18歳未満の児童で20歳まで延長が可能である。対象疾患と

しては、悪性新生物、慢性腎疾患、喘息、慢性心疾患、内分泌疾患、膠原病、糖尿病、先天性代謝性疾患、血液疾患、神経・筋疾患である。保険福祉センターなどで申請でき、医療費公費負担を全額受けることができる。

⑦特別児童扶養手当[8]

身体に重度および中等度の障害または長期にわたる安静を要する状態にある20歳未満のこどもを養育している場合対象となる。申請は市町村役所窓口で行ない、給付内容は、重度障害児は月額51,550円が、中等度障害児では月額34,330円が支給される。

⑧障害年金[9]

病気やけがによる障害がある場合、所得を保障する制度である。対象者は次の条件全てを満たすもので、①障害の原因となった病気・けがの初診日に、国民年金または厚生年金に加入していること、②初診日前日までの被保険者期間のうち、保険料納付済期間が3分の2以上あること(ただし平成18年まで特例措置あり)、③障害認定日において障害の程度が障害年金受給の等級基準に該当することである。ここで、障害認定日とは、病気やけがが治っていない(障害が固定していない)場合は、初診から1年6ヶ月を経過した日、もしくはその期間内に傷病が治った(障害が固定した)日を示す。

申請窓口は、区町村国民年金課や社会保険事務所であり、必要書類は、障害年金裁定請求書、診断書、申立書、戸籍謄本、住民票(写し)等である。障害年金の給付としては、基礎年金として1級が996,300円、2級が787,000円である。厚生年金は障害基礎年金に上乗せされるが、1、2級以外に3級、手当てもある。

【脳腫瘍患者における問題点】

これまで説明した入院や通院などの医療費と所得保障、在宅生活支援サービスを一覧にする(図1)。これによると20歳～40歳における在宅療養における社会資源として療養支援が不足していることがわかる。とくに小さい子供を抱え経済的に所得も決して多くないこの世代において、脳腫瘍を患い何らかの神経学的脱落症状を伴った場合、介護を行っていくにはかなり厳しい現状であることがわかる。

症例①では、小児悪性新生物であったことから小児特定疾患として扱われ、また四肢麻痺から身体障害者手帳が申請できるため、かなりの援助を受けることができた。この他にも小児では、乳幼児医療により、0歳児～1歳児では所得制限なく医療費助成が受けられる。しかし、1歳～4歳では、自治体により所得制限を設けているところがあるので、注意しておく必要がある。

症例②のように運動麻痺が主な障害である場合、身体障害者手帳を申請することで居宅生活支援サービスを受けることができる。しかし、医療費は身障手帳が1級ないし2級でなければ助成を受けることができず、また所得保障に関しては、進行性病変である脳腫瘍においては、頭蓋底腫瘍術後で再発の恐れがなければ障害が固定した時点ですぐに申請が可能であるが、悪性腫瘍であれば、障害が固定せず、治療開始から1年6ヶ月の療養期間を待たなければならず、脳腫瘍の平均生存期間を考慮すれば現実には保障がないのと同じである。

もっとも問題となるのが症例③のように、運動麻痺もなく記銘力障害といった社会復帰が困難である場合であろう。この場合は、居宅生活支援サービスは受けられるが、医療費とくに入院治療費の助成がないことや何よりも在宅での介護におけるマンパワー不足により家族の身体的、精神的負担は計り知れない。また精神障害者保険福祉手帳でホームヘルプ事業を申請しても、実際には患者に触れるケアができないといった問題もある。

また、40歳以下の若年脳腫瘍患者の在宅療養の現状において、介護保険が申請できないため、ケアマネージャーが関わることが事実上できない。つまり、病院が直接訪問看護ステーションと連携をとる必要性が生じる(図2)。また、訪問看護における自己負担額をみても差があることが判る。介護保険利用できる場合(40歳以上)1時間まで864円であるが、介護保険が利用できない場合(40歳未満)、医療保険で利用することになり、通常週3回までの利用で、医療保険3割負担例では1時間まで初回4,155円＋交通費となり、2回目以降で2,770円＋交通費となる。また、訪問看護指示書を作成すること(診断名に末期悪性腫瘍の記載)で、毎日訪問看護を受けられるようになるが、この場合も重度障害者医療費助成制度が利用できないと自己負担が生じるので留意しておく必要がある。しかも、これにより訪問看護を利用できるが、在宅療養における家族の介護負担が軽減できるわけではない。

【まとめ】

悪性脳腫瘍において、望ましい治療は脳腫瘍そのものの外科的治療およびその後の化学療法や放射線治療だけでなく、同時に個々の患者のQOLを考慮したケアを行うことと患者と家族の医学的、心理的、社会的、経済的といった様々な側面からの支援がなされることであることは明らかである[10]。

そのためには、看護相談や医療相談を専門に行う看護師やソーシャルワーカーの存在は重要であり、本学ではすでに地域連携を含めたメディカル・サポート・センターを設置(図2)し、患者および家族の問題や、実際に往診する開業医との連携、また受持医や主治医の、社会的資源や在宅療養全体における認

識の不足から生じる問題の解決に対応するチーム医療を実施している。脳腫瘍患者の治療では、早期から関わるように、治療方針を立て説明するようにしていくべきである。そして、脳腫瘍の治療に携わる我々脳神経外科医も、これまで述べたような社会的資源について、その問題点を含め知っておくことは重要であると思われた。

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13

Development of Oncolytic Replication-Competent Herpes Simplex Virus Vectors

The G207 Paradigm

Tomoki Todo and Samuel D. Rabkin

1. INTRODUCTION

Oncolytic virus therapy is a promising new strategy for treating cancer that involves replication-competent virus vectors that can replicate *in situ* in tumor cells, exhibit oncolytic activity by direct cytocidal effects, and then spread throughout the tumor. In addition, replication-competent virus vectors are capable of transferring and expressing foreign genes in host cells. These virus vectors are either genetically engineered (e.g., herpes simplex virus type 1 [HSV-1], adenovirus, vaccinia virus), naturally attenuated (e.g., Newcastle disease virus), or nonpathogenic in humans (e.g., reovirus), so they replicate selectively in tumor cells, but do not harm normal tissues *(1)*.

HSV-1 in particular has many features that make it attractive for cancer therapy *(2)*: (1) HSV-1 infects most tumor cell types; (2) its life cycle is well studied *(3)*; (3) the HSV-1 genome has been sequenced; (4) the functions of the majority of genes have been identified *(4)*; (5) genes can be manipulated; and (6) the large size of the genome (153 kb) provides space for insertion of large amounts of deoxyribonucleic acid (DNA) *(4)*. Furthermore, HSV-1 has the following features that are well suited for clinical application: (1) total tumor cell killing in vitro can be achieved at a relatively low multiplicity of infection (MOI); (2) antiviral drugs are available that enable optional termination of the therapy *(5)*; (3) animal models are available for preclinical evaluation of safety and efficacy; (4) the viral genome does not integrate into the host cell genome; and (5) it can exist in a latent state without causing detectable damage to the infected cell *(6)*. HSV-1 is a neurotropic virus, and many of the genes necessary for neuropathogenicity are nonessential and can be mutated *(7)*. Therefore, the use of HSV-1 is especially advantageous for brain tumor therapy.

Research on oncolytic HSV-1 therapy has advanced rapidly from a basic concept to clinical studies. In the early days, replication-competent HSV-1 vectors were genetically engineered to have mutations in one nonessential gene associated with either virulence or viral DNA synthesis to restrict viral replication to transformed cells *(2,8)*. These so-called first-generation vectors demonstrated that HSV-1 vectors could in fact efficiently inhibit the growth of tumors without lethally harming the host animal. They also showed that oncolytic HSV-1 therapy could be applied not only to brain tumors, but also to a broad range of solid tumors *(9)*. There were concerns, however, regarding the use of these first-generation vectors in humans because their pathogenicity may not have been sufficiently attenuated, and a single mutation could potentially revert to wild type. To address these concerns, so-called second-generation vectors were developed that had genetically engineered mutations in two different genes.

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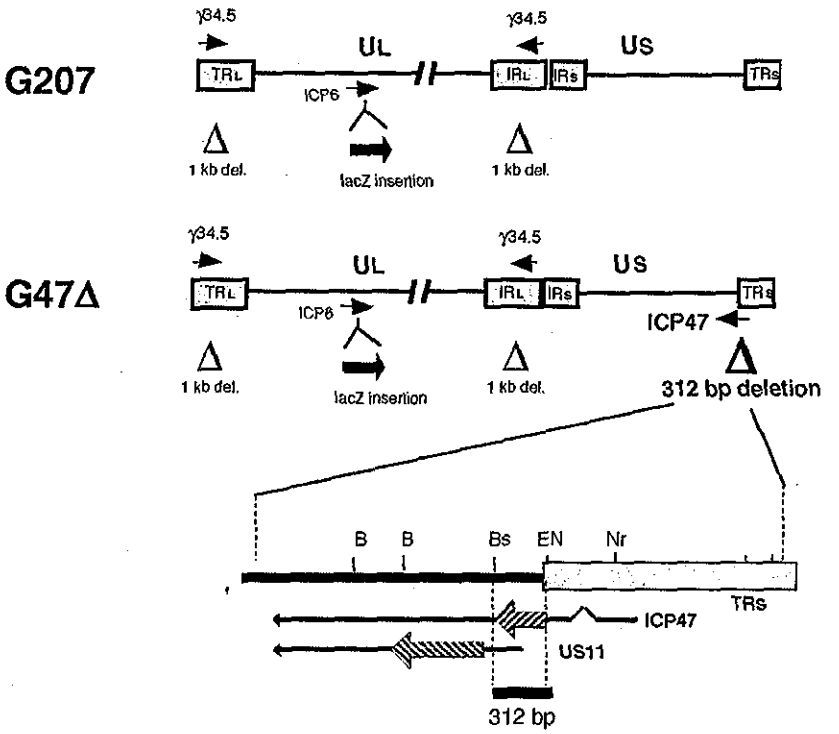

Fig. 1. Structures of G207 and G47Δ. The HSV-1 genome consists of long and short unique regions U_L and U_S, respectively, each bounded by terminal (T) and internal (I) repeat regions R_L and R_S, respectively. G207 was engineered from wild-type HSV-1 strain F by deleting 1 kb within both copies of the *γ34.5* gene and inserting the *E. coli lacZ* gene into the ICP6 coding region. G47Δ was derived from G207 by deleting 312 bp from the ICP47 locus. Because of the overlapping 3' coterminal transcripts of US11 and ICP47, the deletion also places the late gene US11 under control of the ICP47 immediate-early promoter. The ICP47 transcript contains an intron (indicated by Λ). Restriction site abbreviations: B, BamHI; Bs, BstEII; E, EcoRI, EN, EcoNI, Nr, NruI. (Modified from ref. *80*.)

2. G207

G207 was the first of the second-generation HSV-1 vectors *(10)*. It was originally designed for clinical application in patients with brain tumors, with an emphasis on employing ample safeguards. G207 has deletions in both copies of the γ34.5 gene (Fig. 1), the major determinant of HSV-1 neurovirulence *(11)*. The *γ34.5*-deficient HSV-1 vectors are considerably attenuated in normal cells, but retain their ability to replicate in neoplastic cells *(9)*.

In normal cells, HSV-1 infection induces activation of double-stranded RNA-dependent protein kinase R (PKR), which in turn leads to phosphorylation of the α-subunit of eukaryotic initiation factor 2α (eIF-2α) and a subsequent shutdown of host and viral protein synthesis *(12)*. The product of the *γ34.5* gene antagonizes this PKR activity. However, in tumor cells with an activated Ras signaling pathway, it has been suggested that PKR activity is already inhibited, thereby allowing *γ34.5*-deficient HSV-1 vectors to replicate *(13,14)*. Many of the oncolytic HSV-1 vectors currently used have deletions in the *γ34.5* gene *(8)*, including R3616 *(11)*, the parent of G207, and 1716 *(15)*.

G207 also has an insertion of the *Escherichia coli lacZ* gene in the infected-cell protein 6 (ICP6) coding region (UL39), inactivating ribonucleotide reductase, a key enzyme for viral DNA synthesis in

nondividing cells, but not in dividing cells *(16)*. This double mutation greatly minimizes the chances of G207 reverting to wild type or a pathogenic phenotype. It also confers favorable properties on the virus for treating human cancers; G207 replicates preferentially in tumor cells and is harmless in normal tissue because of attenuated virulence, G207 is about 10-fold more sensitive to ganciclovir/acyclovir than its parent virus R3616, and the reporter gene *lacZ* allows easy histochemical detection of G207-infected cells *(10)*. 3616UB is a similar, second-generation vector except uracil DNA glycosylase was inactivated instead of ICP6 *(17)*.

2.1. *Antitumor Efficacy*

G207 has been tested in more than 60 different cell lines, which revealed that the vast majority, although not all, of human tumor cell lines are susceptible to G207 infection and replication *(18)*. In human glioma and malignant meningioma cell lines, for example, G207 can achieve destruction of the entire cell population in culture within 2 to 6 days at an MOI of 0.1 *(10,19)*. In contrast, at the same MOI, G207 manifests no effect on primary cultures of rat cortical astrocytes or cerebellar neurons *(10)*.

This difference in G207 cytopathic effect observed in vitro between tumor cells and normal cells is directly reflected in the results of in vivo studies. In athymic mice harboring U87MG glioma or F5 malignant meningioma tumors intracranially or subcutaneously, a single intraneoplastic inoculation of G207 significantly inhibited tumor growth and prolonged animal survival *(10,19)*. Prominent *lacZ* expression from G207 replication within tumors could still be observed 24 days postinoculation *(19)*.

Besides brain tumors, G207 has proven efficacious in a variety of other animal tumor models in which human, mouse, rat, or hamster tumors have been generated subcutaneously or in various organs, including the liver, peritoneum, sciatic nerve, urinary bladder, and cheek pouch *(18)*.

In addition to direct intratumoral inoculation, G207 has been successfully administered intravenously *(20–22)*, via portal vein *(23)*, intraarterially *(24)*, and intraperitoneally *(25,26)*.

2.2. *Safety*

Because HSV-1 is the most common viral cause of fatal encephalitis *(27)* and G207 was the first replication-competent HSV-1 vector, along with 1716 *(28)*, to be used in human brains, it was extensively evaluated for its toxicity in the brain. In BALB/c mice, the highest dose of G207 (10^7 plaque forming units [pfu]) caused no symptoms for over 20 weeks when inoculated intracerebrally or intraventricularly *(29)*. In A/J mice, one of the most susceptible mouse strains to HSV-1 infection *(30)*, intracerebral inoculation of clinical-grade G207 at 2×10^6 pfu caused only a temporary and slight hunching in 2/8 mice *(31)*. Furthermore, in BALB/c mice that survived an intracerebral inoculation of wild-type HSV-1 (strain KOS) at an LD_{50} dose (~10^3 pfu), a subsequent challenge with an intracerebral inoculation of G207 (10^7 pfu) at the same stereotactic coordinates did not result in reactivation of latent HSV-1 *(29)*.

Aotus nancymae (New World owl monkeys) are among the most sensitive nonhuman primates to HSV-1 infection *(32,33)*. A total of 22 *Aotus* primates have been used for safety evaluation of G207 (intracerebral and/or intraprostatic inoculation) *(34–36)*. In *Aotus*, a single intracerebral inoculation of G207, up to 10^9 pfu or repeat inoculations of 10^7 pfu, caused neither virus-related disease nor detectable changes in the brain as assessed by magnetic resonance imaging (MRI) and pathological studies *(34)*.

In contrast, an intracerebral inoculation of 10^3 pfu of wild-type HSV-1 (strain F) caused acute viral encephalitis, with the animal becoming moribund within 5 days of inoculation. Four *Aotus* were used to evaluate the shedding and biodistribution of G207 after intracerebral inoculation of clinical-grade, column-purified G207 (3×10^7 pfu) *(35)*. Using polymerase chain reaction analyses and viral culture, neither infectious virus nor viral DNA was detected from tear, saliva, vaginal secretion, blood, or urine samples at any time-point up to 1 month postinoculation. Analyses of tissues obtained at necropsy at 1 month showed G207 DNA distribution restricted to the brain, with no infectious

virus isolated. Histopathology revealed normal brain tissues, including the sites of inoculation *(35)*. All *Aotus* receiving an intracerebral G207 inoculation showed an increase in serum anti-HSV-1 antibody titers as early as 21 days postinoculation *(34,35)*.

2.3. Clinical Trial

A phase I clinical trial of G207 for recurrent malignant glioma was performed in 21 patients at two institutions in the United States *(37)*. This dose escalation study started at 10^6 pfu and increased to 3×10^9 pfu, with three patients at each dose. G207 was inoculated stereotactically into an enhancing region of the tumor, visualized by computerized tomographic scan with contrast enhancement. No acute, moderate-to-severe, adverse events attributable to G207 were observed *(37)*. Minor adverse events included seizure (2 cases) and brain edema (1 case). Among 7 biopsied or resected tumors analyzed, specimens from 2 patients were positive for G207 DNA by polymerase chain reaction analysis (56 and 157 days postinoculation). Of 19 patients, 5 were negative for serum anti-HSV-1 antibody prior to G207 treatment, and despite corticosteroid treatment of these patients, 1 patient seroconverted after G207 inoculation *(37)*.

The tools to evaluate efficacy included Karnofsky performance score and serial MRI *(37)*. An improvement in Karnofsky score was observed in 6 of 21 patients (29%) at some time after G207 inoculation. Of 20 patients that had serial MRI evaluations, 8 had a decrease in tumor volume (enhancing area) between 4 days and 1 month postinoculation. All patients, except 1 who died from cerebral infarction 10 months after G207 treatment, eventually showed tumor progression. Interestingly, this glioblastoma patient had no evidence of residual tumor at autopsy. Autopsy was performed in 5 cases, and histology of the brains showed no evidence of encephalitis, white matter degeneration, or inflammatory changes, and all were negative for HSV-1 immunoreactivity. In 3 cases, the tumor was localized to one region of the brain without significant tumor cell invasion into the surrounding brain tissue as usually observed with typical glioblastoma cases.

Overall, the phase I clinical trial confirmed the safety of G207 inoculated into the brain at doses up to 3×10^9 pfu. Currently, a phase Ib clinical trial for recurrent malignant glioma was performed [NIH 481 (2001-07)], and a phase II trial is planned. Similar results were obtained in phase I trials for glioma with 1716 in the United Kingdom *(28,38)*; 1716, which only contains deletions of $\gamma 34.5$ *(15)*, was tested at a lower dose range (up to 10^5 pfu) *(28,38)*.

3. USE OF ONCOLYTIC HSV VECTORS FOR IMMUNE THERAPY

Although G207 proved safe in glioma patients and efficacious in animal tumor models, G207 is considerably attenuated, not only for pathogenicity, but also in its tumor cell-killing capability compared to wild-type HSV-1. One way to improve the efficacy of oncolytic HSV therapy would be to harness antitumor immune responses induced in the course of the oncolytic activity of HSV vectors.

3.1. Antitumor Immune Responses

A difficulty in investigating the immune effects of oncolytic HSV therapy has been the lack of suitable animal tumor models susceptible to HSV-1 infection. Many mouse strains and a majority of murine cell lines are relatively resistant to HSV-1 *(18,30)*. It was not recognized until development of immunocompetent mouse tumor models suitable for HSV-1 evaluation that the host immune response plays an important role in the antitumor activity of oncolytic HSV-1 vectors both in the brain and in the periphery *(39,40)*. Initially, murine N18 neuroblastoma cells, one of the more susceptible murine cell lines tested for G207 susceptibility, were used in syngeneic A/J mice. In A/J mice harboring established N18 tumors subcutaneously or in the brain, intraneoplastic inoculation with G207 caused a significant reduction in tumor growth or prolongation of survival *(39)*. Moreover, in A/J mice bearing bilateral subcutaneous N18 tumors, intraneoplastic G207 inoculation into one tumor alone caused growth reduction and/or regression of both the inoculated and the noninoculated contralateral

tumor, indicating induction of systemic antitumor immunity *(39)*. This inhibition of noninoculated tumor growth was also seen in animals bearing intracerebral brain tumors after subcutaneous tumor inoculation. Animals that were cured of their subcutaneous tumors by G207 were protected against tumor rechallenge, in either the periphery or the brain. Antitumor immunity was associated with cytotoxic T lymphocyte (CTL) activity that was specific to N18 tumor cells and persisted for at least 13 months.

G207-induced, systemic antitumor immunity was also observed in BALB/c mice bearing subcutaneous CT26 (colon carcinoma) tumors and DBA/2 mice bearing subcutaneous M3 (melanoma) tumors *(40)*. In the CT26 model, intraneoplastic inoculation of G207 induced CTL activity that recognized a dominant, tumor-specific, major histocompatibility complex (MHC) class I-restricted epitope (AH1) from CT26 cells. Similar systemic antitumor immunity induction by G207 was observed in Syrian hamsters bearing subcutaneous KIGB-5 (gallbladder carcinoma) tumors *(41)* and BALB/c mice bearing CT26 liver metastases *(42)*. Thus, in an immunocompetent condition, the oncolytic activity of G207 can be augmented by induction of specific and systemic antitumor immunity effective both in the periphery and in the brain.

When high-dose dexamethasone was given to A/J mice bearing subcutaneous N18 tumors for an extensive period (16 days), G207 retained antitumor activity and caused a significant suppression of tumor growth when inoculated into the tumors *(43)*. However, all immunosuppressed (dexamethasone-treated) mice treated with G207 displayed tumor regrowth despite initial shrinkage, whereas 50% of the G207-treated mice not immunosuppressed were cured. Dexamethasone administration significantly reduced neutralizing serum antibodies against G207 after intraneoplastic G207 inoculation, but this did not affect the amount of infectious G207 isolated from tumors. The most striking effect of dexamethasone administration was the abolition of G207-induced CTL activity against N18 cells *(43)*. These results further support the importance of tumor-specific CTL induction in the course of oncolytic HSV-1 antitumor activity.

The effect of circulating anti-HSV-1 antibodies on the efficacy of oncolytic HSV-1 therapy has been investigated because the majority of the population is HSV-1 seropositive *(44,45)*. A/J and BALB/c mice were immunized by repeated intraperitoneal inoculations of wild-type HSV-1 (strain KOS) and then the antitumor efficacy of G207 on established subcutaneous N18 and CT26 tumors was determined *(46)*. In both tumor models, the antitumor efficacy of G207 was the same whether the mice were immunized or not for HSV-1.

In a study using intraocular immunization, treatment of M3 melanoma tumors in DBA/2 mice with HSV-1 1716 was actually more effective than in nonimmunized mice *(47)*. Because HSV-1 predominantly spreads cell to cell, circulating antibodies known to neutralize free virus may have little effect on HSV-1 directly inoculated into tumors. When NV1020, at a low dose (10^6 pfu), was administered intravenously to immunized BALB/c mice with CT26 tumors in the liver, there was a detectable decrease in efficacy *(48)*. This efficacy attenuation with intravenous delivery was overcome by administering a higher dose (10^7 pfu) of NV1020.

3.2. Third-Generation Oncolytic HSV-1 Vector

The therapeutic benefits of oncolytic HSV-1 vectors depend on the extent of both intratumoral viral replication and induction of host antitumor immune responses. We are developing new generations of HSV-1 vectors by enhancing these properties and retaining the safety features of G207. G47Δ is one such vector created from G207 by introducing another genetic alteration, deletion of the *α47* gene and the overlapping *US11* promoter region *(31)* (Fig. 1). Because the *α47* gene product (ICP47) inhibits transporter associated with antigen presentation, which translocates peptides across the endoplasmic reticulum, the downregulation of MHC class I that normally occurs in human cells after infection with HSV-1 does not occur *(49)*. G47Δ-infected human cells in fact presented higher levels of MHC class I than cells infected with other HSV-1 vectors *(31)*. Further, human melanoma

cells infected with G47Δ were better at stimulating their matched tumor-infiltrating lymphocytes in vitro than those infected with G207. Unfortunately, the interaction of ICP47 with transporter associated with antigen presentation is species specific and is exceedingly inefficient in rodent cells *(50)*. Therefore, it is not possible to test the immune effects in vivo in mouse tumor models.

The deletion also places the late *US11* gene under control of the immediate-early *α47* promoter, which results in suppression of the reduced growth phenotype of *γ34.5*-deficient HSV-1 mutants *(51)*, including G207. In the majority of cell lines tested in vitro, G47Δ replicated better than G207, resulting in the generation of higher virus titers, and exhibited greater cytopathic effect *(31)*. In athymic mice bearing subcutaneous U87MG human glioma tumors and A/J mice bearing subcutaneous Neuro2a neuroblastoma tumors, G47Δ was significantly more efficacious than G207 at inhibiting tumor growth when inoculated intraneoplastically *(31)*.

Improved antitumor efficacy of G47Δ has also been shown in other immunocompetent mouse tumor models, including prostate and breast cancer *(65)*. Nevertheless, this deletion does not suppress the attenuated pathogenicity of *γ34.5* deletion mutants *(52)*, and the safety of G47Δ remained unchanged from G207 following injection into the brains of HSV-1-sensitive A/J mice *(31)*.

Thus, compared with the parental virus G207, G47Δ demonstrated (1) better induction of human antitumor immune cells; (2) better growth properties, leading to higher virus yields and increased cytopathic effect in vitro; (3) better antitumor efficacy in both immunocompetent and immunoincompetent animals; and (4) preserved safety. These features make G47Δ highly attractive for clinical application.

3.3. *Combination With Immune Gene Therapy*

Our experience using various HSV-1 vectors to treat tumors, including wild-type HSV-1, indicates that there is a limit to improving the antitumor efficacy of oncolytic vectors by simply bringing the replication capability closer to that of wild-type viruses, putting aside the difficulty of doing so without increasing pathogenicity. In developing new vectors, therefore, more emphasis is currently placed on enhancing the ability to induce antitumor immunity. The combination of oncolytic HSV-1 vectors with defective vectors expressing immunostimulatory molecules can improve therapeutic efficacy significantly (Fig. 2) *(53–55)*. In this approach, the oncolytic HSV-1 vector acts as a helper virus for the propagation of plasmid-based defective vectors *(56)*. An advantage of this approach is that different defective vectors can be generated with different oncolytic helper viruses for a multiplicity of combinations without creating new vectors.

We have developed an immune gene therapy strategy that would work for brain tumors as well as other cancers. The brain is considered an immune-privileged site, and patients with brain tumors are often under an immune-suppressed condition because of immunosuppressive factors secreted by the brain tumor and/or corticosteroid administration. On the other hand, a robust, nonspecific inflammatory response in the brain can cause undesirable brain edema.

To meet these requirements, we created a defective HSV vector (dvB7Ig) expressing a soluble form of B7-1, one of the most potent costimulatory molecules, and used it in combination with G207 *(54)*. Soluble B7-1 was designed as a fusion protein of the extracellular domain of B7-1 and the Fc portion of immunoglobulin G, so that it is secreted by tumor cells rather than expressed on the cell surface. Secreted soluble B7-1 should provide antigen-presenting cells increased T-cell stimulatory activity, activate T cells in an anergic state, and because it is in a dimeric form, provide a strong stimulation to T cells by crosslinking neighboring CD28.

The in vivo efficacy was tested in the poorly immunogenic murine neuroblastoma Neuro2a in A/J mice. Intraneoplastic inoculation of dvB7Ig/G207 at a low titer successfully inhibited the growth of established subcutaneous tumors, despite the expression of B7-1-immunoglobulin detected in only 1% or fewer tumor cells at the inoculation site, and prolonged the survival of mice bearing intracerebral tumors *(54)*. Inoculation of dvB7Ig/G207 induced a significant influx of $CD4^+$ and $CD8^+$ T cells in the tumor. In vivo depletion of immune cell subsets further revealed that the antitumor effect

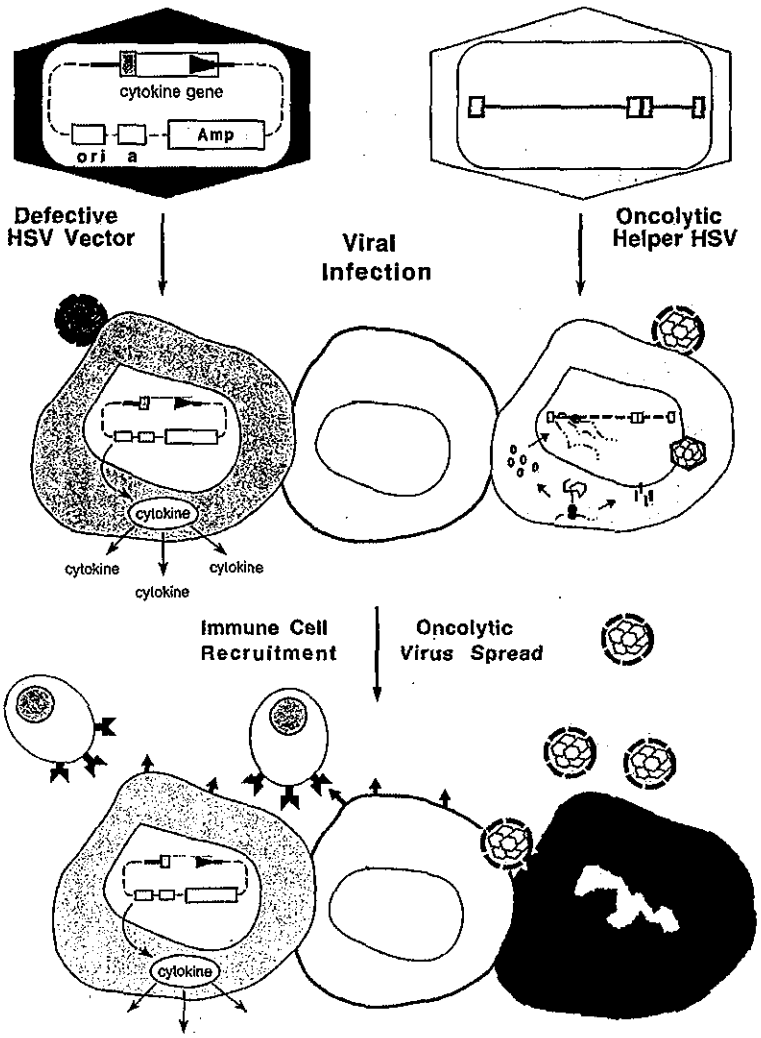

Fig. 2. Schematic diagram of immunomodulatory gene therapy using oncolytic HSV-1 vectors (like G207) as helper virus in combination with a defective HSV-1 vector expressing an immunomodulatory molecule. Defective HSV vector stocks are a mixture of defective particles (upper left) containing tandem repeats of an amplicon plasmid and HSV helper viruses (upper right) *(81)*. The amplicon plasmid consists of the cytokine/immunomodulatory gene, an HSV origin of replication (ori), and an HSV cleavage/packaging signal (a), but no viral coding sequences, and is packaged as a full viral genome length (~150 kb). Any conditional-lethal or replication-competent HSV mutant can be used as helper virus. When a mixture of helper and cytokine-expressing defective vector is inoculated into a tumor, the helper virus replicates, kills the infected cell, and spreads to other tumor cells (right side). On the other hand, tumor cells infected with the defective vector produce the cytokine and recruit immune cells (left side) that augment the antitumor immune response elicited by the oncolytic helper virus.

required $CD8^+$ T cells, but not $CD4^+$ T cells *(54)*. DvB7Ig/G207 treatment conferred tumor-specific protective immunity on cured animals. Thus, this approach proved to be a potent and clinically applicable means of treating brain tumors and other cancers.

A defective HSV vector expressing murine interleukin 12 (IL-12) in combination with G207 was very effective in treating subcutaneous CT26 tumors in BALB/c mice and inducing a tumor cell-specific $CD8^+$ CTL response *(53)*. An IL-2-expressing defective vector in combination with G207 had enhanced efficacy in murine squamous cell carcinoma and rat hepatocellular carcinoma models compared to G207 alone *(55,57)*. However, granulocyte-macrophage colony-stimulating factor (GM-CSF) expression from a defective vector did not have any increased efficacy over G207 alone in treating CT26 tumors (M. Toda and S. D. Rabkin, unpublished results, 1998). Whereas GM-CSF expression from replication-deficient HSV vectors did significantly enhance antitumor activity *(58, 59)*, as a tumor vaccine, GM-CSF-transduced cells have been found to be among the most effective *(60)*. This suggests that HSV infection may be inducing an immune response similar to that of GM-CSF expression, and that the spectrum of cytokines that will be effective in combination with oncolytic HSV vectors will be different from those used in tumor vaccines.

Replication-competent HSV-1 vectors that contain immunostimulatory molecule transgenes (i.e., IL-4, IL-10, IL-12, GM-CSF) have been created *(61–63)*. In particular, replication-competent HSV-1 vectors that express IL-12 have been shown in several animal tumor models to manifest direct oncolytic activity and express sufficient amounts of IL-12, which significantly augments antitumor activity without increasing toxicity, compared with the parental HSV-1 vectors *(62–65)*.

4. FUTURE DIRECTIONS

Now that it has been demonstrated in several clinical trials that oncolytic HSV-1 vectors can be administered safely in humans *(28,37,38,66)*, further development of oncolytic HSV-1 vectors will be directed toward improving antitumor efficacy. Doing so without compromising the safety of the vectors is the key to prevailing in this type of therapy. G47Δ is a good example of providing such an improvement in efficacy yet retaining safety features. A syncytial mutant (Fu-10) generated from G207, which forms tumor cell syncytium, was more efficacious in a lung metastases model than the parent, G207 *(22)*.

Expression of foreign transgenes, for example, "suicide" or immunostimulatory molecules, is another promising method to augment the activity of oncolytic HSV-1 vectors. A number of suicide genes, cytochrome P450 (CYP2B1) and cytosine deaminase (CD), have been incorporated into oncolytic HSV-1 vectors, and treatment with prodrugs significantly improved efficacy *(67,68)*. With the addition of foreign transgenes, it is important to be aware that they may increase the toxicity of the vector, decrease safety, and/or interfere with viral replication and decrease efficacy.

A practical method for improving the efficacy of oncolytic HSV-1 vectors is to combine them with conventional therapies. For example, a combination with cisplatin was shown to enhance the antitumor effect of G207 against human head and neck cancer *(69)*, and mitomycin C with 1716 was more effective than either treatment alone against human non-small cell lung cancer *(70)*.

Others have shown that ionizing radiation amplifies the replication of HSV-1 R3616 *(71)*, leading to improved survival of athymic mice bearing intracerebral U87MG tumors *(72)* and NV1020 (R7020) in some hepatoma tumor cell lines *(73)*. Although we did not observe such an enhancing effect of ionizing radiation with G207 in prostate cancer *(74)*, others have shown such an effect with G207 and cervical cancer *(75)*. Systemic delivery to brain tumors after intracarotid artery infusion can be enhanced by disruption of the blood–brain barrier using mannitol, bradykinin, or RMP-7 *(76–78)*. The replication and spread of oncolytic HSV-1 vector hrR3 in brain tumors after RMP-7 can be further enhanced by intraperitoneal administration of cyclophosphamide *(79)*. The combination of oncolytic HSV-1 vectors with established therapies should be rapidly translatable to the clinic.

5. CONCLUSION

Oncolytic virus therapy is an attractive treatment strategy because it is based on a new concept that the antitumor agent can amplify specifically at the tumor site after administration. This strategy

also has features that make it attractive for clinical application: (1) tumor cells are targeted irrespective of their genetic makeup; (2) it can be combined with conventional therapies such as surgery, radiation therapy, and chemotherapy; (3) combination with immunotherapy has potential synergistic effects; and (4) it can act as a vehicle for gene delivery in vivo. An increasing number of clinical trials using oncolytic viruses have been initiated or planned in recent years. We anticipate that oncolytic virus therapy will be established as an important modality of cancer treatment in the near future.

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