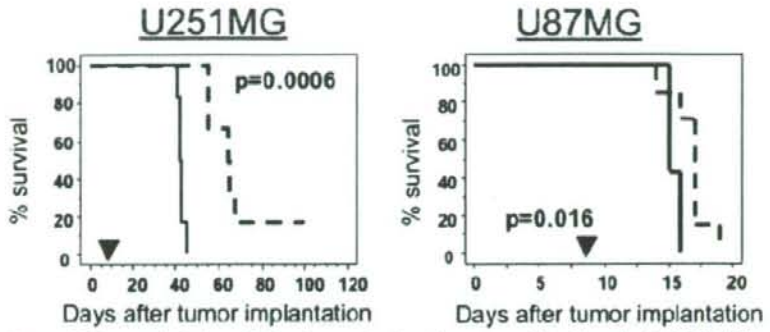

FIG. 5. Kaplan–Meier survival plots of 12 nude rats (rnu/rnu, homozygous) implanted with U-251MG tumor cells and 14 Fischer 344/NJcl-rnu/rnu (nude) rats implanted with U-87MG tumor cells and divided into 2 groups: a sham surgery group (*solid lines*), and a group that received CED of PLD (0.2 mg/ml doxorubicin; *dotted lines*). Seven days after tumor cell implantation, CED of 20-μl PBS or drug infusion was performed. Rats were killed after developing neurological symptoms indicative of tumor progression. The survival of the rats that received 0.2 mg/ml doxorubicin as PLD was significantly longer than those that received mock surgery ($p = 0.0006$ in U-251MG model, $p = 0.016$ in U-87MG model; log-rank test).

the initial distribution area in the brain tissue exposed to free doxorubicin, whereas only slight tissue damage was observed adjacent to the needle tract in the brain tissue exposed to PLD (Fig. 2). Sixty-three days after infusion, however, the 100% PLD infusion (2 mg/ml doxorubicin) had damaged the tissue throughout the hemisphere (Fig. 4), indicating the slow release capacity of liposomes. Fluorescence generated from doxorubicin could be detected at least 30 days after infusion (Fig. 3). These findings indicate that PLD achieved wide distribution and sustained release of doxorubicin in the brain parenchyma.

In the survival studies 10% PLD was used, containing 0.2 mg/ml doxorubicin, which was defined as the safe dose for local administration. Significant prolongation of survival was observed in the intracranial U-251MG xenograft model.[18] Similar but less significant survival prolongation was observed in the intracranial U-87MG xenograft model. This difference may depend on the cellular sensitivity to doxorubicin, or on the life spans of the rats in these models. The life expectancy in the U-251MG xenograft model rats was 42.7 ± 1.4 days, whereas that in the U-87MG model rats was 15.4 ± 0.5 days. Therefore, the life span of the U-87MG rats might not allow testing of the efficacy of the proposed treatment. Humans with gliomas have a much longer life expectancy, so this treatment strategy may achieve better efficacy.

Conclusions

Convection-enhanced delivery of PLD achieved wide distribution and slow release of the encapsulated doxorubicin in the brain parenchyma of the rat. Polyethylene glycol–coated liposomal doxorubicin administered at a dose safe to the normal brain tissue demonstrated significant survival prolongation effects in rodent intracranial glioma xenograft models. Convection-enhanced delivery of PLD is a promising treatment for malignant gliomas.

Disclaimer

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

References

1. Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH: Convection-enhanced delivery of macromolecules in the brain. **Proc Natl Acad Sci U S A 91:**2076–2080, 1994
2. Brem H, Piantadosi S, Burger PC, Walker M, Selker R, Vick NA, et al: Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. **Lancet 345:**1008–1012, 1995
3. Fleming AB, Saltzman WM: Pharmacokinetics of the carmustine implant. **Clin Pharmacokinet 41:**403–419, 2002
4. Groothuis DR: The blood-brain and blood-tumor barriers: a review of strategies for increasing drug delivery. **Neuro Oncol 2:**45–59, 2000
5. Hau P, Fabel K, Baumgart U, Rummele P, Grauer O, Bock A, et al: Pegylated liposomal doxorubicin-efficacy in patients with recurrent high-grade glioma. **Cancer 100:**1199–1207, 2004
6. Krauze MT, Saito R, Noble C, Tamas M, Bringas J, Park JW, et al: Reflux-free cannula for convection-enhanced high-speed delivery of therapeutic agents. **J Neurosurg 103:**923–929, 2005
7. Mamot C, Nguyen JB, Pourdehnad M, Hadaczek P, Saito R, Bringas JR, et al: Extensive distribution of liposomes in rodent brains and brain tumors following convection-enhanced delivery. **J Neurooncol 68:**1–9, 2004
8. O'Shaughnessy JA: Pegylated liposomal doxorubicin in the treatment of breast cancer. **Clin Breast Cancer 4:**318–328, 2003
9. Ozawa T, Wang J, Hu LJ, Bollen AW, Lamborn KR, Deen DF: Growth of human glioblastomas as xenografts in the brains of athymic rats. **In Vivo 16:**55–60, 2002
10. Perez-Lopez ME, Curiel T, Gomez JG, Jorge M: Role of pegylated liposomal doxorubicin (Caelyx) in the treatment of relapsing ovarian cancer. **Anticancer Drugs 18:**611–617, 2007
11. Saito R, Bringas JR, McKnight TR, Wendland MF, Mamot C, Drummond DC, et al: Distribution of liposomes into brain and rat brain tumor models by convection-enhanced delivery monitored with magnetic resonance imaging. **Cancer Res 64:** 2572–2579, 2004

12. Saito R, Krauze MT, Bringas JR, Noble C, McKnight TR, Jackson P, et al: Gadolinium-loaded liposomes allow for real-time magnetic resonance imaging of convection-enhanced delivery in the primate brain. **Exp Neurol 196**:381–389, 2005
13. Saito R, Krauze MT, Noble CO, Drummond DC, Kirpotin DB, Berger MS, et al: Convection-enhanced delivery of Ls-TPT enables an effective, continuous, low-dose chemotherapy against malignant glioma xenograft model. **Neuro Oncol 8**: 205–214, 2006
14. Saito R, Krauze MT, Noble CO, Tamas M, Drummond DC, Kirpotin DB, et al: Tissue affinity of the infusate affects the distribution volume during convection-enhanced delivery into rodent brains: implications for local drug delivery. **J Neurosci Methods 154**:225–232, 2006
15. Siegal T, Horowitz A, Gabizon A: Doxorubicin encapsulated in sterically stabilized liposomes for the treatment of a brain tumor model: biodistribution and therapeutic efficacy. **J Neurosurg 83**:1029–1037, 1995
16. Voulgaris S, Partheni M, Karamouzis M, Dimopoulos P, Papadakis N, Kalofonos HP: Intratumoral doxorubicin in patients with malignant brain gliomas. **Am J Clin Oncol 25**: 60–64, 2002
17. Westphal M, Hilt DC, Bortey E, Delavault P, Olivares R, Warnke PC, et al: A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. **Neuro Oncol 5**:79–88, 2003
18. Yamashita Y, Saito R, Krauze MT, Kawaguchi T, Noble CO, Drummond DC, et al: Convection-enhanced delivery of liposomal doxorubicin in intracranial brain tumor xenografts. **Targeted Oncol 1**:79–85, 2006

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テクニカル・ノート◆Technical Note

パルスレーザージェットメス—神経膠腫手術への臨床応用*

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Clinical Application of Pulsed Laser-induced Liquid Jet: Preliminary Report in Glioma Surgery

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Key words:
glioma,
microsurgery,
minimally invasive neurosurgery,
water jet

Purpose: Both maximum resection of tumor and preservation of fine vessels are conflicting aims, but important factors to improve outcome in glioma surgery. Water jet dissection has been reported to dissect tissue while ensuring preservation of fine vessels. However, it was difficult to apply conventional water jet device in microsurgery due to the use of high pressure and continuous water a flow. To overcome these issues, we have developed pulsed holmium: yttrium-aluminum-garnet (YAG) laser-induced liquid jet (LILJ) for microsugical use and applied it in glioma surgery.

Methods: LILJ was generated by irradiating pulsed Ho: YAG laser (3 Hz, pulse laser energy 233-300 mJ/pulse) within a stainless tube (outer diameter (OD): 1.26 mm inner diameter (ID): 0.90 mm) filled with cold (4°C) lactated Ringer's solution. The laser beam was conducted through optical quartz fiber (core diameter: 400 μm). The jet generated was ejected from a stainless nozzle (OD: 1.06 mm, ID: 0.70 mm). To avoid splash and air bubbles within the surgical field, the nozzle was placed inside a stainless suction tube (OD: 3.06 mm, ID: 2.64 mm). LILJ was ejected randomly toward blood vessels and tissue simultaneously after removal of arachnoid membrane by microsurgical technique, and the quality of the dissection and the visual field were evaluated in 4 patients with supratentorial glioma.

Results: Restoration of small arteries (diameter: 100 to 200 microns) was accomplished. There was no significant occurrence of splash or air bubbles under the microscopic view.

Conclusion: Present results showed that the pulsed LILJ system may safely be used for microsurgical procedures, and may be useful for glioma resection where preservation of fine vessels is required.

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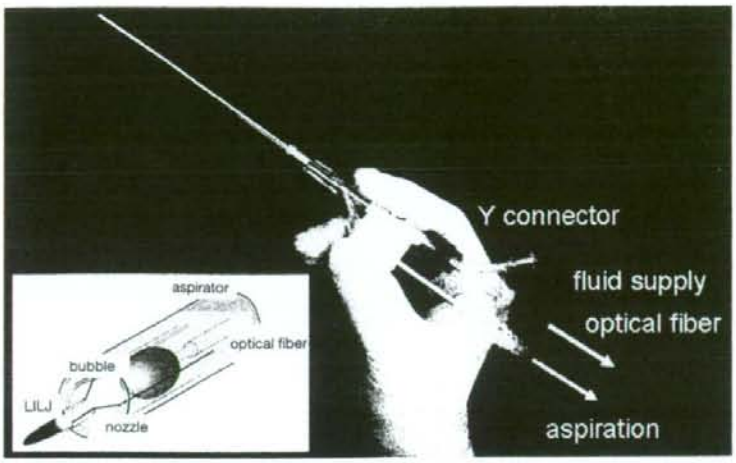

Fig. 1 Overview of the LILJ system. LILJ was generated by irradiating pulsed Ho: YAG laser within a stainless tube, filled with cold (4℃) lactated Ringer's solution. The laser was conducted through optical quartz fiber. Transient expansion of laser-induced bubble pushes the liquid distal to the tip of the optical fiber. The jet generated was ejected from a stainless nozzle. To avoid splash and air bubbles within the surgical field, the nozzle was placed inside a stainless suction tube.

I. はじめに

液体（水）ジェットメスは水流により組織を線状に切開，もしくは体積をもつ塊として破砕するデバイスである．最大の特徴は熱損傷がない点と高い組織選択性をもち細血管（200 μm 程度）温存下に組織切開・破砕が可能である点である[21,26]．こうした特性は早くから注目され，25 年以上前から腹部外科を中心に臨床応用されてきた[21]．特に，脳と同様に細血管からの出血や胆管損傷の処置に難渋する肝切除術では，既存の手術デバイス（超音波手術装置）と比較して手術時間短縮と出血量減少効果が報告されている[7]．その一方，従来の液体ジェットメスでは術野外に飛沫が飛散することによる播種や医療従事者への感染の危険性，術野内での気泡発生や水分貯留による視野悪化，微調整や小型化が困難といった問題から顕微鏡手術機器としては普及していないのが実情である．

工学的には，前述の問題点は高圧連続流の利用が少なからず関与しているものと考えられる[2]．パルスレーザージェットメスは，holmium：yttrium-aluminum-garnet（以下 Ho：YAG，ホルミウムヤグ）レーザーを駆動源として，実効水量が極めて微量の高速液体ジェットをパルス状に発生させ組織の切開・破砕を行うデバイスである．これまでの基礎実験では，効果ならびに安全性に関して従来の液体ジェットメスと同等以上であることを報告してきた[3-6,10,13-15,20,24]．2007 年 3 月に東北大学病院倫理委員会の承認を得て同年 5 月から臨床応用を開始し，これまでテント上神経膠腫 4 例において使用した経験を報告する．

II. 方　法

対象は 2007 年 5 ～ 7 月に東北大学病院で摘出術を施行したテント上神経膠腫 4 例である．パルスレーザージェットメスを用いて，切除予定脳内もしくは腫瘍内で径 2 mm 以下の動脈を含む領域内で液体ジェットを照射し，操作性と血管温存能力について評価を行った．

パルスレーザージェットメスは，液体ジェット発生部と吸引システムから構成される（**Fig. 1**）．液体ジェット発生部は吸引管（11 G ステンレス製，外径 3.06 mm，内径 2.64 mm）内腔に配置しており，液体ジェット射出管（18 G ステンレス製，

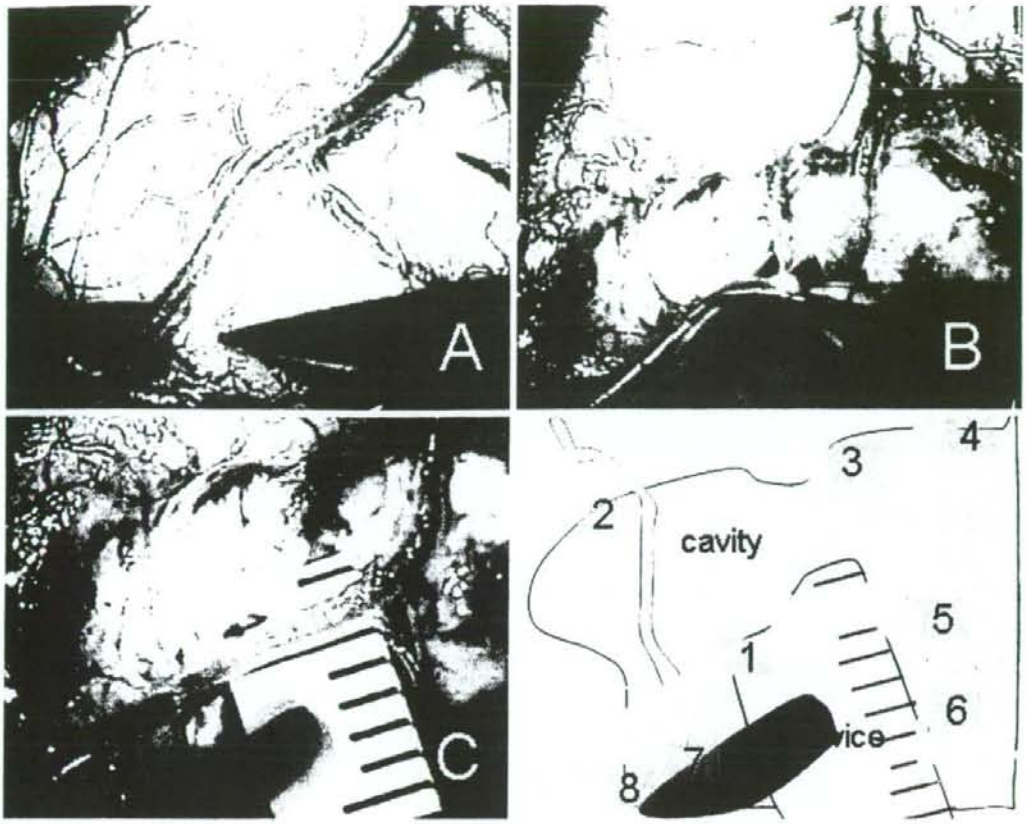

Fig. 2 Restoration of fine arteries after removal of brain tissue (diameter: 100 to 200 μm). Under the microscopic view, there was no significant occurrence of splash and air bubbles. **A**: Before removal. **B**: The arterial component is preserved even after direct ejection of LILJ toward a fine artery. **C**: After removal of brain tissue around the artery showing preservation of the fine arteries. **D**: Schematic diagram describing **C**, preservation of fine arteries in the removed cavity (1 to 8). Scale shows 1 mm. Smallest diameter of a preserved artery was 100 to 200 μm.

外径 1.26 mm，内径 0.90 mm），Y コネクター（アンプラスト®C Y コネクター AP-YC15S；テルモ㈱，東京），石英光ファイバー（無水石製，コア径 400 μm）で構成され，石英光ファイバーは Y コネクターにより液体ジェット射出管内に固定される．パルスレーザー光源は波長 2.1 μm の Ho：YAG レーザー（スパークリングフォトン㈱，東京；SLS-HO）で，レーザー光は石英光ファイバーにより液体ジェット発生部まで導光される．液体ジェット発生部には Y コネクターを介して 4℃に冷却された輸液（ラクテック®注；㈱大塚製薬工場，徳島）が供給され，管内部は常時満たされた状態となっている．液体ジェット射出管内にレーザー光がパルス照射されると一過性に蒸気泡が発生・膨張し，気泡の前方にある液体がノズル（19 G ステンレス製，外径 1.06 mm，内径 0.70 mm）方向へ押し出され，パルス状の液体ジェットが射出される．

液体ジェットによる組織深達度は液体ジェットの噴出圧，初速に比例し，レーザーエネルギー，standoff distance（光ファイバー出射端とノズル先端間距離），ノズル（アスペクト）比による調節が可能であるが[3-6,10,13-15,20,24]，今回の検討では，簡便性の点からレーザーエネルギーによる調節を行い（233 ～ 300 mJ/pulse），standoff distance は 10 cm で固定した．吸引システムは通常の手術吸

引装置に接続して使用した．操作は超音波手術装置と同じ要領で，レーザーのフットスイッチを踏み込むとパルス液体ジェットがノズルから射出され，飛沫，余剰水分，破砕片は吸引管から回収されるようになっている．

III. 結　果

液体ジェットが線維成分に富むくも膜は貫通しないことは，ブタを用いた詳細な検討で報告されているが[16]，今回の検討でも同様で膜成分を超えて深達することはなく，マイクロ鑷子にて別に剝離した．今回使用したエネルギーの範囲内では，低エネルギーでは連続照射により神経膠腫・正常脳組織を線状に切開することが可能で，エネルギーの増加に伴い組織を一塊として破砕することも可能であった．動脈に関しては径100～200 μm程度の血管までの温存が可能であったが，同径の静脈温存効果は認められなかった．操作性に関しては顕微鏡視野で問題となるような飛沫，気泡の発生は認められず，パルスレーザージェットメスの使用による合併症は認められなかった．これまでのところいずれの症例においても播種は認められていない．

症例呈示

〈症　例〉 65歳　女性

診　断 右側頭葉～島部神経膠芽腫

手術操作 通常の右前頭側頭開頭を行った．顕微鏡下操作に入り，深部の腫瘍摘出に先立ち右側頭葉部分切除を行う際にパルスレーザージェットメスを使用した．切除予定脳表領域内部で径1 mm程度の動脈を含む2×3 cm，深さ1 cm程度の領域(**Fig. 2A**)の切開・破砕をレーザージェットメスで行った．液体ジェットはくも膜は貫通しなかったため，マイクロ鑷子を用いて脳表血管のくも膜を剝離した．その後，径1 mm程度の動脈と脳実質を一塊として液体ジェットを照射したところ，脳実質のみこそげ落ちるように破砕されたが，動脈は温存された(**Fig. 2B**)．続いて分枝している動脈と脳実質を一塊として照射した．今回のデバイスでは吸引の微調整が十分ではなく，吸引管径が大きいために吸引管による視野が妨げられ，いったん温存した後に吸引管で損傷する場面もあった．その一方で，特に血管の走行を意識することなく液体ジェットを射出したにもかかわらず，径100～200 μm程度の分枝までは温存された(**Fig. 2C, D**)．また，顕微鏡操作に支障を来す飛沫や気泡の発生は認められず，血管の間隙や血管の深部での破砕操作も可能であった．温存された動脈と同径の細静脈は液体ジェットによる物理的損傷により温存は困難であった．

IV. 考　察

神経膠腫摘出術では，病変の摘出率向上と術後神経学的後遺症を出さないこととの両立が求められる[1,9]．1990年代以降，脳機能マッピング，ニューロナビゲーションシステムなどの導入により摘出術の際の機能温存方法に関しては格段に進歩した．しかし神経膠腫摘出術中の穿通枝の温存については克服すべき課題が多い．例えば島部神経膠腫，前頭弁蓋部腫瘍摘出術における外側線条体動脈，髄質動脈は血管径が1 mm以下と非常に細いにもかかわらず，損傷が重篤な麻痺につながることが報告されている[11,12]．

これまで，腫瘍摘出術に際しては電気，超音波，レーザー，電磁式手術デバイスなどを適宜組み合わせ行っているが，調節性，操作性に関しては優れている反面，熱損傷の防止と細血管の温存に関しては限界がある．このため，液体ジェットメスは以前より従来の手術デバイスの短所を補い得るものとして期待されてきた(**Table**)．近年，高圧連続流を用いるタイプの液体ジェットメスも改良され，欧州の一部脳神経外科施設で臨床応用されており[8,16-19,22,23,25,27]，熱損傷がなく細血管が温存されること，超音波手術装置との比較を行ったところ，手術時間の延長はなく，出血量減少効果があったとされている[22,23]．その一方で，前述した高圧連続流の使用による諸問題の解消には限界があり，既存の手術デバイスとの比較において際立った優位性を打ち出せず，普及していないのが実情である．今回高速パルス液体ジェットを用いた結果，第一に微調整が可能で穿通枝レベルの血管

Table Summary of advantages and disadvantages found between the current devices and present pulsed LILJ system

	Electro-coagulation	Ultrasound	Conventional water jet	Present device
fine control	fair	excellent	fair	excellent
vessel preservation	difficult	possible	excellent	excellent (100-200 μm)
volume reduction	excellent	excellent	fair	possible
heat injury	+	+	−	−
operative field	excellent	excellent	(splash/aerosol)	fair
endoscope encorporation	difficult	difficult	difficult	excellent

The latter is superior to conventional devices in terms of preservation of fine vessels (diameter up to 100 to 200 μm), and absence of heat injury. The size is smaller and has the advantage of being able to be incorporated into a endoscopic instrument.

の温存下に腫瘍の破砕が可能な点，第二に照射される実効水量が著しく減少し，飛沫，気泡の発生も顕微鏡操作に支障のない操作が可能である点が確認できた．また，構造が単純であり，カテーテル内[27]，内視鏡[15,19,20,25]への導入も期待できることから，従来デバイスと比較して十分な優位性があるものと考えられる（**Table**）．

われわれは微量の高速パルスジェット発生原理の開発にあたり，Ho：YAG レーザーを選択した．このレーザーは，パルス発振と光ファイバーによる導光が可能な中近赤外レーザーの 1 つで，その波長（2.1 μm）が水分子の光吸収スペクトルのピークの 1 つ（1.9 μm）の近傍に存在することから効率よく水分子に吸収される[3]．高速度写真による解析の結果，レーザーが水中で照射されると水は瞬時に気化し蒸気泡を形成し，膨張に伴う細管腔内での押し出し効果によって微量かつ高速の液体ジェットが発生する．レーザーエネルギーは液体ジェットの運動エネルギーに変換され，直接の光凝固・蒸散作用は起こらない[15]．

これまでの基礎実験の結果，われわれは次の点を明らかにした．まず液体ジェットのパルス化により実効射出量は 2 μL 以下[21]と極めて少量で，吸引管の装着により飛沫，気泡の発生は顕微鏡下で問題にならない程度に減少する[15]．また，4℃の冷却輸液を供給することによりジェットの温度は 41℃以下にコントロール可能で，熱損傷は認められなかった[16]．さらに温存血管径に関して，ビーグル犬脳表，ブタ肝臓での検討では 100 ～ 200 μm 程度まで可能であった[15]．従来の液体ジェットと同じく模擬モデルを用いた高速度写真による解析から，組織選択性は液体ジェットが線維成分の多い応力の高い構造物を避けて進達する性質があることによるものと考えられている[2,10,13]．

これらの結果は，今回の摘出術中にも再現された一方で，今後の臨床応用に際しての課題も明らかにした．今回使用した吸引管径は外径が大きく，操作部が死角となってしまい，温存した血管も管角で損傷することもあり，先端径の縮小を含めた改良が必要である．また，高エネルギーでは mass reduction が可能であったが，従来デバイスとの性能の比較を含めて今後の検討課題であると考えられる．

今後改善すべき課題はあるものの，パルスレーザージェットメスは既存の手術デバイスでは両立が困難であった細血管温存と腫瘍摘出を実現するための有用な手法となり得る可能性があるものと考えられた．

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文 献

1) Brown PD, Maurer MJ, Rummans TA, Pollock BE, Ballman KV, Sloan JA, Boeve BF, Arusell RM, Clark MM, Buckner JC：A prospective study of quality of life in adults with newly diagnosed high-grade gliomas：the impact of the extent of resection on quality of life and survival. Neurosurgery **57**：495-504, 2005
2) Fletcher DA, Planker DV：Pulsed liquid microjet for microsurgery. Appl Phys Lett **78**：1933-1935, 2001
3) Hirano T, Komatsu M, Saeki T, Uenohara H, Takahashi A, Takayama K, Yoshimoto T：Enhancement of fibrinolytics with a laser-induced liquid jet. Lasers Surg Med **29**：360-368, 2001
4) Hirano T, Komatsu M, Uenohara H, Takahashi A, Takayama K, Yoshimoto T：A novel method of drug delivery for fibrinolysis with Ho：YAG laser-induced liquid jet. Lasers Med Sci **17**：165-172, 2002
5) Hirano T, Uenohara H, Komatsu M, Nakagawa A, Satoh M, Ohyama H, Takayama K, Yoshimoto T：Holmium：YAG laser-induced liquid jet dissector：a novel prototype device for dissecting organs without impairing vessels. Minim Invasive Neurosurg **46**：121-125, 2003
6) Hirano T, Nakagawa A, Uenohara H, Ohyama H, Jokura H, Takayama K, Shirane R：Pulsed liquid jet dissector using holmium：YAG laser-a novel neurosurgical device for brain incision without impairing vessels. Acta Neurochir (Wien) **145**：401-406, 2004
7) Izumi R, Yabushita K, Shimizu K, Yagi M, Yamaguchi A, Konishi K, Nakagawa T, Miyazaki I：Hepatic resection using a water jet dissector. Surg Today **23**：31, 1993
8) Jakob S, Kehler U, Reusche E, Friedrich HJ, Arnold H：Endoscopic use of the waterjet dissector in the cerebral ventricle system- and experimental study. Zentralbl Neurochir **61**：14-21, 2000
9) Keles GE, Lamborn KR, Berger MS：Low-grade hemispheric gliomas in adults：a critical review of extent of resection as a factor influencing outcome. J Neurosurg **95**：735-745, 2001
10) Kodama T, Takayama K, Uenohara H：A new technology for revascularization of cerebral embolism using liquid jet impact. Phys Med Biol **42**：2355-2367, 1997
11) 隈部俊宏，冨永悌二：血管温存に留意した神経膠腫摘出術．No Shinkei Geka **33**：19-27, 2005
12) Kumabe T, Higano S, Takahashi S, Tominaga T：Ischemic complications associated with resection of opercular glioma. J Neurosurg **105**：263-269, 2007
13) Nakagawa A, Hirano T, Komatsu M, Sato M, Uenohara H, Ohyama H, Kusaka Y, Shirane R, Takayama K, Yoshimoto T：Holmium：YAG laser-induced liquid jet knife：possible novel method for dissection. Lasers Surg Med **31**：129-135, 2002
14) Nakagawa A, Kusaka Y, Hirano T, Saito T, Shirane R, Takayama K, Yoshimoto T：Application of shock waves as a treatment modality in the vicinity of brain and skull. J Neurosurg **99**：156-162, 2003
15) Nakagawa A, Hirano T, Jokura H, Uenohara H, Ohki T, Hashimoto T, Menezes V, Sato Y, Kusaka Y, Ohyama H, Saito T, Takayama K, Shirane R, Tominaga T：Pulsed holmium：yttrium-aluminum-garnet laser-induced liquid jet as a novel dissection device in neuroendoscopic surgery. J Neurosurg **101**：145-150, 2004
16) Oertel J, Gaab MR, Knapp A, Essig H, Warzok R, Piek J：Water jet dissection in neurosurgery：experimental results in the porcine cadaveric brain. Neurosurgery **52**：153-159, 2003
17) Oertel J, Gaab MR, Pillich DT, Schroeder HW, Warzok R, Piek J：Comparison of waterjet dissection and ultrasonic aspiration：an *in vivo* study in the rabbit brain. J Neurosurg **100**：498-504, 2004
18) Oertel J, Wagner W, Piek J, Schroeder H, Gaab M：Waterjet dissection of gliomas-experience with 51 procedures. Minim Invasive Neurosurg **47**：154-159, 2004
19) Oertel J, Gen M, Krauss JK, Zumkeller M, Gaab MR：The use of waterjet dissection in endoscopic neurosurgery. Technical note. J Neurosurg **105**：928-931, 2006
20) Ohki T, Nakagawa A, Hirano T, Hashimoto T, Menezes V, Jokura H, Uenohara H, Sato Y, Saito T, Shirane R, Tominag T：Experimental application of pulsed Ho：YAG laser-induced liquid jet as a novel device for rigid neuroendoscope. Lasers Surg Med **34**：227-234, 2004
21) Papachristou DN, Barters R：Resection of the liver with a water jet. Br J Surg **69**：93-94, 1982
22) Piek J, Wille C, Warzok R, Gaab MR：Waterjet dissection of the brain-experimental and first clinical results. J Neurosurg **89**：861-864, 1998
23) Piek J, Oertel J, Gaab MR：Waterjet dissection in neurosurgical procedures-clinical results in 35 patients. J Neurosurg **96**：690-696, 2002
24) Sato Y, Nakagawa A, Hirano T, Ohki T, Uenohara H, Takahashi A, Takayama K, Tominaga T：Pulsed laser-induced liquid jet microcatheter system for rapid and reliable fibrinolysis in acute cerebral embolisms：experiments on safety and preliminary application in porcine cranial vessels. Min Invasive Neurosurg **50**：212-218, 2007
25) Schurr MO, Wehrmann M, Kunert W, Melzer A, Lirici MM, Trapp R, Kanehira E, Buess G：Histologic effects of different technologies for dissection in endoscopic surgery：ND：YAG laser；high frequency and water-jet. Endosc Surg Allied Technol **2**：195-201, 1994
26) Terzis AJ, Nowak G, Rentzsch O, Arnold H, Diebold J, Baretton G：A new system for cutting brain tissue preserving vessels：water jet cutting Br J Neurosurg **3**：361-366, 1989

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Special Focus: Glioma Therapy

"Armed" oncolytic herpes simplex viruses for brain tumor therapy

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Abbreviations: HSV-1, herpes simplex virus type 1; PKR, double-stranded RNA-dependent protein kinase; ICP6, infected-cell protein 6; pfu, plaque-forming units; IL, interleukin; GM-CSF, granulocyte macrophage colony-stimulating factor; GALV.fus, gibbon ape leukemia virus envelope fusogenic membrane glycoprotein; yCD, yeast cytosine deaminase; TNFα, tumor necrosis factor alpha

Key words: oncolytic virus therapy, gene therapy, herpes simplex virus, viral vectors, G47Δ, G207, antitumor immunity

Genetically engineered, conditionally replicating herpes simplex viruses type 1 (HSV-1) are promising therapeutic agents for brain tumors and other solid cancers. They can replicate in situ, spread and exhibit oncolytic activity via a direct cytocidal effect. One of the advantages of HSV-1 is the capacity to incorporate large and/or multiple transgenes within the viral genome. Oncolytic HSV-1 can therefore be "armed" to add certain functions. Recently, the field of armed oncolytic HSV-1 has drastically advanced, due to development of recombinant HSV-1 generation systems that utilize bacterial artificial chromosome and multiple DNA recombinases. Because antitumor immunity is induced in the course of oncolytic activities of HSV-1, transgenes encoding immunomodulatory molecules have been most frequently used for arming. Other armed oncolytic HSV-1 include those that express antiangiogenic factors, fusogenic membrane glycoproteins, suicide gene products, and proapoptotic proteins. Provided that the transgene product does not interfere with viral replication, such arming of oncolytic HSV-1 results in augmentation of antitumor efficacy. Immediate-early viral promoters are often used to control the arming transgenes, but strict-late viral promoters have been shown useful to restrict the expression in the late stage of viral replication when desirable. Some armed oncolytic HSV-1 have been created for the purpose of noninvasive in vivo imaging of viral infection and replication. Development of a wide variety of armed oncolytic HSV-1 will lead to an establishment of a new genre of therapy for brain tumors as well as other cancers.

Introduction

Oncolytic virus therapy is an attractive and rapidly developing means for treating cancer.[1] Genetically engineered viruses, such as herpes simplex virus type 1 (HSV-1) and adenovirus, are designed so that virus replication is restricted to tumor cells and therefore infection causes no harm to normal tissues. In principle, infected tumor cells are destroyed by a direct oncolytic activity of the viruses. Importantly, oncolytic viruses can also act as vectors that provide amplified transgene delivery.

HSV-1, especially in comparison with adenovirus, has suitable features for cancer therapy: (1) HSV-1 infects most tumor cell types. (2) A relatively low multiplicity of infection is needed for total cell killing. (3) Anti-viral drugs are available. (4) A large genome (~152 kb) allows the insertion of large and/or multiple transgenes. (5) The host immune reactions enhance antitumor effects. (6) Circulating anti-HSV-1 antibodies do not affect cell-to-cell spread of the virus. (7) There are HSV-1 sensitive mouse and nonhuman primate models for preclinical evaluation. (8) Viral DNA is not integrated into the host genome. HSV-1 is neurotropic and the genes necessary for neuropathogenicity have been identified and can be mutated. Therefore, the use of HSV-1 is especially advantageous for brain tumor therapy.

In order to target HSV-1 replication to tumor cells, viral genes that are essential for viral replication in normal cells but dispensable in tumor cells are inactivated or deleted.[2] This principle uses features common for all types of cancer, therefore the application of oncolytic HSV-1 is not restricted to brain tumors, but also includes a wide variety of cancer. The key for successful and practical development of oncolytic HSV-1 is to achieve a wide therapeutic window by the use of genetic engineering technology.

Second-Generation Oncolytic HSV-1

G207 was the first oncolytic HSV-1 used in a clinical trial in the United States.[3] This second-generation oncolytic HSV-1 has double mutations created in the HSV-1 genome.[4] G207 has deletions in both copies of the *γ34.5* gene, the major determinant of HSV-1 neurovirulence.[5] *γ34.5*-deficient HSV-1 vectors are considerably attenuated in normal cells, but retain their ability to replicate within neoplastic cells. In normal cells, HSV-1 infection induces activation of double-stranded RNA-dependent protein kinase (PKR), which in turn leads to phosphorylation of the α-subunit of eukaryotic initiation factor 2 and a subsequent shutdown of host and viral protein synthesis.[6] The product of the *γ34.5* gene antagonizes this PKR activity. However, tumor cells have low PKR activities, thereby allowing *γ34.5*-deficient HSV-1 vectors to replicate.[7,8] G207 also has an insertion of the *E. coli lacZ* gene in the infected-cell protein 6

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(*ICP6*) coding region (UL39), inactivating ribonucleotide reductase, a key enzyme for viral DNA synthesis in non-dividing cells but not in dividing cell.[9]

In preclinical studies using immunocompetent animals, the most remarkable finding with G207 was that it induced systemic antitumor immunity in the course of oncolytic activity.[10,11] For example, in A/J mice bearing bilateral subcutaneous N18 (syngeneic neuroblastoma) tumors, intraneoplastic G207 inoculation into the left tumor alone caused growth reduction not only of the inoculated tumors but also of the non-inoculated contralateral tumors. The antitumor immunity was associated with an elevated cytotoxic T lymphocyte activity specific to N18 tumor cells that persisted for at least 13 months.

After an extensive in vivo safety evaluation using HSV-1-susceptible mice and non-human primates, the G207 phase I clinical trial was performed between 1998 and 2000 at two institutions.[3] Twenty-one patients with recurrent malignant glioma were treated, and G207 was administered directly into the tumor via stereotactic inoculation. This dose escalation study started from 10^6 plaque-forming units (pfu) and increased to 3×10^9 pfu, with three patients at each dose. As a result, no acute, moderate to severe adverse events attributable to G207 were observed. Eight of 20 patients that had serial MRI evaluations had a decrease in tumor volume between four days and one month post-inoculation and two patients survived for more than five years.

Third-Generation Oncolytic HSV-1

The phase I clinical trial proved the safety of G207 and hinted its efficacy for human brain tumors. However, in order to further improve the efficacy without compromising its safety, a third-generation oncolytic HSV-1 termed G47Δ was newly created from G207 by introducing another genetic alteration, i.e., the deletion of the *α47* gene and the overlapping *US11* promoter region, in the G207 genome.[12] Because the *α47* gene product 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 when the *α47* gene is deleted.[13] G47Δ-infected human cells in fact presented higher levels of MHC class I expression than cells infected with other HSV-1 vectors.[12] Further, human melanoma cells infected with G47Δ were better at stimulating their matched tumor-infiltrating lymphocytes in vitro than those infected with G207. 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 including G207.[14] In the majority of cell lines tested, G47Δ replicated better than G207, resulting in the generation of higher virus titers, and exhibiting greater cytopathic effect.[12] In athymic mice bearing subcutaneous U87MG human glioma and A/J mice bearing subcutaneous Neuro2a neuroblastoma, G47Δ was significantly more efficacious than G207 at inhibiting the tumor growth when inoculated intraneoplastically.[12] G47Δ was also more efficacious than G207 in athymic mice bearing intracerebral U87MG tumors (Ino Y et al., manuscript in preparation). Nevertheless, the safety of G47Δ remained unchanged from G207 following injection into the brain of HSV-1-sensitive A/J mice.[12] In Japan, a clinical trial of G47Δ in recurrent glioblastoma patients is underway. G47Δ has been shown efficacious in animal tumor models of a variety of cancers including brain tumors, prostate cancer, breast cancer and neurofibroma.[12,15-17]

Construction of "Armed" Oncolytic HSV-1

One of the advantages of HSV-1 is the capacity to incorporate large and/or multiple transgenes within the viral genome. Certain antitumor functions may be added to oncolytic activities of HSV-1. The use of replication-competent HSV-1 for transgene expression has multiple attractive advantages over replication-incompetent or defective HSV-1 vectors: (1) A continuous generation of a high-titer, homogenous vector stock is possible, which allows manufacturing of a large amount with a better quality control. (2) An amplified gene delivery can be obtained in vivo. And, (3) transgene expression may lower administering doses required, therefore decrease toxicity. On the other hand, potential demerits of using replication-competent viruses for expressing foreign proteins are that the transgene expression may increase the toxicity of the vector, and may also interfere with viral replication. Transgene expression by armed oncolytic HSV-1 could be shorter in duration than replication-incompetent vectors due to destruction of the host cell by viral replication. However, we have observed that, because continuous viral spread and infection occur within the tumor, a larger number of tumor cells consequently express the transgene, therefore a much higher total amount of transgene product is achieved compared with non-replicating vectors.

In the past, a recombinant HSV-1 was constructed by conventional homologous recombination techniques that required selection of a correctly structured clone from millions of candidates. It often took a few years until the intended HSV-1 was obtained. In order to circumvent the time-consuming processes, we have developed an innovative "armed" oncolytic HSV-1 construction system using G47Δ as the backbone.[18] Besides its favorable features for human cancer therapy, including the safety, high yields of virus, improved oncolytic activity and potent stimulation of antitumor immune cells, G47Δ is especially suited as a replication-competent backbone for expressing any foreign protein molecules, because of the wide therapeutic window and preclusion of the shutoff of protein synthesis in the infected host cells. The system, termed T-BAC system, utilizes bacterial artificial chromosome and two DNA recombinase systems (Cre/loxP and FLP/FRT) (Fig. 1). It allows (1) a construction of armed oncolytic HSV-1 in a short period (usually 3–4 months), (2) a simultaneous construction of multiple vectors, (3) an accurate insertion of a desired transgene into the deleted *ICP6* locus, (4) an insertion of multiple transgenes using the same effort as inserting a single transgene, and (5) a direct comparison of multiple "armed" oncolytic HSV-1 with the same backbone. A similar system, termed HSVQuik system, has been also developed using a G207-like backbone.[19,20]

Oncolytic HSV-1 Armed with Immunostimulatory Genes

Aside from the extent of replication capability within the tumor, the efficacy of an oncolytic HSV-1 depends on the extent of antitumor immunity induction.[10,11] Therefore, while any transgene that does not interfere with HSV-1 replication may be used, the genes encoding immunomodulatory molecules would be reasonable candidates for arming oncolytic HSV-1. Immunostimulatory functions should augment the antitumor immunity induction that adds to

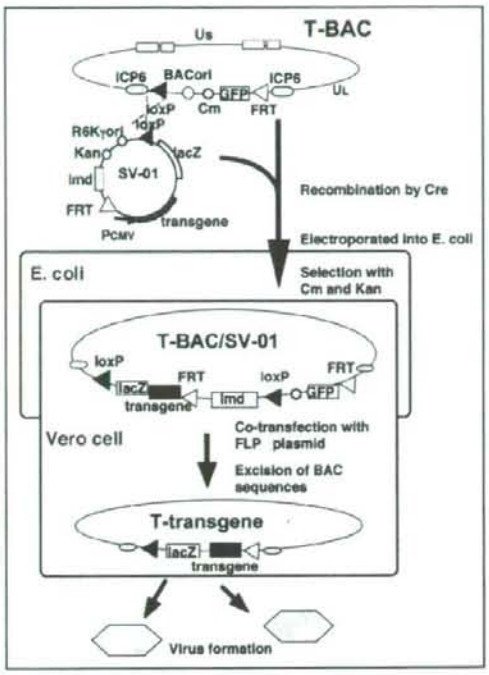

Figure 1. A schema describing the T-BAC system for constructing "armed" oncolytic HSV-1 with the G47Δ backbone. The desired transgene for "arming" is inserted into the multiple cloning site of the shuttle vector (SV-01). The first step is to insert the entire sequence of the shuttle vector into the loxP site of T-BAC by a Cre-mediated recombination, followed by an electroporation into *E. coli*. The second step is to co-transfect the co-integrate with a plasmid expressing FLP onto Vero cells to excise the BAC sequence flanked by the FRT sites. The objective armed oncolytic HSV-1 appear as GFP-negative and lacZ-positive virus plaques. Non-recombined viruses do not appear, due to the presence of the lambda stuffer sequence (Imd) causing an oversize of the genome.[18]

direct oncolytic activity of the virus, resulting in enhanced antitumor activities (Fig. 2).[2] *γ34.5* -deficient HSV-1 containing the murine interleukin 4 (IL-4) gene displayed a significantly higher antitumor activity and prolonged survival of mice with intracranial tumors compared with its parental virus or the one expressing IL-10.[21] First-generation oncolytic HSV-1 expressing IL-12 (M002 and NV1042) showed improved in vivo efficacy against 4C8 glioma in syngeneic B6D2F mice[22] and brain tumors of Neuro2a neuroblastoma in syngeneic A/J mice,[23] and also against murine squamous cell carcinoma[24] and murine colorectal tumor.[25] Immunohistochemical analyses of tumors treated with these IL-12-expressing HSV-1 revealed a significant influx of $CD4^+$, $CD8^+$ T cells and macrophages. The oncolytic HSV-1 expressing IL-12 (NV1042) was more efficacious than the one expressing granulocyte macrophage colony-stimulating factor (GM-CSF) in the same backbone (NV1034) in mice with subcutaneous squamous cell carcinoma.[24] The mice cured by NV1042 had a higher rate of rejecting rechallenged tumor cells than those cured by NV1034.[24]

The HSVQuik was used to create G207-like second-generation oncolytic HSV-1 armed with murine IL-4, CD40 ligand or 6CK (Fig. 3).[20] In BALB/c mice bearing 4T1 breast cancer in the brain, all of these armed HSV-1 showed better antitumor efficacy than the control virus. Using the HSVQuik system, we also created oncolytic HSV-1 armed with IL-12, IL-18 or soluble B7-1.[19] All of these armed HSV-1 demonstrated replicative capabilities similar to the parental virus in vitro. The in vivo efficacy was tested in A/J mice harboring subcutaneous tumors of syngeneic and poorly immunogenic Neuro2a neuroblastoma. IL-12 was the most efficacious among the immunostimulatory molecules investigated when expressed by the G207-like HSV-1. The triple combination of the three armed viruses exhibited the highest efficacy amongst all single viruses or combinations of two viruses. Combining 1×10^5 pfu each of the three armed viruses showed stronger antitumor activities than any single armed virus at 3×10^5 pfu in inoculated tumors as well as non-inoculated remote tumors.

Using the Neuro2a subcutaneous tumor model, another research group demonstrated that the antitumor efficacy of M002, a first-generation *γ34.5*-deficient HSV-1 that expresses IL-12, could be augmented when used in combination with M010, the same backbone HSV-1 that expresses chemokine CCL2.[26] The group also demonstrated that the virus selected after in vivo serial passage of M002 in tumors of a D54-MG human malignant glioma cell line improved survival in two independent murine brain tumor models compared to the parent M002.[27] This enhanced antitumor efficacy was not due to restoration of protein synthesis or early *US11* expression.

Recently, using the T-BAC system, we generated a G47Δ-backbone oncolytic HSV-1 armed with mouse fusion-type IL-12, termed T-mflL12 (Fig. 3). In A/J mice bearing bilateral subcutaneous Neuro2a tumors, intraneoplastic inoculation with T-mflL12 into the left tumor alone led to a significantly better antitumor activity than the unarmed control virus, T-01, not only in the inoculated left tumors but also in the non-inoculated remote tumors (Miyamoto S, et al., manuscript in preparation). We also created a G47Δ-backbone HSV-1 armed with both IL-18 and soluble B7-1.[18] This double-armed oncolytic HSV-1 showed a significant enhancement of antitumor efficacy via T-cell mediated immune responses in A/J mice with subcutaneous Neuro2a tumors as well as in C57BL/6 mice bearing subcutaneous TRAMP-C2 prostate cancer.

An armed oncolytic HSV-1 has not been tested in patients with brain tumors, however a phase I clinical trial with a second-generation oncolytic HSV-1 expressing GM-CSF was conducted in patients with cutaneous or subcutaneous deposits of breast, head and neck and gastrointestinal cancers and recurrent malignant melanoma.[28] $OncoVEX^{GM\text{-}CSF}$ has a deletion in the *α47* gene and the *γ34.5* gene replaced with the *GM-CSF* gene driven by a CMV promoter (Fig. 3). A single dose (13 patients) or multiple doses (17 patients), ranging from 10^6 to 10^8 pfu/ml/dose, were injected intratumorally. Local inflammation, erythema and febrile responses were the main side effects, and the local reaction to injection was dose limiting in HSV-1-seronegative patients at 10^7 pfu/ml. Some of biopsy specimens after treatment showed areas of necrosis that strongly stained for HSV-1. Three patients had stable disease, six patients showed flattened injected and/or uninjected tumors, and four patients showed inflammation of uninjected tumors.

Armed Oncolytic HSV-1 with Other Antitumor Functions

Various types of transgenes other than immunomodulatory genes have been used to arm oncolytic HSV-1. Theoretically, antiangiogenic factors can augment the antitumor activities of oncolytic HSV-1 without compromising the viral replication and antitumor immunity induction. Early generation oncolytic HSV-1, such as G207, was shown to retain the ability of wild type HSV-1 to increase infected tissue vascularity, whereas third-generation G47Δ showed suppressed vascularity in infected tumors.[29] By using the G47Δ-BAC system, a preliminary version of the T-BAC system, G47Δ-backbone oncolytic HSV-1 armed with Platelet Factor 4 or dominant negative fibroblast growth factor receptor have been created.[30,31] Both of these armed oncolytic HSV-1 were more efficacious in inhibiting the tumor growth and angiogenesis than the control virus in both human U87MG glioma and mouse 37-3-18-4 malignant peripheral nerve sheath tumor models. By using the HSVQuik system, an oncolytic HSV-1 armed with tissue inhibitor of metalloproteinases 3, termed rQT3, has been created.[32] In athymic mice bearing human neuroblastoma or malignant peripheral nerve sheath tumor, treatment with rQT3 caused delayed tumor growth, increased peak levels of infectious virus, and immature collagen extracellular matrix. Remarkably, rQT3 treatment caused reduced tumor vascular density, which was associated with reduced circulating endothelial progenitors.

Another approach for arming oncolytic HSV-1 is the use of fusogenic membrane glycoproteins. Expression of fusogenic proteins by infected tumor cells could cause involvement of surrounding uninfected cells to form syncytium and facilitate tumor cell killing, but might also increase toxicity in the normal tissue. Fu et al. constructed an oncolytic HSV-1 armed with a truncated form of the gibbon ape leukemia virus envelope fusogenic membrane glycoprotein (*GALV.fus*).[55] In athymic mice bearing human Hep 3B hepatocellular carcinoma xenografts, the expression of *GALV.fus* significantly enhanced the antitumor effect of the virus. Furthermore, by using a strict late viral promoter instead of a CMV promoter, *GALV.fus* glycoprotein could be expressed only in tumor cells and not in normal non-dividing cells.

So-called suicide genes have been used from early stages of armed oncolytic HSV-1 development. Expression of a suicide gene by an infected tumor cell should elicit bystander killing of surrounding uninfected tumor cells via extracellular diffusion of activated prodrug, but premature killing of the host cell could also suppress viral replication. HSV-1 naturally expresses thymidine kinase that activates the prodrug ganciclovir. However, a combination with systemic ganciclovir administration did not significantly enhance the efficacy of G207 in A/J mice with intracerebral N18 neuroblastoma.[34] rRp450 was engineered by replacing the *lacZ* gene within the *ICP6* locus of the first-generation oncolytic HSV-1, hrR3, with the gene encoding rat cytochrome P450 2B1 (CYP2B1), a member of the cytochrome P450 family responsible for activating the prodrug cyclophosphamide.[35] In rat 9L and human U87ΔEGFR glioma models, systemic administration of both cyclophosphamide and ganciclovir in combination with rRp450 showed the most efficacy compared with any other combinations.[36] By using the HSVQuik system, an oncolytic HSV-1, termed MGH2, was created that expressed both CYP2B1 and secreted human intestinal carboxylesterase.[37] The latter enzyme converts irinotecan into an active metabolite. In athymic mice bearing Gli36ΔEFGR glioma in the brain, MGH2 displayed increased antitumor efficacy when combined with cyclophosphamide and irinotecan. The researchers found that, unlike ganciclovir, cyclophosphamide, irinotecan or the combination of both did not significantly affect virus replication. HSV1yCD was created by replacing the *ICP6* gene of HSV-1 with the gene encoding yeast cytosine deaminase (yCD).[38] yCD converts the prodrug 5-fluorocytosine (5-FC) to a cytotoxic agent, 5-fluorouracil. This research group also observed that the approach enhanced cytotoxicity without significantly reducing viral replication and oncolysis. In BALB/c mice bearing subcutaneous tumors or diffuse liver metastases of MC26 colon cancer, anti-neoplastic activity of HSV1yCD combined with systemic 5-FC administration was greater than HSV1yCD alone. By utilizing the same backbone as OncoVEX$^{GM\text{-}CSF}$ (Fig. 3), an oncolytic HSV-1 termed OncoVEX$^{GALV/CD}$ double-armed with yCD/uracil phosphoribosyltransferase fusion and GALV.fus has been created.[39] In Fischer f344 rats bearing subcutaneous 9L glioma, OncoVEX$^{GALV/CD}$ proved most efficacious compared with the control viruses (OncoVEX, OncoVEX^{GALV} or OncoVEX^{CD}) when combined with systemic 5-FC administration.

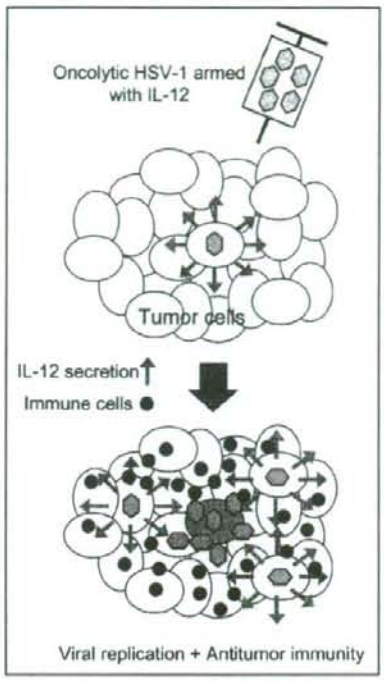

Figure 2. Concept of antitumor efficacy augmentation using oncolytic HSV-1 armed with an immunostimulatory gene. When oncolytic HSV-1 armed with the *IL-12* gene infects tumor cells, IL-12 is secreted in the course of viral replication and stimulates the immune cells. In addition to direct tumor cell killing via viral replication and spread, tumor cells are destroyed by augmented antitumor immune responses, resulting in enhanced antitumor activities.

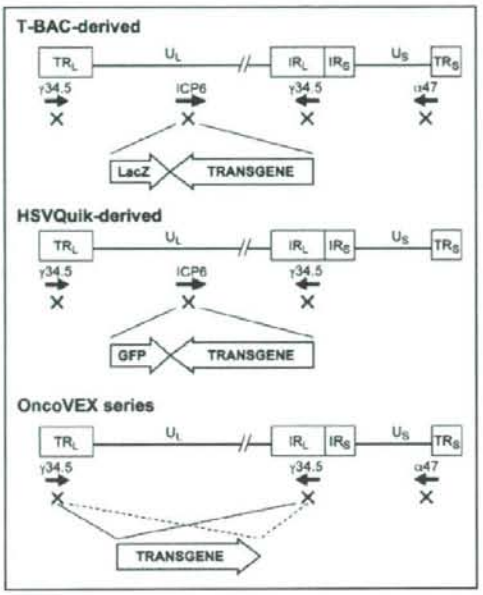

Figure 3. Structures of representative armed oncolytic HSV-1. The HSV-1 genome consists of long and short unique regions (U_L and U_S) each bounded by terminal (T) and internal (I) repeat regions (R_L and R_S). Armed oncolytic HSV-1 created by using the T-BAC (or G47Δ-BAC) system has the backbone structure of G47Δ, a third-generation oncolytic HSV-1. It has triple deletions in the *γ34.5*, *ICP6* and *α47* genes. The transgene is inserted into the deleted *ICP6* locus. As a marker, it also expresses the *lacZ* gene driven by the *ICP6* promoter. Armed oncolytic HSV-1 created by using the HSVQuik system has the backbone structure similar to G207 or MGH1, second-generation oncolytic HSV-1. It has double deletions in the *γ34.5* and *ICP6* genes. The transgene is inserted into the deleted *ICP6* locus. As a marker, it also expresses the *GFP* gene driven by the *ICP6* promoter. The OncoVEX series has the backbone structure of a second-generation oncolytic HSV-1 with double deletions in the *γ34.5* and *α47* genes. The transgene is inserted into the deleted *γ34.5* loci.

Han et al. recently created an oncolytic HSV-1, with double deletions in the *γ34.5* and *α47* genes, armed with tumor necrosis factor alpha (TNFα).[40] TNFα is a cytokine with a potent antitumor activity, but a local delivery of TNFα is known to cause toxicity, and its ability to induce tumor cell apoptosis could interfere with viral replication. To avoid these problems, they used the *US11* true late HSV-1 promoter to drive the *TNFα* gene. Whereas the virus armed with *US11*-driven TNFα expressed lower amounts of TNFα, it exhibited higher antitumor effects and less toxicity than the virus that used the immediate-early CMV promoter.

Armed Oncolytic HSV-1 for in vivo Imaging

With the advancement of oncolytic virus therapy development, there has been an increasing need for non-invasive methods of imaging or monitoring of viral infection and replication. Oncolytic HSV-1 can be armed not only for the purpose of augmenting the therapeutic efficacy but also for realizing such non-invasive in vivo imaging. In preclinical settings, one approach is to utilize a luciferase-based bioluminescent system. Two HSVQuik-based oncolytic HSV-1 were generated that express firefly luciferase under the control of the *immediate-early (IE) 4/5* promoter or *gC* promoter.[41] The *IE4/5* promoter acts immediately after viral infection, whereas the strict late *gC* promoter acts in the late stage of the replication cycle. When athymic mice bearing subcutaneous tumors of Gli36ΔEGFR glioma were observed under a supersensitive charged coupled device camera, the expression of luciferase controlled by the *IE4/5* promoter correlated with viral infection and that by the *gC* promoter with viral replication.

Systemic Delivery of Armed Oncolytic HSV-1

Whereas the most common route of delivery of oncolytic HSV-1 has been a direct intratumoral inoculation, an intravenous delivery would further broaden the clinical application of oncolytic HSV-1 if proven effective. The main hurdle for intravenous delivery is that only a small percentage of the administered virus reaches the tumor. By arming of oncolytic HSV-1, a large antitumor effect can be induced from a small number of virus that initiates replication at the tumor. We observed that intravenous delivery of IL-12-expressing T-mflL12 caused a significant inhibition of tumor growth compared with mock and the unarmed control virus (T-01) treatments in A/J mice bearing subcutaneous Neuro2a tumors (Guan et al., manuscript in preparation). When A/J mice bearing intracerebral tumors were treated by repeated intravenous injections, T-mflL12, but not T-01, significantly prolonged the survival compared with mock. Also, in a renal cancer lung metastases model using BALB/c mice and syngeneic RenCa cells, intravenous administrations of T-mflL12 significantly inhibited the number of metastases compared with mock and T-01 treatments (Tsurumaki et al., manuscript in preparation).

Summary

In summary, "arming" of oncolytic HSV-1 with transgenes leads to development of a variety of oncolytic HSV-1 with certain functions resulting in enhancement of antitumor efficacy and/or in vivo imaging capability. In the future, a series of armed oncolytic HSV-1 suited for certain tumor types or certain administration routes may be used differentially or in combination according to conditions of patients. Armed oncolytic HSV-1 has high potential as a new genre of therapy for brain tumors as well as other cancers.

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References

1. Aghi M, Martuza RL. Oncolytic viral therapies—the clinical experience. Oncogene 2005; 24:7802-16.
2. Todo T. Oncolytic virus therapy using genetically engineered herpes simplex viruses. Front Biosci 2008; 13:2060-4.
3. Markert JM, Medlock MD, Rabkin SD, Gillespie GY, Todo T, Hunter WD, Palmer CA, Feigenbaum F, Tornatore C, Tufaro F, Martuza RL. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial [see comments]. Gene Ther 2000; 7:867-74.
4. Mineta T, Rabkin SD, Yazaki T, Hunter WD, Martuza RL. Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. Nat Med 1995; 1:938-43.
5. Chou J, Kern ER, Whitley RJ, Roizman B. Mapping of herpes simplex virus-1 neurovirulence to gamma 34.5, a gene nonessential for growth in culture. Science 1990; 250:1262-6.
6. He B, Chou J, Brandimarti R, Mohr I, Gluzman Y, Roizman B. Suppression of the phenotype of gamma(1)34.5-herpes simplex virus 1: failure of activated RNA-dependent protein kinase to shut off protein synthesis is associated with a deletion in the domain of the alpha47 gene. J Virol 1997; 71:6049-54.

7. Leib DA, Machalek MA, Williams BR, Silverman RH, Virgin HW. Specific phenotypic restoration of an attenuated virus by knockout of a host resistance gene. Proc Natl Acad Sci USA 2000; 97:6097-101.
8. Farassati F, Yang AD, Lee PW. Oncogenes in Ras signalling pathway dictate host-cell permissiveness to herpes simplex virus 1. Nat Cell Biol 2001; 3:745-50.
9. Goldstein DJ, Weller SK. Herpes simplex virus type 1-induced ribonucleotide reductase activity is dispensable for virus growth and DNA synthesis: isolation and characterization of an ICP6 lacZ insertion mutant. J Virol 1988; 62:196-205.
10. Todo T, Rabkin SD, Sundaresan P, Wu A, Meehan KR, Herscowitz HB, Martuza RL. Systemic antitumor immunity in experimental brain tumor therapy using a multimutated, replication-competent herpes simplex virus. Hum Gene Ther 1999; 10:2741-55.
11. Todo T, Rabkin SD, Chahlavi A, Martuza RL. Corticosteroid administration does not affect viral oncolytic activity, but inhibits antitumor immunity in replication-competent herpes simplex virus tumor therapy. Hum Gene Ther 1999; 10:2869-78.
12. Todo T, Martuza RL, Rabkin SD, Johnson PA. Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. Proc Natl Acad Sci USA 2001; 98:6396-401.
13. York IA, Roop C, Andrews DW, Riddell SR, Graham FL, Johnson DC. A cytosolic herpes simplex virus protein inhibits antigen presentation to CD8+ T lymphocytes. Cell 1994; 77:525-35.
14. Mohr I, Gluzman Y. A herpesvirus genetic element which affects translation in the absence of the viral GADD34 function. Embo J 1996; 15:4759-66.
15. Fukuhara H, Martuza RL, Rabkin SD, Ito Y, Todo T. Oncolytic herpes simplex virus vector G47Δ in combination with androgen ablation for the treatment of human prostate adenocarcinoma. Clin Cancer Res 2005; 11:7886-90.
16. Liu R, Varghese S, Rabkin SD. Oncolytic herpes simplex virus vector therapy of breast cancer in C3(1)/SV40 T-antigen transgenic mice. Cancer Res 2005; 65:1532-40.
17. Messerli SM, Prabhakar S, Tang Y, Mahmood U, Giovannini M, Weissleder R, Bronson R, Martuza R, Rabkin S, Breakefield XO. Treatment of schwannomas with an oncolytic recombinant herpes simplex virus in murine models of neurofibromatosis type 2. Hum Gene Ther 2006; 17:20-30.
18. Fukuhara H, Ino Y, Kuroda T, Martuza RL, Todo T. Triple gene-deleted oncolytic herpes simplex virus vector double-armed with interleukin 18 and soluble B7-1 constructed by bacterial artificial chromosome-mediated system. Cancer Res 2005; 65:10663-8.
19. Ino Y, Saeki Y, Fukuhara H, Todo T. Triple combination of oncolytic herpes simplex virus-1 vectors armed with interleukin-12, interleukin-18 or soluble B7-1 results in enhanced antitumor efficacy. Clin Cancer Res 2006; 12:643-52.
20. Terada K, Wakimoto H, Tyminski E, Chiocca EA, Saeki Y. Development of a rapid method to generate multiple oncolytic HSV vectors and their in vivo evaluation using syngeneic mouse tumor models. Gene Ther 2006; 13:705-14.
21. Andreansky S, He B, van Cott J, McGhee J, Markert JM, Gillespie GY, Roizman B, Whitley RJ. Treatment of intracranial gliomas in immunocompetent mice using herpes simplex viruses that express murine interleukins. Gene Ther 1998; 5:121-30.
22. Hellums EK, Markert JM, Parker JN, He B, Perbal B, Roizman B, Whitley RJ, Langford CP, Bharara S, Gillespie GY. Increased efficacy of an interleukin-12-secreting herpes simplex virus in a syngeneic intracranial murine glioma model. Neuro Oncol 2005; 7:213-24.
23. Parker JN, Gillespie GY, Love CE, Randall S, Whitley RJ, Markert JM. Engineered herpes simplex virus expressing IL-12 in the treatment of experimental murine brain tumors. Proc Natl Acad Sci USA 2000; 97:2208-13.
24. Wong RJ, Patel SG, Kim S, DeMatteo RP, Malhotra S, Bennett JJ, St-Louis M, Shah JP, Johnson PA, Fong Y. Cytokine gene transfer enhances herpes oncolytic therapy in murine squamous cell carcinoma. Hum Gene Ther 2001; 12:253-65.
25. Bennett JJ, Malhotra S, Wong RJ, Delman K, Zager J, St-Louis M, Johnson P, Fong Y. Interleukin 12 secretion enhances antitumor efficacy of oncolytic herpes simplex viral therapy for colorectal cancer. Ann Surg 2001; 233:819-26.
26. Parker JN, Meleth S, Hughes KB, Gillespie GY, Whitley RJ, Markert JM. Enhanced inhibition of syngeneic murine tumors by combinatorial therapy with genetically engineered HSV-1 expressing CCL2 and IL-12. Cancer Gene Ther 2005; 12:359-68.
27. Shah AC, Price KH, Parker JN, Samuel SL, Meleth S, Cassady KA, Gillespie GY, Whitley RJ, Markert JM. Serial passage through human glioma xenografts selects for a Deltagamma134.5 herpes simplex virus type 1 mutant that exhibits decreased neurotoxicity and prolongs survival of mice with experimental brain tumors. J Virol 2006; 80:7308-15.
28. Hu JC, Coffin RS, Davis CJ, Graham NJ, Groves N, Guest PJ, Harrington KJ, James ND, Love CA, McNeish I, Medley LC, Michael A, Nutting CM, Pandha HS, Shorrock CA, Simpson J, Steiner J, Steven NM, Wright D, Coombes RC. A phase I study of OncoVEXGM-CSF, a second-generation oncolytic herpes simplex virus expressing granulocyte macrophage colony-stimulating factor. Clin Cancer Res 2006; 12:6737-47.
29. Aghi M, Rabkin SD, Martuza RL. Angiogenic response caused by oncolytic herpes simplex virus-induced reduced thrombospondin expression can be prevented by specific viral mutations or by administering a thrombospondin-derived peptide. Cancer Res 2007; 67:440-4.
30. Liu TC, Zhang T, Fukuhara H, Kuroda T, Todo T, Canron X, Bikfalvi A, Martuza RL, Kurtz A, Rabkin SD. Dominant-negative fibroblast growth factor receptor expression enhances antitumoral potency of oncolytic herpes simplex virus in neural tumors. Clin Cancer Res 2006; 12:6781-9.
31. Liu TC, Zhang T, Fukuhara H, Kuroda T, Todo T, Martuza RL, Rabkin SD, Kurtz A. Oncolytic HSV armed with platelet factor 4, an antiangiogenic agent, shows enhanced efficacy. Mol Ther 2006; 14:789-97.
32. Mahller YY, Vaikunth SS, Ripberger MC, Baird WH, Saeki Y, Cancelas JA, Crombleholme TM, Cripe TP. Tissue inhibitor of metalloproteinase-3 via oncolytic herpesvirus inhibits tumor growth and vascular progenitors. Cancer Res 2008; 68:1170-9.
33. Fu X, Tao L, Jin A, Vile R, Brenner MK, Zhang X. Expression of a fusogenic membrane glycoprotein by an oncolytic herpes simplex virus potentiates the viral antitumor effect. Mol Ther 2003; 7:748-54.
34. Todo T, Rabkin SD, Martuza RL. Evaluation of ganciclovir-mediated enhancement of the antitumoral effect in oncolytic, multimutated herpes simplex virus type 1 (G207) therapy of brain tumors. Cancer Gene Ther 2000; 7:959-66.
35. Chase M, Chung RY, Chiocca EA. An oncolytic viral mutant that delivers the CYP2B1 transgene and augments cyclophosphamide chemotherapy. Nature Biotechnol 1998; 16:444-8.
36. Aghi M, Cheu TC, Suling K, Breakefield XO, Chiocca EA. Multimodal cancer treatment mediated by a replicating oncolytic virus that delivers the oxazaphosphorine/rat cytochrome P450 2B1 and ganciclovir/herpes simplex virus thymidine kinase gene therapies. Cancer Res 1999; 59:3861-5.
37. Tyminski E, Leroy S, Terada K, Finkelstein DM, Hyatt JL, Danks MK, Potter PM, Saeki Y, Chiocca EA. Brain tumor oncolysis with replication-conditional herpes simplex virus type 1 expressing the prodrug-activating genes, CYP2B1 and secreted human intestinal carboxylesterase, in combination with cyclophosphamide and irinotecan. Cancer Res 2005; 65:6850-7.
38. Nakamura H, Mullen JT, Chandrasekhar S, Pawlik TM, Yoon SS, Tanabe KK. Multimodality therapy with a replication-conditional herpes simplex virus 1 mutant that expresses yeast cytosine deaminase for intratumoral conversion of 5-fluorocytosine to 5-fluorouracil. Cancer Res 2001; 61:5447-52.
39. Simpson GR, Han Z, Liu B, Wang Y, Campbell G, Coffin RS. Combination of a fusogenic glycoprotein, prodrug activation, and oncolytic herpes simplex virus for enhanced local tumor control. Cancer Res 2006; 66:4835-42.
40. Han ZQ, Assenberg M, Liu BL, Wang YB, Simpson G, Thomas S, Coffin RS. Development of a second-generation oncolytic Herpes simplex virus expressing TNFalpha for cancer therapy. J Gene Med 2007; 9:99-106.
41. Yamamoto S, Deckter LA, Kasai K, Chiocca EA, Saeki Y. Imaging immediate-early and strict-late promoter activity during oncolytic herpes simplex virus type 1 infection and replication in tumors. Gene Ther 2006; 13:1731-6.

脳腫瘍のウイルス療法

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治療技術の進歩にもかかわらず悪性脳腫瘍の治療成績はここ半世紀横ばいであり，革新的な治療法が切望される．ウイルス療法は腫瘍細胞で選択的に複製する増殖型ウイルスを用いて，ウイルスの直接的な殺細胞効果により腫瘍細胞を破壊する治療法である．とくにウイルスゲノムの操作により人為的に治療域の拡大を図る遺伝子組換えウイルスは，新しいがん治療薬としてその有用性と発展性に期待がかけられ，脳腫瘍に対してはアデノウイルスや単純ヘルペスウイルス1型(HSV-1)がすでに臨床で試されている．わが国では第三世代遺伝子組換えHSV-1のG47 Δを用いて，再発膠芽腫患者を対象とした臨床研究の準備が進んでいる．ウイルス療法は脳腫瘍にかぎらずあらゆる固形がんに対して効果が期待できることから，トランスレーショナルリサーチが強力に推進されることが望まれる．

Key Words
トランスレーショナルリサーチ
脳腫瘍
ウイルス療法
遺伝子治療
ヘルペスウイルスベクター

はじめに

膠芽腫(glioblastoma)は脳実質から発生する脳腫瘍のうち最も頻度が高くかつ最も予後が悪い．集学的治療にもかかわらず膠芽腫の生存期間中央値は約1年であり，それはこの半世紀ほぼ横ばいである．化学療法薬として有効なエビデンスを示して注目されるテモゾロミドも放射線治療併用により生存期間を2ヵ月延長したに過ぎない[1]．革新的な治療法が切望される．

悪性腫瘍にウイルスを感染させて治療する試みは古くは20世紀初頭から報告がある[2]．散発的な症例報告から動物実験や臨床試験へと進んだが，いずれもウイルスの病原性を制御できないことから実用化には至らなかった[3]．近年，遺伝子工学の進歩に伴い，ウイルスゲノムの遺伝子操作により腫瘍細胞特異的なウイルス複製を人工的に生じることが可能となり[4]，ウイルスを用いた腫瘍治療の開発がベンチからベッドサイドへと飛躍的に進歩した[5][6]．脳腫瘍をはじめ，種々のがんに対して臨床応用が期待される．

1．ウイルス療法とは

ウイルス療法(oncolytic virus therapy)とは，増殖型ウイルス(replication-competent virus)を腫瘍細胞へ感染させ，ウイルス複製に伴う直接的な殺細胞効果を利用した腫瘍の治療法である．治療遺伝子をベクターに組み込んで細胞内で発現させ，その効果を期待する遺伝子治

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表 1　脳腫瘍のウイルス療法のうち論文発表された代表的な臨床試験結果

試験相 実施国	ウイルス	投与量						
第 I 相 米国	ONYX-015 (adenovirus)	10^7~10^{10} pfu	腫瘍内	24	0/24	24/24	4.9(GBM) 11.3(AA,AO)	なし
第 I 相 米国	G207 (HSV-1)	10^9~3×10^9 pfu	腫瘍内	21	0/21	21/21	15.9(GBM) 40.5(AA)	なし
第 I 相 イギリス	1716 (HSV-1)	10^5 pfu	腫瘍内	12	2/12	10/12	3/12 alive at 15,18,22	なし
第 I / II 相 イスラエル	OV001 (NDV)	10^8~10^{10} IU/q.d.× 5 days/week × 1~2 weeks	静脈内	7	1/7	5/7	N/S	なし
第 I / II 相 イスラエル	NDV-HUJ (NDV)	up to 5.5×10^{10} dose escalation 1.1×10^{10} IU/week × 3weeks	静脈内	11	0/11	11/11	8	なし

pfu : plaque forming units, IU : infectious units, N/S : not stated, GBM : glioblastoma, AA : anaplastic astrocytoma, AO : anaplastic oligodendroglioma　(Aghi M *et al*, 2005[21] より改変引用)

療とは区別される．レオウイルス(reovirus)やニューカッスル病ウイルス(Newcastle disease virus：NDV)などヒトを宿主としないウイルスを用いる方法と，単純ヘルペスウイルスⅠ型(herpes simplex virus type1：HSV-1)，アデノウイルス(adenovirus)，ワクシニアウイルス(vaccinia virus)など，おもにヒトを宿主とするウイルスを用いる方法に大別される．後者の場合，ウイルスゲノムに遺伝子工学的な改変を加えてウイルス複製に腫瘍特異性をもたせる．悪性脳腫瘍に対してはNDVや遺伝子組換えHSV-1，遺伝子組換えアデノウイルスが臨床で試されている[7]~[11](**表1**[12])．

腫瘍治療用の増殖型ウイルスは腫瘍細胞に感染すると複製し，その過程で宿主となった腫瘍細胞を死滅させる．増えたウイルスは周囲の腫瘍細胞に広がって再び感染し，複製，細胞破壊，伝播をくり返す．一方正常細胞には感染しても複製できないため正常組織は傷害されず，したがって治療域(therapeutic window)が広く保たれる(**図1**)．とくにHSV-1は，ウイルスによる腫瘍細胞破壊に伴って特異的抗腫瘍免疫が誘導されることや，ゲノムサイズが大きいために治療遺伝子を組み込んで発現させることも可能で，複合的に抗腫瘍効果を発揮することができる．従来の放射線治療や化学療法との併用で治療効果を増強することもできる新しい治療アプローチとして期待される．遺伝子組換えウイルスを用いたウイルス療法の1998年の最初の臨床試験から11年が経過し，現在では世界で35を超える種々のがんを対象とした臨床試験がおこなわれている[13]．2005年にはE1B領域を欠失させた遺伝子組換えアデノウイルス(H101)が世界初のウイルス療法製剤として中国で認可された[14]．悪性脳腫瘍に対してはアデノウイルスよりHSV-1のほうが治療効果が高いと報告された[15]．本稿ではわれわれが研究を進める遺伝子組換えHSV-1を用いたウイルス療法を中心に解説する．

2．HSV-1を用いたウイルス療法の基礎理論

HSV-1はウイルス療法の開発をおこなううえで以下のような利点をもちあわせている．①あらゆる種類の腫瘍細胞に感染する．②ゲノムの配列やおもな遺伝子の機能が解明されている．③ゲノムサイズが大きく(152kb)，複数の外来遺伝子が挿入できる．④抗ウイルス薬が存在する．⑤殺細胞作用が比較的強い．⑥ウイルスゲノムが宿主細胞のゲノムに組み込まれない．⑦血中抗体がウイルスの細胞間伝播に影響しない．⑧宿主免疫が抗腫瘍効果に有利にはたらく．

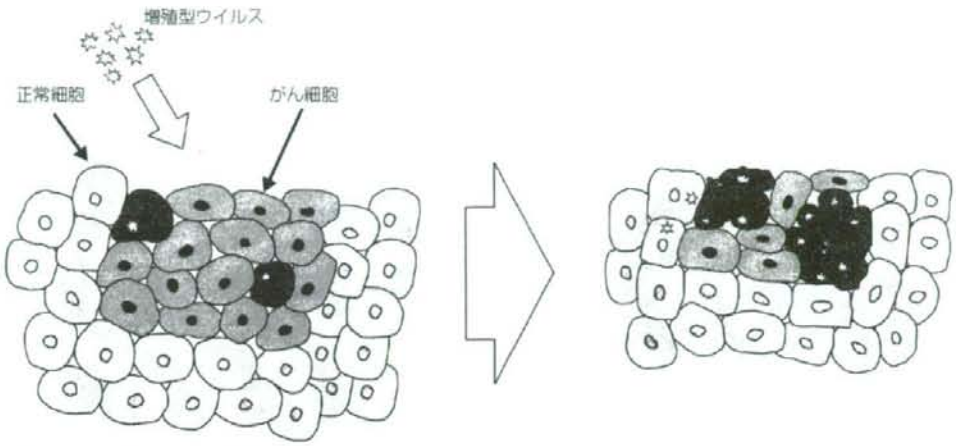

図1　ウイルス療法の概念(筆者作成)
ウイルスは腫瘍細胞に感染し，ウイルス複製をおこなって宿主細胞を死滅させる．増えたウイルスは周囲の細胞へ広がり再びウイルス複製，殺細胞，周囲への伝播をくり返す．正常細胞ではウイルス複製ができないため，正常組織は傷害されない．

腫瘍治療用のHSV-1(oncolytic HSV-1)は以下のような機序で腫瘍特異的に増殖するように工夫されている．

リボヌクレオチド還元酵素(ribonucleotide reductase：RR)やチミジンキナーゼ(thynidine kinase：TK)はウイルスのDNA合成に必須であるため，これらをコードするウイルス遺伝子を失活させると正常細胞で複製できない．しかし，腫瘍細胞ではこれらの酵素活性が上昇しているため，失活したウイルス遺伝子機能が代償され複製できる．

また，正常細胞ではウイルス感染に呼応して二本鎖RNA依存プロテインキナーゼ(protein kinase R：PKR)のリン酸化が誘導される．リン酸化PKRはeukaryotic initiation factor 2α(eIF-2α)のαサブユニットをリン酸化して宿主とウイルスの蛋白合成を遮断する．HSV-1のγ*34.5*遺伝子産物はリン酸化PKRに拮抗することで蛋白合成を可能にする．したがってγ*34.5*遺伝子を欠失させるとHSV-1は正常細胞で複製できないが，腫瘍細胞では一様にPKR活性がもともと低いためγ*34.5*遺伝子がなくても複製できる[9]．

3．遺伝子組換えHSV-1の開発：第一世代から第三世代まで

最初に開発された遺伝子組換えHSV-1は*tk*遺伝子を不活化させた*dl*sptkで，Martuzaら[10]が1991年に報告した．その後γ*34.5*遺伝子を欠失させたR3616や1716，RRの大サブユニットをコードする*ICP6*遺伝子を不活化したhrR3などが開発された．1716は英国で膠芽腫を対象に複数の第Ⅰ相臨床試験がおこなわれ，1.0×10^5から1.0×10^9 pfu(plaque forming units)までの量の腫瘍内投与で安全性が確認された[10][17]．HSV-1のゲノムの一部をHSV-2の遺伝子に置換したNV1020は，大腸がんの肝転移を対象に第Ⅰ相臨床試験がおこなわれた．これら第一世代HSV-1は，強い殺細胞効果を有する反面弱毒化が不十分で，変異が1ヵ所のため野生株にもどる可能性があるなど，安全性に課題を残した．

臨床応用を見据えて，安全性を重視した第二世代HSV-1のG207が開発された[11]．G207はγ*34.5*遺伝子欠失と*ICP6*遺伝子不活化の二重変異を有する．野生株へもどる可能性がほぼなくなり，複製の腫瘍特異性が高まるなどの特徴を示した．米国2施設で21例の再発悪性グリオーマ患者に対して第Ⅰ相臨床試験がおこなわれ，3.0×10^9 pfuまでの脳腫瘍内投与の安全性が確認された．G207は高い安全性を確保した一方で，野生型HSV-1にくらべ極端にウイルス複製能が減弱した．そこでG207の安全性を維持したまま，より強い抗腫瘍作用を示す遺伝子組換えHSV-1の開発が求められた．

G47Δは G207 のゲノムから α47 遺伝子とそれに重なる *US11* プロモータを欠失させた，三重変異を有する第三世代 HSV-1 である[19]．α47 遺伝子産物は抗原提示関連トランスポータ（transporter associated with antigen presentation：TAP）を阻害して主要組織適合性抗原（major histocompatibility complex：MHC）class Ⅰの発現を抑えることで，宿主の免疫サーベイランスから逃れるはたらきをもつ．したがって，α47 遺伝子を欠失する G47Δは感染細胞による免疫細胞刺激能が増強される．また，G47Δでは *US11* 遺伝子の発現時期が早まり，これがγ34.5 遺伝子欠失によって減弱したウイルス複製能を腫瘍細胞にかぎって復元する．

G47Δはマウス脳腫瘍モデルで，G207 の安全性を維持したまま抗腫瘍効果を格段に改善した[19]．マウスの前立腺がんモデル[20]，乳がん転移性脳腫瘍モデル[21]，神経線維腫モデル，乳がんトランスジェニックマウス[22]などでも既存の遺伝子組換え HSV-1 にくらべてすぐれた治療効果が示されている．腫瘍血管新生を抑制する効果や[23]，テモゾロミド併用による増強効果も示された[24]．現在わが国で再発膠芽腫を対象とした臨床研究の準備が進んでいる．

4．G47Δのトランスレーショナルリサーチ

臨床応用のポテンシャルを有する基礎研究成果を，実際に治療法として患者に届けることは容易ではなく，科学とは異なる多くのハードルを乗り越えなければならない．最大の難関は多大な資金と労力を要する点にある．G47Δは世界初の第三世代遺伝子組換え HSV-1 で，文部科学省の研究支援を得てわが国でがんの新しい治療薬開発としてトランスレーショナルリサーチ（TR）を実践している．

TR でまず重要になるのは知的財産権の確保であり，G47Δに関してもその特許権の整備と強化，特許マップの作成などから開始した．製剤開発には大量調整法の開発が重要であり，ウイルス生産はスケールアップや精製の技術開発が必ずしも容易ではない．調整技術を確立したうえで，master cell bank と master virus seed stock を作製し，それぞれから working cell bank と working virus seed stock を作製してウイルスの bulk harvest をおこない，精製・分注を経て最終的なバイアルに入った製剤となる．この生産工程のうち最低 4 工程で Good Laboratory Practice（GLP）に則った詳細な品質テストをおこなう必要があり，各ウイルス製剤ロットにつきそれだけで現在は数千万円の資金を要する．G47Δは東京大学の試験物製造施設において，標準作業手順書（standard operating procedure：SOP）を作成したうえで，current Good Manufacturing Practice（cGMP）に則った製剤生産がおこなわれている．製剤についてはさらに，ウイルスのゲノム構造の確認とゲノムの安定性の確認，種々の温度における製剤の安定性試験を実施する．非臨床安全性試験は，HSV-1 に感受性を有する A/J マウスを用いて，種々の投与経路で実施された．G47Δの最初の臨床研究は，再発膠芽腫患者を対象として計画され，第Ⅰ～Ⅱ相の試験デザインの臨床プロトコルおよび関連文書が作成された．ウイルス療法は厳密には遺伝子治療の定義にはあてはまらないが，わが国では「遺伝子治療臨床研究に関する指針」に該当すると厚生労働省の判断があった．それにしたがい，臨床研究実施計画は学内の遺伝子治療臨床研究審査委員会の承認を得たうえで，厚生労働省で審議される．また遺伝子組換え HSV-1 は，「遺伝子組換え生物等の使用等の規制による生物の多様性の確保に関する法律」（カルタヘナ法）の対象となるため，ウイルス製剤生産や非臨床試験の段階ではその第二種使用について，臨床研究ではその第一種使用について，それぞれ文部科学省と厚生労働省に拡散防止措置の大臣確認を得る必要がある．これらをすべて経たうえで，Good Clinical Practice（GCP）で臨床研究を実施すべく，適格性判定委員会，データセンター，臨床研究コーディネータ，統計解析者，独立データモニタリング委員会などの臨床実施体制を整備する．G47Δは 2008 年中の臨床研究開始が見込まれている．

5．遺伝子組換え HSV-1 の発展性

近年 HSV-1 のゲノムに直接治療遺伝子を組み込んで，ウイルス複製に伴う殺細胞作用に，治療遺伝子発現による特殊な機能を付加した「武装」HSV-1 が開発されている．抗腫瘍免疫惹起能強化を目的として IL-12 や IL-18，可溶型 B7-1 を発現するものや[24][25]，自殺遺伝

子としてシトシン酸アミノ酵素を発現するものなどが報告されている[26]．*Musashi 1* や *Nestin* のプロモータでウイルス遺伝子を制御してウイルス複製に組織特異性をもたせる試みや[27][28]，サイトメガロウイルス(CMV)のPKR回避遺伝子を組み込んで抗腫瘍効果を高めたCMV/HSV-1キメラウイルスの作製など，新しい方向性の開発もあいついでいる[29]．従来はHSV-1の遺伝子組換えに多大な労力と時間が必要であったが，細菌人工染色体(bacterial artificial chromosome：BAC)とDNA組換え酵素を利用して，G47Δのゲノムに短期間で的確に外来遺伝子を組み込むことができる遺伝子組換えHSV-1作製システムなどが開発され，更なる研究の促進に拍車をかけている[30]．

6．その他のウイルスを用いた脳腫瘍のウイルス療法

遺伝子組換えアデノウイルスは，HSV-1に約5年遅れて腫瘍治療用ウイルスとしての開発がはじまった．ゲノムが36kbの二重鎖DNAで組換えが比較的容易でありベクターとしてすでに広く研究者に普及していたことや，動物モデルに乏しいことから，遺伝子組換えHSV-1とほぼ同時期に臨床に試されている．高い力価(～1.0×10^{12} pfu)が得られることが利点だが，レセプター・coxackievirus and adenovirus receptor(CAR)をもつ細胞にしか感染しないことや，ウイルス自体の免疫原性が高く抗体により抗腫瘍効果が下がること，ウイルス自体の毒性や殺細胞作用が比較的低いことなど，腫瘍治療用ウイルスとして不向きな点も少なくない．アデノウイルスのE1B領域にコードされる蛋白(E1B 55K)はp53と結合してこれを不活化させ，宿主細胞がアポトーシスを起こすのを防いでウイルス複製を可能とする．遺伝子組換えアデノウイルスONYX-015はE1B領域に欠失変異をもち，正常細胞では複製できないが，*p53* 経路に異常がある腫瘍ではウイルスは複製可能となり殺細胞効果を現す．この理論にもとづき，40％以上のがん種が *p53* 異常を示すことから，複数のONYX-015の臨床試験がおこなわれた．再発膠芽腫患者24例を対象とした第Ⅰ相臨床試験では，1.0×10^7 ～ 1.0×10^{10} pfuまで段階的に用量を増加させて腫瘍摘出腔壁に投与した[11]．重大な有害事象は認めなかったが，無再発期間中央値が46日，生存期間中央値は6.2ヵ月にとどまった．ONYX-015と同じ構造をもつH101が最近中国で肺がんや頭頸部がんなどを適応症とする世界初のウイルス製剤として製品化された．

NDVの臨床応用の例としては，イスラエルでおこなわれた再発膠芽腫を対象としたNDV-HUJの第Ⅰ/Ⅱ相試験がある．0.1 billion infectious units(BIU)にはじまり55 BIUまで段階的用量増加がおこなわれ，その後11例に対して11 BIUが3回静脈内投与された．グレード3以上の有害事象は認められず，1例が完全寛解(complete responce：CR)を得たと報告された[7]．

おわりに

悪性脳腫瘍は進行が早く，随伴する脳機能の障害により生活の質が低下することも多い．革新的な治療アプローチのニーズに応えるものとしてウイルス療法開発の期待が高い．脳腫瘍にかぎらずあらゆる固形がんに対して効果が期待できることから，わが国におけるこの分野のTRが強力に推進されることが望まれる．

文　献

1) Stupp R *et al*：Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 352：987-996, 2005
2) Kelly E *et al*：History of oncolytic viruses：genesis to genetic engineering. *Mol Ther* 15：651-659, 2007
3) Pack GT：Note on the experimental use of rabies vaccine for melanomatosis. *AMA Arch Derm Syphilol* 62：694-695, 1950
4) Martuza RL：Conditionally replicating herpes vectors for cancer therapy. *J Clin Invest* 105：841-846, 2000
5) Chiocca EA：Oncolytic viruses. *Nat Rev Cancer* 2：938-950, 2002
6) Vähä-Koskela MJ *et al*：Oncolytic viruses in cancer therapy. *Cancer Lett* 254：178-216, 2007
7) Freeman AI *et al*：Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. *Mol Ther* 13：221-228, 2006
8) Markert JM *et al*：Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma：results of a phase I trial. *Gene Ther* 7：867-874, 2000
9) Papanastassiou V *et al*：The potential for efficacy of the modified (ICP 34.5(−)) herpes simplex virus

HSV1716 following intratumoural injection into human malignant glioma : a proof of principle study. *Gene Ther* 9 : 398-406, 2002

10) Harrow S *et al* : HSV1716 injection into the brain adjacent to tumour following surgical resection of high-grade glioma : safety data and long-term survival. *Gene Ther* **11** : 1648-1658, 2004

11) Chiocca EA *et al* : A phase I open-label, dose-escalation, multi-institutional trial of injection with an E1B-Attenuated adenovirus, ONYX-015, into the peritumoral region of recurrent malignant gliomas, in the adjuvant setting. *Mol Ther* **10** : 958-966, 2004

12) Aghi M *et al* : Oncolytic viral therapies-the clinical experience. *Oncogene* **24** : 7802-7816, 2005

13) Yu W *et al* : Clinical trials with oncolytic adenovirus in China. *Curr Cancer Drug Targets* **7** : 141-148, 2007

14) Hoffmann D *et al* : Comparison of herpes simplex virus- and conditionally replicative adenovirus-based vectors for glioblastoma treatment. *Cancer Gene Ther* **14** : 627-639, 2007

15) Farassati F *et al* : Oncogenes in Ras signalling pathway dictate host-cell permissiveness to herpes simplex virus 1. *Nat Cell Biol* **3** : 745-750, 2001

16) Martuza RL *et al* : Experimental therapy of human glioma by means of a genetically engineered virus mutant. *Science* **252** : 854-856, 1991

17) Rampling R *et al* : Toxicity evaluation of replication-competent herpes simplex virus (ICP34.5 null mutant 1716) in patients with recurrent malignant glioma. *Gene Ther* **7** : 859-866, 2000

18) Mineta T *et al* : Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. *Nat Med* **1** : 938-943, 1995

19) Todo T *et al* : Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. *Proc Natl Acad Sci U S A* **98** : 6396-6401, 2001

20) Fukuhara H *et al* : Oncolytic herpes simplex virus vector g47delta in combination with androgen ablation for the treatment of human prostate adenocarcinoma. *Clin Cancer Res* **11** : 7886-7890, 2005

21) Liu R *et al* : Intracarotid delivery of oncolytic HSV vector G47Delta to metastatic breast cancer in the brain. *Gene Ther* **12** : 647-654, 2005

22) Liu R *et al* : Oncolytic herpes simplex virus vector therapy of breast cancer in C3(1)/SV40T-antigen transgenic mice. *Cancer Res* **65** : 1532-1540, 2005

23) Aghi M *et al* : Angiogenic response caused by oncolytic herpes simplex virus-induced reduced thrombospondin expression can be prevented by specific viral mutations or by administering a thrombospondin-derived peptide. *Cancer Res* **67** : 440-444, 2007

24) Parker JN *et al* : Engineered herpes simplex virus expressing IL-12 in the treatment of experimental murine brain tumors. *Proc Natl Acad Sci U S A* **97** : 2208-2213, 2000

25) Ino Y *et al* : Triple combination of oncolytic herpes simplex virus-1 vectors armed with interleukin-12, interleukin-18, or soluble B7-1 results in enhanced antitumor efficacy. *Clin Cancer Res* **12** : 643-652, 2006

26) Guffey MB *et al* : Engineered herpes simplex virus expressing bacterial cytosine deaminase for experimental therapy of brain tumors. *Cancer Gene Ther* **14** : 45-56, 2007

27) Kanai R *et al* : Augmented therapeutic efficacy of an oncolytic herpes simplex virus type 1 mutant expressing ICP34.5 under the transcriptional control of musashi1 promoter in the treatment of malignant glioma. *Hum Gene Ther* **18** : 63-73, 2007

28) Kambara H *et al* : An oncolytic HSV-1 mutant expressing ICP34.5 under control of a nestin promoter increases survival of animals even when symptomatic from a brain tumor. *Cancer Res* **65** : 2832-2839, 2005

29) Shah AC *et al* : Enhanced antiglioma activity of chimeric HCMV/HSV-1 oncolytic viruses. *Gene Ther* **14** : 1045-1054, 2007

30) Fukuhara H *et al* : Triple gene-deleted oncolytic herpes simplex virus vector double-armed with interleukin 18 and soluble B7-1 constructed by bacterial artificial chromosome-mediated system. *Cancer Res* **65** : 10663-10668, 2005

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