各1表-4.　悪性神経膠腫に対する放射線治療の分割方法・総線量の検討

報告者	治療方法	症例数	対　象	放射線治療	生存期間中央値	生存率（%）	PFS中央値	5年PFS（%）
Tanaka[69]	standard dose RT	94	GBM：64% AA：36%	2Gy/fr×1/day total 60 Gy	GBM：12.4カ月 AA：22.3カ月	GBM：11.4/2年 AA：14.7/5年	GBM：7.2カ月 AA：17カ月	
	high dose conformal RT	90	GBM：68% AA：32%	2Gy/fr×1/day total 80～90 Gy	GBM：16.2カ月 AA：未到達	GBM：38.4/2年 AA：51.3/5年	GBM：7カ月 AA：37.5カ月	
Chang[70]	3D conformal IMRT	34	GBM：97% AA：3%	2Gy/fr×1/day total 90 Gy	11.7カ月	47.1/1年 12.9/2年		
Mizoe[71]	ACNU ＋X-ray RT ＋CRT	48	GBM：67% AA：33%	X-ray RT： 2Gy/fr×1/day total 50 Gy CRT：16.8～24.8 GyE	GBM：17カ月 AA：35カ月		GBM：7カ月 AA：18カ月	
Buckner[72] NCCTG93-72-52/SWOG9503	BCNU ＋standard RT	98	GBM：96.9% gliosarcoma：3.1%	1.8Gy/fr×1/day total 64.8 Gy	10.4カ月	16.8/2年	5.2カ月	20.4
	BCNU ＋accelerated RT	103	GBM：96.1% gliosarcoma：3.9%	1.6 Gy/fr×2/day total 48 Gy	10.1カ月	9.1/2年	5.5カ月	16.7
	cisplatin＋BCNU ＋standard RT	100	GBM：99% gliosarcoma：1%	1.8 Gy/fr×1/day total 64.8 Gy	12.0カ月	18.3/2年	6.2カ月	15.2
	cisplatin＋BCNU ＋accelerated RT	100	GBM：99% gliosarcoma：1%	1.6 Gy/fr×2/day total 48 Gy	11.6カ月	17.5/2年	6.6カ月	17.3

PFS：progression free survival，GBM：glioblastoma，AA：anaplastic astrocytoma
IMRT：intensity modulated radiation therapy，CRT：carbon ion radiotherapy

carbon ionを用いた重粒子線による悪性神経膠腫の臨床第Ⅰ/Ⅱ層試験の報告がMizoeら[71]により2007年に報告されている（**各1表-4**）．nimustineによる化学療法とX線による50 Gy/25回分割/5週間の照射のあと重粒子線による16.8～24.8 Gy相当の追加を8回分割/2週間で追加している．生存期間の中央値は退形成性星細胞腫で35カ月および膠芽腫で17カ月であった．今後の研究による対象の選択と最適な治療方法の開発が期待されている．

分割照射方法の検討では，1回線量を少なくし照射回数を増加することにより，正常組織の遅発性反応を低減し総線量を増加する多分割照射を応用する試みが1990年代の後半より検討されてきた．その後，照射期間の短縮を目的とした加速分割照射の臨床試験が加わった．2006年にBucknerら[72]により1回線量を1.6 Gyとし1日2回照射する，加速分割照射による臨床試験の結果がNorth Central Cancer Treatment GroupとSouthwest Oncology Groupの共同臨床試験として報告されている．この臨床試験では化学療法をBCNU単剤群とcisplatin＋BCNUの2剤併用群とし，放射線治療は標準分割照射64.8 Gy/36回分割/7.2週と加速分割照射48.0 Gy/30回分割/3週で比較している．生物学的等効果線量を計算すると標準分割照射群が77 Gy_{10}および104 Gy_3となるのに対し，加速分割照射群では56 Gy_{10}および74 Gy_3となり加速分割照射群で少ない線量となるが，生存期間の中央値と有害事象は両群間に有意差なく（**各1表-4**），cisplatinを含む化学療法を行った群で有害事象が多いという結果となっている．加速分割照射の有用性に関しては，小細胞肺がんなど一定の評価を受けていた領域でも総線量増加した標準分割照射と比較試験が施行されており，その有用性については未だ検討中といわざるを得ない．

悪性神経膠腫の放射線治療における照射体積の検討

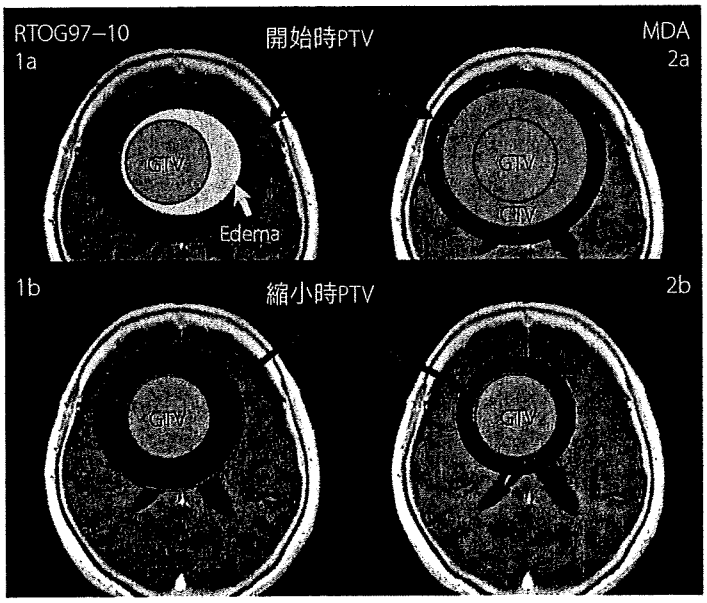
放射線治療は，以前は全脳照射が行われていた時期もあったが，現在は画像上腫瘍本体周囲の脳浮腫領域（CTであれば低吸収域，MRIであればT2強調画像の高輝度領域まで）に1～2 cm程度外側を含む領域に対しての局所照射が行われることが多い．Hochbergら[73]によると星細胞腫Grade 4の再発は原発巣から2 cm以内の局所再発が90%を占めると報告している．腫瘍周囲の脳浮腫領域に関しては腫瘍細胞の浸潤とともに，mass-effectや血管透過性亢進などさまざまな成因が考えられている[73,74]．脳浮腫領域が広い場合は照射体積も大きくなるため，遅発性反応としての放射線壊死が問題となってくる．

Emaniら[75]は，照射線量と照射体積が放射線壊死の発生頻度に関連しているとの報告を行っている．総線量が60 Gy照射される体積が大脳の1/3の場合，放射線壊死の可能性は5年で5%であり，照射体積・

照射線量が増加するとともに放射線壊死が増加する．脳の遅発性放射線反応である放射線壊死は照射後数カ月から数年で発生し，照射野に含まれた脳組織の一部もしくは全部が壊死・浮腫を起こす．主な症状としては放射線壊死を起こした部位によって起こるさまざまな神経症状（巣症状）や浮腫によって引き起こされる頭蓋内圧亢進症状がある．放射線壊死の範囲が小さければ経過観察のみにて軽快することもあるが，一般に重症化し致死的となる場合も多い．ただし，画像上では腫瘍の再発と区別することが困難な場合や，再発とみなして壊死部位を切除せざる得ない場合もある．

悪性神経膠腫の放射線治療計画においてはCTを用いた3D-CRTが必要であり，従来より利用されてきたMRIのみでなくPETやMR spectroscopyとのfusionにより治療成績が向上するとした報告もされている[76~78]．Radiation Therapy Oncology Group（RTOG）の臨床試験では従来脳浮腫領域周囲に2 cmのmarginを設定し治療を開始し，途中でGTV周囲に2.5 cmのmarginをとった照射体積に縮小してきた．しかし，Chang[79]らによるとM. D. Anderson Cancer CenterではGTVに2 cmのmarginを設定し臨床標的体積（Clinical Target Volume：CTV）とし，さらに5 mmのmarginを設定し計画標的体積（Planning Target Volume：PTV）としてきた．さらに縮小照射野はGTVに0.5 cmのmarginを設定しPTVとしてきた．この方法では開始時の照射体積はGTVより3 cm程度，縮小時は1 cm程度となる．すなわち脳浮腫領域を考慮しない照射体積の設定であるが，再発形式を両者で比較したところ同様であり，脳浮腫領域と再発形式に関連がないことを示している点で興味深い．M. D. Anderson Cancer Center方式では照射体積が小さくなり，照射線量増加と有害事象軽減に有用である可能性が示唆されている（**各1図-4**）．

定位放射線治療が悪性神経膠腫の放射線治療に応用されるようになり，多くの報告がなされてきた．しかし，2005年に発表された米国放射線腫瘍学会のevidence-based reviewでは[80]，BCNUと外部照射に定位手術的照射を追加した場合の生存率や局所制御およびQOLに関する有用性は明らかでなく，有害事象が増加するとされた．2004年にRTOG93-05の結果がSouhamiら[81]により報告されたが，定位手術的照射の有無で生存期間に有意差なく再発形式も変わらなかった（**各1表-5**）．その後のRTOG00-23では，分割照射による定位放射線治療を施行し摘出率のよい症例で生存率の延長が報告されている[82]．悪性神経膠腫の放射線治療における役割に関しては，その対象やタイミングなど検討が必要とされている．



各1図-4．悪性神経膠腫の放射線治療計画

（左）RTOG97-10の照射体積
　（1a）開始時．照射野の辺縁はedema＋2 cmマージン
　（1b）縮小時．照射野の辺縁はGTV＋2 cmマージン
（右）M. D. Anderson Cancer Center（MDA）の照射体積
　（2a）開始時のPTV＝CTV（GTV＋2 cmマージン）＋0.5 cm
　　照射野の辺縁はPTVにMLCマージン0.5 cmが加わりGTVより3 cmとなる
　（2b）縮小時のPTV＝GTV＋0.5 cmマージン
　　照射野の辺縁はPTVにMLCマージン0.5 cmが加わりGTVより1 cmとなる

各1表-5.　悪性神経膠腫に対する定位放射線照射の検討

報告者	治療方法	症例数	対　象	放射線治療	生存期間の中央値	生存率(%)	PFS中央値	1年PFS(%)
Souhami[81] RTOG93-05	conventional EBRT＋BCNU	97	GBM≦4 cm	2 Gy/fr×1/day total 60 Gy	13.5カ月	19/2年 13/3年		
	SRS＋conventional EBRT＋BCNU	89	GBM≦4 cm	EBRT：2 Gy/fr×1/day total 60 Gy SRT：15～24 Gy	13.6カ月	21/2年 9/3年		
Cardinale[82] RTOG00-23	conventional EBRT＋FSRT boost＋BCNU	76	GBM＜6 cm	EBRT：2 Gy/fr×2/day total 50 Gy FSRT boost：5 or 7 Gy/fr total 20～28 Gy	12.5カ月		5.7カ月	

PFS：progression free survival，GBM：glioblastoma，EBRT：external beam radiation therapy
SRS：stereotactic radiosurgery，FSRT：fractionated stereotactic radiotherapy

❹ 転移性脳腫瘍の放射線治療

悪性腫瘍の約10～30%に転移性脳腫瘍が生じるとされているが，薬物療法の進歩により進行症例の予後が延長するにつれ，またMRIなど画像診断の進歩により小さな病巣の診断が可能となったこともあり，転移性脳腫瘍と診断される症例は増加している．転移性脳腫瘍はがんによる死因の1つであるとともに，脳の圧迫による神経障害が発生することより，がん患者のQOLを著しく低下させる原因であることが問題である．転移性脳腫瘍の原発巣の頻度は，日本脳腫瘍統計[61]では肺がん60.1%，消化器系腫瘍15.7%，乳がん10.6%，腎泌尿器系の腫瘍6.4%，婦人科系腫瘍2.2%とされている．また，転移の発生部位は脳の体積と一致し，大脳半球が約80%で，小脳が約15%，脳幹が5%程度とされる．

単発の場合は手術や放射線治療のよい適応とされ，腫瘍摘出術（＋全脳照射）または腫瘍径が3 cm未満の場合，定位放射線照射が選択されている．多発性の場合は単発に比べて予後不良とされるが，全脳照射や定位放射線照射が実施されている．

Patchellらは1998年に単発の転移性脳転移に対して，腫瘍摘出術単独と腫瘍摘出術＋全脳照射（50.4 Gy）の無作為化比較試験の結果を報告している[83]．この結果，全生存期間に有意差はみられないものの，脳内再発が腫瘍摘出術＋全脳照射群（49例）で18%であったのに対し，腫瘍摘出術単独群（46例）では70%と多かった．この結果より原疾患がコントロールされている場合，単発の脳腫瘍に対する欧米での標準治療は腫瘍摘出術＋全脳照射と考えられてきた．しかし，わが国では腫瘍摘出術＋全脳照射を実施されている症例はさほど多くなく，腫瘍摘出術単独や，腫瘍摘出術＋局所照射，腫瘍摘出後再発時に定位放射線照射を行う，などの治療が各施設の方針に基づいて行われており，標準治療についてのコンセンサスが存在していない．そこで日本臨床腫瘍研究グループ（JCOG）脳腫瘍グループでは，2005年より頭蓋内の転移個数が4個以下で最大病変の腫瘍径が3 cm以上の転移巣が1個のみの転移性脳腫瘍を対象として，第Ⅲ相試験を開始している．すなわち，標準治療とグループで考えられた腫瘍摘出術＋全脳照射群と，腫瘍摘出術後に全脳照射を行わず残存病変および新病変に対して定位放射線照射行う群とで有効性の比較（非劣性）を行う「転移性脳腫瘍に対する，腫瘍摘出術＋全脳照射と腫瘍摘出術＋Salvage Radiation Therapyとの無作為化比較試験」であり，腫瘍摘出術後の標準治療の確立に向けた前向き臨床試験となっている．

転移性脳腫瘍の治療で問題となることの多い認知障害にはさまざまな要素が関係している．すなわち，腫瘍そのものや全脳照射，脳外科手術，化学療法および抗けいれん薬やステロイドを含む薬物，そして悪性腫瘍による腫瘍随伴症状である[84]．全脳照射は転移性脳腫瘍，特に多発時には標準的な治療方法として広く使用されてきた．神経症状の改善とともにQOLの改善に有効であり，術後や定位放射線照射時の併用療法としても局所および新たな脳転移出現対策として実施されている．しかし，わが国では全脳照射を標準治療と位置づける欧米に比較し，術後併用療法を検討する脳外科医を含む臨床医全般に，全脳照射の副作用を考慮し回避しようとする考え方も存在するとされている（JCOG脳腫瘍グループ調査結果）．定位放射線照射や手術などの局所療法と比較し，全脳照射では嘔気や頭痛などの急性反応が知られているが，重篤度や期間は限られている[85]．これに対しより問題となるのが遅発性放射線反応であり，より重篤かつ

進行性であり，不可逆性の変化を生ずる．ミエリン障害を主体とする白質の構造的変化をきたすためleukoencephalopathyと称される．不注意や記憶障害，感情失禁などより痴呆や昏睡に至るさまざまな状況が生じうることが知られており，通常は照射後6ないし24カ月にわたり進行する．神経障害の程度に関与する可能性のある因子として，全脳照射の総線量と1回線量・照射期間が指摘されてきた[86]．最もよく使用されている全脳照射の方法は，30 Gy/10回分割であるが，長期の予後が期待される場合は1回線量を2.5 Gyにするなどの配慮が行われている．さらに患者自身の年齢や糖尿病の影響も考えられているが，その影響を予測する情報は未だ得られていない[87,88]．RTOG0118は多発性脳転移に対する全脳照射群と全脳照射＋thalidomide群による比較試験であるが[89]，前向き試験として認知機能とQOLを調査している．生存期間や局所制御では有意差を認めていないこの臨床試験の結果において，当初のQOLの差異が予後に相関していることと認知機能が低下してもQOLが安定していたことが報告されており，今後の転移性脳腫瘍の臨床試験における認知機能とQOL調査の方法論として興味深い．放射線治療による神経障害対策としてはdonepezil[90]など薬物の開発が進行中であり，今後の臨床試験による評価が待たれる．

定位放射線照射は，手術に比べて侵襲が少なく新たな病巣出現時に繰り返し施行可能であり，手術が不可能な部位でも施行可能であるといった利点がある．しかし対象が一定の大きさを超えると線量の均一性が保てなくなり，3 cm以上の大きな病変ではよい線量分布の作成が困難な場合がある．副作用の頻度は低いが，腫瘍径と有害事象の相関が報告されている[91]．腫瘍内出血や照射後の浮腫が報告されている[92,93]．

2005年に発表された米国放射線腫瘍学会のevidence-based reviewでは[94]，4 cm以下の3～4個までの転移性脳腫瘍の場合，全脳照射に定位手術的照射を追加することにより，局所制御を向上させることが示され，単発性の場合は生存率の向上にも寄与する．しかし，全脳照射と定位放射線照射の組み合わせの意義および実施のタイミングについては，未だcontroversialな状況と言わざるを得ない．比較的大規模な全脳照射と定位手術的照射の組み合わせに関する無作為化比較試験がRTOGとわが国で実施された(**各1表-6**)．共に全体の解析では全脳照射±定位手術的照射および定位手術的照射±全脳照射のいずれについても生存率に有意差を認めなかった．Andrewsら[95]によると，RTOGのサブグループ解析では転移個数が1個の場合では生存期間の中央値は全脳群4.9カ月に対して全脳＋定位手術的照射群6.5カ月と上回っていた．さらに全体での6カ月時点におけるKPS改善・維持割合が全脳群27%に対し全脳＋定位手術的照射群43%

各1表-6．転移性脳腫瘍に対する定位手術的照射と全脳照射の臨床試験

		Andrews[95]	RTOG9508	Aoyama[96]	JROSG99-1
治療方法		WBRT only	WBRT＋SRS	SRS only	WBRT＋SRS
症例選択		18歳以上 脳転移1～3個 最大径4 cm（ほかは≦3 cm） KPS≧70		18歳以上 脳転移1～4個 腫瘍径≦3 cm KPS≧70	
症例数		167	164	67	65
平均年齢		59.9歳	58.8歳	62.1歳	62.5歳
腺がん		47%	51%	64%	66%
原発巣制御		75%	77%	49%	46%
脳以外の転移なし		31%	32%	57%	63%
KPS90～100		63%	57%	66%	52%
治療方法		WBRT 37.5 Gy/15 fr/3 wks		WBRT30 Gy/10 fr/2～2.5 wks	
			SRS： ～2 cm＝24 Gy 2～3 cm＝18 Gy 3～4 cm＝15 Gy	SRS： ～2 cm＝22～25 Gy 2 cm～＝18～20 Gy	30% SRS dose reduction
生存期間の中央値	全症例	5.7カ月	6.5カ月	8.0カ月	7.5カ月
	単発例	4.9カ月	6.5カ月	－	－
	多発例	6.7カ月	5.8カ月	－	－
	RPA class1	9.6カ月	11.6カ月	－	－
1年生存率		－	－	28.4%	38.5%
1年時の局所制御		71%	82%	72.5%	88.7%
1年時の脳転移再発		－	－	76.4%	46.8%
1年時の新脳転移		－	－	63.7%	41.5%

と有意に優っていた．有害事象に関しては，有意差はないとしている．また，日本放射線腫瘍学研究グループ（JROSG）では定位放射線照射単独と全脳＋定位放射線照射の比較試験を行った．Aoyama ら[96]の報告では，1年生存割合は定位放射線照射群 28.4％に対して全脳＋定位放射線照射群 38.5％であり全脳＋定位放射線照射群で生存が良い傾向があるが統計的有意差を認めなかった．生存期間の中央値も定位放射線照射群 8 カ月に対して全脳＋定位放射線照射群 7.5 カ月と有意差を認めていない．1年脳転移再発率は定位放射線照射群 76.4％に対して全脳＋定位放射線照射群 46.8％であり，全脳照射により新病変の出現が減少していた．有害事象は有意差ないと報告されているが，2007 年に報告された神経機能に関する検討では[85]，Mini-Mental State Examination（MMSE）の経過を解析している．結論として転移性脳腫瘍症例の神経機能については腫瘍の制御が最も影響するため，全脳照射の神経機能への影響が明らかでないと報告されている．遅発性有害事象の長期的な観察結果は，その適切な評価方法も含めて検討の必要があるといえよう．

悪性神経膠腫や転移性脳腫瘍に対する放射線治療の役割は，今後集学的治療の一環としてさらに重要性を増すことが推測される．定位放射線照射や強度変調放射線治療（Intensity Modulated Radiation Therapy：IMRT）などを含む高精度放射線治療の適応を含め，治療の最適化を図るためには，よく検討された臨床試験により evidence を集積していくことが重要と考えられる．

［角　美奈子］

治療方針のまとめ

エビデンスのレベルの高いものから低いものと，著者らの経験を総合した現時点での脳腫瘍の治療指針を示す．

【神経膠腫】

	治療方針	その他の選択肢
膠芽腫	全摘，あるいは可能な限りの減量手術．その後に補助化学放射線[注1]と化学療法[注2]	
退形成性星細胞腫	全摘，あるいは可能な限りの減量手術．その後に補助（化学）放射線[注1]と化学療法[注2]	切除後待期的に（化学）放射線と化学療法
星細胞腫	全摘，あるいは可能な限りの減量手術．その後悪化したら放射線療法	
希突起膠細胞腫	全摘，あるいは可能な限りの減量手術．その後悪化したら放射線と化学療法[注3]	切除不能の 1p19q 欠損症例には化学療法を先行させてもよい．その後切除または放射線
上衣腫	全摘，あるいは可能な限りの減量手術．その後に補助放射線	病理学的に完全に切除されたら放射線は待期的に施行してもよい
KPS 不良例	施行可能な治療を施行するが治療選択の基準となるガイドラインは存在しない．また緩和医療と何らかの積極的な治療との比較試験は存在せず，どちらにするかは症例毎に判断する	

【転移性脳腫瘍】

	治療方針	その他の選択肢
予後良好群 KPS≧70％，コントロールされた原病巣，単発もしくは 2〜3 個の脳転移巣	切除または SRS．これにて十分局所が根治的に治療された場合は WBRT は待期的に施行してよい．残存病変が存在する場合は WBRT を追加	WBRT または定位照射
予後不良群 KPS≦60％，手術適応のない多発性脳転移	WBRT または緩和医療（ステロイドを含む）	
がん性髄膜炎	感受性のある腫瘍には髄腔内治療[注4]	WBRT，緩和医療，適応症例には定位照射

WBRT：全脳照射

注 1）・temozolomide 75 mg/m² 照射 1〜2 時間前 1 日 1 回を放射線照射日 day1 から照射終了まで（照射しない休日も投与）に連日投与（最大 49 日間）．temozolomide 開始早期には制吐剤を併用するが，嘔気がなければその後中止してよい．
・放射線局所照射（病巣部照射）は 1 日 2 Gy でトータル 60 Gy．

注 2)・上記照射終了 4 週間後，初回 150 mg/m² を制吐剤とともに 5 日間連続投与．耐えられれば 2 サイクル目から 200 mg/m² を 28 日周期で 5 サイクル（トータル 6 サイクル）．胃内の食物が吸収を阻害するため，空腹時に投与するが食事内容の制限は必要ない．投与量が多いため 75 mg/m² のときより催吐作用が強いので，5HT₃ 拮抗薬を含む制吐剤を適宜併用する．就寝時の投与または 1 日 2〜3 回の分割投与にて，嘔気の軽減が可能な場合がある．通常 1 サイクル目投与開始から 3 週後（day 22）に血算と生化学検査を測定し，骨髄抑制の程度をチェックする．もし好中球数が 1,500/μl または血小板が 10 万/μl 以下になっていれば，その後毎週チェックしてそれ以上に回復するまで次サイクルを開始しない．好中球数 1,000/μl または血小板数 5 万以下になったら次サイクルから 50 mg/m² 減量する．しかし，100 mg/m² が最低推奨量となっている．カリニ肺炎の予防と 6 サイクル以上投与するかについては本文参照．

注 3)・化学療法：temozolomide または PCV
・temozolomide は上記と同じ投与法．
・PCV レジメン：わが国では CCNU がないため ACNU にて代用されることが多い[77]．
procarbazine 60 mg/m² 経口，day 8 から day 21
ACNU 70 mg/m² 静注，day 1
vincristine 1.4 mg/m²（最大 2 mg/body）静注 day 8 と day 29
毒性をみて 6 週間毎に 6 サイクル．ACNU と procarbazine は催吐性が強いので制吐剤を併用する．ニトロソウレア系の殺細胞薬は投与 4〜6 後に骨髄抑制をきたすので注意（遅発性骨髄抑制）．

注 4)・腰椎穿刺または Ommaya リザーバーから下記を投与：
methotrexate 10〜15 mg（12 mg が多い）/body 単剤または cytarabine 30〜50 mg. body 単剤を週 2 回投与．悪性細胞が消失したら週 1 回に間隔を空け 4 回施行，その後月に 1 回最長 1 年まで投与し終了．長期投与のデータはなく，症例毎に適宜投与間隔と期間を調節．methotrexate の髄腔内投与に際して，骨髄抑制が懸念される場合に少量の leucovorin 救済療法が投与されるが，ルーチン使用を推奨する専門家もいる．リンパ腫の髄膜浸潤と予防では methotrexate 15 mg，cytarabine 40 mg，ハイドロコルチゾン 50 mg の髄腔内投与が同時に投与されるレジメンもある．悪性リンパ腫の髄膜浸潤の予防には通常 4 回の投与が必要．

【脳腫瘍の放射線治療】

	1st line，および代表的な 2nd line の治療方法・レジメン	文献
悪性神経膠腫	生検を含む腫瘍摘出＋放射線治療＋化学療法（下記の選択肢）	63, 64)
	・temozolomide	65)
	・ACNU	62)
	腫瘍摘出＋放射線治療	66)
	・best supportive care	
転移性脳腫瘍	全脳照射	83, 94)
	定位放射線照射±全脳照射	94, 95, 96)
	腫瘍摘出術±全脳照射または部分照射または定位放射線照射	83)
	best supportive care	

[参考文献]

1) Perry, JR, Rogers, L, Laperriere, N, et al：PRODIGE：A phase Ⅲ randomized placebo-controlled trial of thromboprophylaxis using dalteparin low molecular weight heparin (LMWH) in patients with newly diagnosed malignant glioma. (Abstract). J Clin Oncol, 25：77s, 2007.

2) Delattre JY, Krol G, Thaler HT, Posner JB：Distribution of brain metastases. Arch Neurol, 45 (7)：741-744, 1988.

3) Patchell RA, Tibbs PA, Walsh JW, Dempsey RJ, Maruyama Y, Kryscio RJ, Markesbery WR, Macdonald JS, Young B：A randomized trial of surgery in the treatment of single metastases to the brain. N Engl J Med, 322 (8)：494-500, 1999.

4) Gaspar L, Scott C, Rotman M, Asbell S, Phillips T, Wasserman T, McKenna WG, Byhardt R：Recursive partitioning analysis (RPA) of prognostic factors in three Radiation Therapy Oncology Group (RTOG) brain metastases trials. Int J Radiat Oncol Biol Phys, 37 (4)：745-751, 1997.

5) Patchell RA, Tibbs PA, Regine WF, Dempsey RJ, Mohiuddin M, Kryscio RJ, Markesbery WR, Foon KA, Young B：Postoperative radiotherapy in the treatment of single metastases to the brain：a randomized trial. JAMA, 280 (17)：1485-1489, 1998.

6) Aoyama H, Shirato H, Tago M, Nakagawa K, Toyoda T, Hatano K, Kenjyo M, Oya N, Hirota S, Shioura H, Kunieda E, Inomata T, Hayakawa K, Katoh N, Kobashi G：Stereotactic radiosurgery plus whole-brain radiation therapy vs stereotactic radiosurgery alone for treatment of brain metastases：a randomized controlled trial. JAMA, 295 (21)：2483-2491, 2006.

7) Mehta MP, Tsao MN, Whelan TJ, Morris DE, Hayman JA, Flickinger JC, Mills M, Rogers CL, Souhami L：The American Society for Therapeutic Radiology and Oncology (ASTRO) evidence-based review of the role of radiosurgery for brain metastases. Int J Radiat Oncol Biol Phys, 63 (1)：37-46, 2005.

8) Addeo R, De Rosa C, Faiola V, Leo L, Cennamo G, Montella L, Guarrasi R, Vincenzi B, Caraglia M, Del Prete S：Phase 2 trial of temozolomide using protracted low-dose and whole-brain radiotherapy for nonsmall cell lung cancer and breast cancer patients with brain metastases. Cancer, 113 (9)：2524-2531, 2008.

9) Namba Y, Kijima T, Yokota S, Niinaka M, Kawamura S, Iwasaki T, Takeda Y, Kimura H, Okada T, Yamaguchi T, Nakagawa M, Okumura Y, Maeda H, Ito M：Gefitinib in patients with brain metastases from non-small-cell lung cancer：review of 15 clinical cases. Clin Lung Cancer, 6 (2)：123-128, 2004, Review.

10) Costa DB, Kobayashi S : Response of intracranial metastases to epidermal growth factor receptor tyrosine kinase inhibitors : it may all depend on EGFR mutations. J Clin Oncol, 26 (4) : 686, 2008.

11) NU Lin, V Dieras, D Paul, D Lossignol, C Christodoulou, D Laessig, H Roché, D Zembryki, CR Oliva, EP Winer, EGF105084 Study Group : EGF105084, a phase II study of lapatinib for brain metastases in patients (pts) with HER2+ breast cancer following trastuzumab (H) based systemic therapy and cranial radiotherapy (RT). Journal of Clinical Oncology, 2007 ASCO Annual Meeting Proceedings Part I. Vol 25, No. 18S (June 20 Supplement), 1012, 2007.

12) Koutras AK, Krikelis D, Alexandrou N, Starakis I, Kalofonos HP : Brain metastasis in renal cell cancer responding to sunitinib. Anticancer Res, 27 (6C) : 4255-4257, 2007.

13) Valcamonico F, Ferrari V, Amoroso V, Rangoni G, Simoncini E, Marpicati P, Vassalli L, Grisanti S, Marini G : Long-lasting successful cerebral response with sorafenib in advanced renal cell carcinoma. J Neurooncol, 2008 [Epub ahead of print]

14) Torp SH, Alsaker M : Ki-67 immunoreactivity, basic fibroblastic growth factor (bFGF) expression, and microvessel density as supplementary prognostic tools in low-grade astrocytomas. An immunohistochemical study with special reference to the reliability of different Ki-67 antibodies. Pathol Res Pract, 198 (4) : 261-265, 2002.

15) van den Bent MJ, Afra D, de Witte O, Ben Hassel M, Schraub S, Hoang-Xuan K, Malmström PO, Collette L, Piérart M, Mirimanoff R, Karim AB : EORTC Radiotherapy and Brain Tumor Groups and the UK Medical Research Council. Long-term efficacy of early versus delayed radiotherapy for low-grade astrocytoma and oligodendroglioma in adults : the EORTC 22845 randomised trial. Lancet 366 (9490) : 985-90, 2005. Erratum in : Lancet, 367 (9525) : 1818, 2006.

16) Walker MD, Green SB, Byar DP, Alexander E Jr, Batzdorf U, Brooks WH, Hunt WE, MacCarty CS, Mahaley MS Jr, Mealey J Jr, Owens G, Ransohoff J 2nd, Robertson JT, Shapiro WR, Smith KR Jr, Wilson CB, Strike TA : Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med, 303 (23) : 1323-1329, 1980.

17) Andersen AP : Postoperative irradiation of glioblastomas. Results in a randomized series. Acta Radiol Oncol Radiat Phys Biol, 17 (6) : 475-484, 1978.

18) Walker MD, Alexander E Jr, Hunt WE, MacCarty CS, Mahaley MS Jr, Mealey J Jr, Norrell HA, Owens G, Ransohoff J, Wilson CB, Gehan EA, Strike TA : Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. J Neurosurg, 49 (3) : 333-343, 1978.

19) Kristiansen K, Hagen S, Kollevold T, et al : Combined modality therapy of operated astrocytomas grade III and IV : Confirmation of the value of postoperative irradiation and lack of potentiation of bleomycin on survival time-A prospective multicentre trial of the Scandinavian Glioblastoma Study Group. Cancer, 52 : 997-1007, 1983.

20) Chang CH, Horton J, Schoenfeld D, Salazer O, Perez-Tamayo R, Kramer S, Weinstein A, Nelson JS, Tsukada Y : Comparison of postoperative radiotherapy and combined postoperative radiotherapy and chemotherapy in the multidisciplinary management of malignant gliomas. A joint Radiation Therapy Oncology Group and Eastern Cooperative Oncology Group study. Cancer, 52 (6) : 997-1007, 1983.

21) Huncharek M, Muscat J, Geschwind JF : Multi-drug versus single agent chemotherapy for high grade astrocytoma : results of a meta-analysis. Anticancer Res, 18 (6B) : 4693-4697, 1998.

22) Stewart LA : Chemotherapy in adult high-grade glioma : a systematic review and meta-analysis of individual patient data from 12 randomised trials. Lancet, 359 (9311) : 1011-1018, 2002.

23) Randomized Trial of Procarbazine, Lomustine, and Vincristine in the Adjuvant Treatment of High-Grade Astrocytoma : A Medical Research Council Trial. J Clin Oncol, 19 : 509-518, 2001.

24) Levin VA, Silver P, Hannigan J, et al : Superiority of post-radiation adjuvant chemotherapy with CCNU, procarbazine and vincristine (PCV) over BCNU for malignant glioma : NCOG 6G61 final report. Int J Radiat Oncol Biol Phys, 18 : 321-324, 1990.

25) Brandes AA, Nicolardi L, Tosoni A, Gardiman M, Iuzzolino P, Ghimenton C, Reni M, Rotilio A, Sotti G, Ermani M : Survival following adjuvant PCV or temozolomide for anaplastic astrocytoma. Neuro-oncol, 8 (3) : 253-260, 2006.

26) Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO : Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med, 352 (10) : 987-996, 2005.

27) Athanassiou H, Synodinou M, Maragoudakis E, Paraskevaidis M, Verigos C, Misailidou D, Antonadou D, Saris G, Beroukas K, Karageorgis P : Randomized phase II study of temozolomide and radiotherapy compared with radiotherapy alone in newly diagnosed glioblastoma multiforme. J Clin Oncol, 23 (10) : 2372-2377, 2005.

28) Franceschi E, Tosoni A, Brandes AA : Adjuvant temozolomide : how long and how much ? Expert Rev Anticancer Ther, 8 (5) : 663-665, 2008.

29) Stupp R, Dietrich PY, Ostermann Kraljevic S, Pica A, Maillard I, Maeder P, Meuli R, Janzer R, Pizzolato G, Miralbell R, Porchet F, Regli L, de Tribolet N, Mirimanoff RO, Leyvraz S : Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide. J Clin Oncol, 20 (5) : 1375-1382, 2002.

30) Brandes AA, Tosoni A, Cavallo G, Bertorelle R, Gioia V, Franceschi E, Biscuola M, Blatt V, Crinò L, Ermani M, GICNO : Temozolomide 3 weeks on and 1 week off as first-line therapy for recurrent glioblastoma : phase II study from gruppo italiano

cooperativo di neuro-oncologia (GICNO). Br J Cancer, 95 (9) : 1155-1160, 2006. Epub 2006 Oct 3.

31) Brandes AA, Tosoni A, Cavallo G, Reni M, Franceschi E, Bonaldi L, Bertorelle R, Gardiman M, Ghimenton C, Iuzzolino P, Pession A, Blatt V, Ermani M, GICNO : Correlations between O6-methylguanine DNA methyltransferase promoter methylation status, 1p and 19q deletions, and response to temozolomide in anaplastic and recurrent oligodendroglioma : a prospective GICNO study. J Clin Oncol, 24 (29) : 4746-4753, 2006.

32) Hegi ME, Liu L, Herman JG, Stupp R, Wick W, Weller M, Mehta MP, Gilbert MR : Correlation of O6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity. J Clin Oncol, 26 (25) : 4189-4199, 2008.

33) Hegi ME, Diserens AC, Gorlia T et al : MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med, 352 : 997-1003, 2005.

34) Brandes AA, Franceschi E, Tosoni A, Blatt V, Pession A, Tallini G, Bertorelle R, Bartolini S, Calbucci F, Andreoli A, Frezza G, Leonardi M, Spagnolli F, Ermani M : MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. J Clin Oncol, 26 (13) : 2192-2197, 2008.

35) Brem H, Piantadosi S, Burger PC, Walker M, Selker R, Vick NA, Black K, Sisti M, Brem S, Mohr G, et al : Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The Polymer-brain Tumor Treatment Group. Lancet, 345 (8956) : 1008-1012, 1995.

36) Valtonen S, Timonen U, Toivanen P, Kalimo H, Kivipelto L, Heiskanen O, Unsgaard G, Kuurne T : Interstitial chemotherapy with carmustine-loaded polymers for high-grade gliomas : a randomized double-blind study. Neurosurgery, 41 (1) : 44-48 ; discussion 48-49, 1997.

37) Westphal M, Hilt DC, Bortey E, Delavault P, Olivares R, Warnke PC, Whittle IR, Jääskeläinen J, Ram Z : A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. Neuro Oncol, 5 (2) : 79-88, 2003.

38) Westphal M, Ram Z, Riddle V, Hilt D, Bortey E : Executive Committee of the Gliadel Study Group. Gliadel wafer in initial surgery for malignant glioma : long-term follow-up of a multicenter controlled trial. Acta Neurochir (Wien), 148 (3) : 269-275, 2006, discussion 275.

39) Cloughesy, TF, Prados, MD, Mikkelsen, T, et al : A phase II randomized, non-comparative trial of the effect of bevacizumab (BV) alone or in combination with irinotecan (CPT) on 6-month progression-free survival (PFS6) in recurrent, treatment-refractory glioblastoma (GBM) (abstract). J Clin Oncol, 26 : 91s, 2008.

40) Vredenburgh JJ, Desjardins A, Herndon JE 2nd, Marcello J, Reardon DA, Quinn JA, Rich JN, Sathornsumetee S, Gururangan S, Sampson J, Wagner M, Bailey L, Bigner DD, Friedman AH, Friedman HS : Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol, 25 (30) : 4722-4729, 2007.

41) Cairncross JG, Ueki K, Zlatescu MC, Lisle DK, Finkelstein DM, Hammond RR, Silver JS, Stark PC, Macdonald DR, Ino Y, Ramsay DA, Louis DN : Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst, 90 (19) : 1473-1479, 1998.

42) van den Bent MJ, Taphoorn MJ, Brandes AA, Menten J, Stupp R, Frenay M, Chinot O, Kros JM, van der Rijt CC, Vecht ChJ, Allgeier A, Gorlia T, European Organization for Research and Treatment of Cancer Brain Tumor Group : Phase II study of first-line chemotherapy with temozolomide in recurrent oligodendroglial tumors : the European Organization for Research and Treatment of Cancer Brain Tumor Group Study 26971. J Clin Oncol, 21 (13) : 2525-2528, 2003.

43) Lindegaard KF, Mörk SJ, Eide GE, Halvorsen TB, Hatlevoll R, Solgaard T, Dahl O, Ganz J : Statistical analysis of clinicopathological features, radiotherapy, and survival in 170 cases of oligodendroglioma. J Neurosurg, 67 (2) : 224-230, 1987.

44) Shaw EG, Scheithauer BW, O'Fallon JR, Tazelaar HD, Davis DH : Oligodendrogliomas : the Mayo Clinic experience. J Neurosurg, 76 (3) : 428-434, 1992.

45) Yeh SA, Lee TC, Chen HJ, Lui CC, Sun LM, Wang CJ, Huang EY : Treatment outcomes and prognostic factors of patients with supratentorial low-grade oligodendroglioma. Int J Radiat Oncol Biol Phys, 54 (5) : 1405-1409, 2002.

46) Karim AB, Afra D, Cornu P, Bleehan N, Schraub S, De Witte O, Darcel F, Stenning S, Pierart M, Van Glabbeke M : Randomized trial on the efficacy of radiotherapy for cerebral low-grade glioma in the adult : European Organization for Research and Treatment of Cancer Study 22845 with the Medical Research Council study BRO4 : an interim analysis. Int J Radiat Oncol Biol Phys, 52 (2) : 316-324, 2002.

47) van den Bent MJ, Afra D, de Witte O, Ben Hassel M, Schraub S, Hoang-Xuan K, Malmström PO, Collette L, Piérart M, Mirimanoff R, Karim AB, EORTC Radiotherapy and Brain Tumor Groups and the UK Medical Research Council : Long-term efficacy of early versus delayed radiotherapy for low-grade astrocytoma and oligodendroglioma in adults : the EORTC 22845 randomised trial. Lancet 366 (9490) : 985-990, 2005. Erratum in : Lancet, 367 (9525) : 1818, 2006.

48) Cairncross G, Berkey B, Shaw E, Jenkins R, Scheithauer B, Brachman D, Buckner J, Fink K, Souhami L, Laperierre N, Mehta M, Curran W : Phase III trial of chemotherapy plus radiotherapy compared with radiotherapy alone for pure and mixed anaplastic oligodendroglioma : Intergroup Radiation Therapy Oncology Group Trial 9402. J Clin Oncol, 24 (18) : 2707-2714, 2006.

49) van den Bent MJ, Carpentier AF, Brandes AA, Sanson M, Taphoorn MJ, Bernsen HJ, Frenay M, Tijssen CC, Grisold W, Sipos L, Haaxma-Reiche H, Kros JM, van Kouwenhoven MC, Vecht CJ, Allgeier A, Lacombe D, Gorlia T : Adjuvant procarbazine,

lomustine, and vincristine improves progression-free survival but not overall survival in newly diagnosed anaplastic oligodendrogliomas and oligoastrocytomas : a randomized European Organisation for Research and Treatment of Cancer phase Ⅲ trial. J Clin Oncol, 24 (18) : 2715-2722, 2006.

50) Cairncross JG, Macdonald D, Ludwin S, et al : Chemotherapy for anaplastic oligodendroglioma. J Clin Oncol, 12 : 2013-2021, 1994.

51) Van den Bent M, Kros JM, Heimans JJ, et al : Response rate and prognostic factors of recurrent oligodendroglioma treated with PCV chemotherapy. Neurology, 51 : 1140-1145, 1998.

52) Paleologos NA, MacDonald DR, Vick NA, et al : Neoadjuvant procarbazine, CCNU, and vincristine for anaplastic and aggressive oligodendroglioma. Neurology, 53 : 1141-1143, 1999.

53) Hoang-Xuan K, Capelle L, Kujas M, Taillibert S, Duffau H, Lejeune J, Polivka M, Crinière E, Marie Y, Mokhtari K, Carpentier AF, Laigle F, Simon JM, Cornu P, Broët P, Sanson M, Delattre JY : Temozolomide as initial treatment for adults with low-grade oligodendrogliomas or oligoastrocytomas and correlation with chromosome 1p deletions. J Clin Oncol, 22 (15) : 3133-3138, 2004.

54) van den Bent MJ, Looijenga LH, Langenberg K, Dinjens W, Graveland W, Uytdewilligen L, Sillevis Smitt PA, Jenkins RB, Kros JM : Chromosomal anomalies in oligodendroglial tumors are correlated with clinical features. Cancer, 97 (5) : 1276-1284, 2003.

55) Mohile NA, Forsyth P, Stewart D, Raizer JJ, Paleologos N, Kewalramani T, Louis DN, Cairncross JG, Abrey LE : A phase Ⅱ study of intensified chemotherapy alone as initial treatment for newly diagnosed anaplastic oligodendroglioma : an interim analysis. J Neurooncol, 89 (2) : 187-193, 2008.

56) Kouwenhoven MC, Kros JM, French PJ, Biemond-ter Stege EM, Graveland WJ, Taphoorn MJ, Brandes AA, van den Bent MJ : 1p 19q loss within oligodendroglioma is predictive for response to first line temozolomide but not to salvage treatment. Eur J Cancer. 42 (15) : 2499-2503, 2006.

57) van den Bent MJ, Chinot O, Boogerd W, Bravo Marques J, Taphoorn MJ, Kros JM, van der Rijt CC, Vecht CJ, De Beule N, Baron B : Second-line chemotherapy with temozolomide in recurrent oligodendroglioma after PCV (procarbazine, lomustine and vincristine) chemotherapy : EORTC Brain Tumor Group phase Ⅱ study 26972. Ann Oncol, 14 (4) : 599-602, 2003.

58) Hukin J, Epstein F, Lefton D, Allen J : Treatment of intracranial ependymoma by surgery alone. Pediatr Neurosurg, 29 (1) : 40-45, 1998.

59) Vernooij MW, Ikram MA, Tanghe HL, Vincent AJ, Hofman A, Krestin GP, Niessen WJ, Breteler MM, van der Lugt A : Incidental findings on brain MRI in the general population. N Engl J Med, 357 (18) : 1821-1828, 2007.

60) JASTRO データベース委員会：全国放射線治療施設の 2005 年定期構造調査報告（第 1 報）. J Jpn Soc Ther Radiol Oncol, 19 : 181-192, 2007.

61) The Committee of Brain Tumor Registry of Japan : Report of brain tumor registry of Japan (1969-1993) 10th edition. Neurol medico-chirurgica, 40 (suppl), 2000.

62) Walker MD, et al : Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med, 303 : 1323-1329, 1980.

63) Fine HA, et al : Meta-analysis of radiation therapy with and without adjuvant chemotherapy for malignant gliomas in adults. Cancer, 71 : 2585-2597, 1993.

64) Stewart LA : Chemotherapy in adult high-grade glioma : a systematic review and meta-analysis of individual patient data from 12 randomised trials. Lancet, 359 : 1011-1018, 2002.

65) Stupp R, et al : Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med, 352 : 987-996, 2005.

66) Keim-Guibert F, et al : Radiotherapy for Glioblastoma in the Elderly. N Engl J Med, 356 : 1527-1535, 2007.

67) Walker MD, et al : An analysis of dose-effect relationship in the radiotherapy of Malignant gliomas. Int J Radiation Oncology Biol Phys, 5 : 1725-1731, 1979.

68) Anderson AP : Postoperative irradiation of glioblastoma. Acta radiol, 17 : 475-484, 1978.

69) Tanaka M, et al : High-dose conformal radiotherapy for supratentorial malignant glioma : a historical comparison. Lancet Oncol, 6 : 953-960, 2005.

70) Chang JL, et al : Survival and failure patterns of high-grade gliomas after three-dimensional conformal radiotherapy J Clin Oncol, 20 : 1635-1642, 2002.

71) Mizoe J, et al : Phase Ⅰ/Ⅱ clinical trial of carbon ion radiotherapy for malignant gliomas : combined X-ray radiotherapy, chemotherapy, and carbon ion radiotherapy. Int J Radiation Oncology Biol Phys, 69 : 390-396, 2007.

72) Buckner JC, et al : Phase Ⅲ trial of carmustine and cisplatin compared with carmustine alone and standard radiation therapy or accelerated radiation therapy in patients with glioblastoma multiforme : North Central Cancer Treatment Group 93-72-52 and Southwest Oncology Group 9503 trials. J Clin Oncol, 24 : 3871-3879 2006.

73) Hochberg FH, et al : Assumptions in the radiotherapy of glioblastomas. Neurology, 30 : 907-911, 1980.

74) Giese A, et al : Glioma invasion-pattern of dissemination by mechanisms of invasion and surgical intervention, pattern of gene expression and its regulatory control by tumor suppressor p.53 and proto-oncogene ETS-1. Acta Neurochir Suppl, 88 : 153-162, 2003.

75) Emami B, et al : Tolerance of normal tissue to therapeutic irradiation. Int J Radiat Oncol Biol Phys, 21 : 109-122, 1991.

76) Douglas JG, et al : [F-18]-fluorodeoxyglucose positron emission tomography for targeting radiation dose escalation for patients with glioblastoma multiforme : clinical outcomes and patterns of failure. Int J Radiat Oncol Biol Phys, 68 : 144-150, 2007.

77) Solberg TD, et al : A feasibility study of 18F-fluorodeoxyglucose positron emission tomography for targeting and simultaneous integrated boost for intensity-modulated radiosurgery and radiotherapy. J Neurosurg, 101 : 381-389, 2004.

78) Chang J, et al : Image-fusion of MR spectroscopic images for treatment planning of gliomas. Med. Phys, 33 : 32-40, 2006.

79) Chang EL, et al : Evaluation of peritumoral edema in the delineation of radiotherapy clinical target volumes for glioblastoma. Int J Radiat Oncol Biol Phys, 68 : 144-150, 2007.

80) Tsao MN, et al : The American Society for Therapeutic Radiology and Oncology (ASTRO) evidence-based review of the role of radiosurgery for malignant glioma. Int J Radiat Oncol Biol Phys, 63 : 47-55, 2005.

81) Souhami I, et al : Randomized comparison of stereotactic radiosurgery followed by conventional radiotherapy with carmustine to conventional radiotherapy with carmustine for patients with glioblastoma multiforme : report of Radiation Therapy oncology Group 93-05 protocol. Int J Radiat Oncol Biol Phys, 60 : 853-860, 2004.

82) Cardinale R, et al : A phase II trial of accelerated radiotherapy using weekly stereotactic conformal boost for supratentorial glioblastoma multiforme : RTOG 0023. Int J Radiat Oncol Biol Phys, 65 : 1422-1428, 2006.

83) Patchell RA, et al : Postoperative radiotherapy in the treatment of single brain metastases to the brain. JAMA, 280 : 1485-1489, 1998.

84) Meyers CA : Neurocognitive dysfunction in cancer patients. Oncology (Huntington), 14 : 75-79, 2000.

85) Aoyama H, et al : Neurocognitive function of patients with brain metastasis who received either whole brain radiotherapy plus stereotactic radiosurgery or radiosurgery alone. Int J Radiat Oncol Biol Phys, 68 : 1388-1395, 2007.

86) Filley CM, et al : Toxic leukoencephalopathy. N Engl J Med, 345 : 425-432, 2001.

87) Crossen JR, et al : Neurobehavioral sequelae of cranial irradiation in adults : A review of radiation-induced encephalopathy. J Clin Oncol, 12 : 627-642, 1994.

88) Lee AW, et al : Factors affecting risk of symptomatic temporal lobe necrosis : Significance of fractional dose and treatment time. Int J Radiat Oncol Biol Phys, 53 : 75-85, 2002.

89) Corn BW, et al : Prospective Evaluation of Quality of Life and Neurocognitive Effects in Patients with Multiple Brain Metastases Receiving Whole-Brain Radiotherapy with or without Thalidomide on Radiation Therapy Oncology Group (RTOG) Trial 0118. Int J Radiat Oncol Biol Phys, 71 (1) : 71-78, Epub 2007.

90) Shaw EG, et al : Phase II study of donepezil in irradiated brain tumor patients. Effect on cognitive function, mood and quality of life. J Clin Oncol, 24 : 1415-1420, 2006.

91) Shaw E, et al : Single dose radiosurgical treatment of recurrent previously irradiated primary brain tumors and brain metastases : final report of RTOG protocol 90-05. Int J Radiat Oncol Biol Phys, 47 : 291-298, 2000.

92) Suzuki H, et al : Spontaneous haemorrhage into metastatic brain tumours after stereotactic radiosurgery using a linear accelerator. J Neurol Neurosurg Psychiatry, 74 : 908-912, 2003.

93) Boyd T.S., et al : Stereotactic radiosurgery for brain metastases. Oncology (Williston Park), 13 : 1397-1409, 1999.

94) Mehta MP, et al : The American Society for Therapeutic Radiology and Oncology (ASTRO) evidence-based review of the role of radiosurgery for brain metastases. Int J Radiat Oncol Biol Phys, 63 : 37-46, 2005.

95) Andrews DW, et al : Whole brain radiation therapy with or without stereotactic radiosurgery boost for patient with one to three brain metastases : phase III result of the RTOG 9508 randomized trial. Lancet, 363 : 1665-1672, 2004.

96) Aoyama H, et al : Stereotactic radiosurgery plus whole-brain radiation therapy vs. stereotactic radiosurgery alone for treatment of brain metastases : A randomized controlled trial. JAMA, 295 : 2483-2491, 2006.

97) 河内正人 他：成人大脳半球膠芽腫に対する PCB, ACNU, VCR-IFN-beta (PAV-IFN) vs. PAV-第Ⅲ相試験. 第 61 回日本脳神経外科学会, 1112-1113, 2002.

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Pulmonary Metastasectomy for Pulmonary Metastases of Head and Neck Squamous Cell Carcinomas

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Background. The lung is the major organ for distant metastasis from head and neck cancers, and pulmonary metastasectomy is indicated for selected cases. The efficacy of surgical treatment for pulmonary metastatic lesions from head and neck cancers has not been thoroughly examined.

Methods. The database developed by the Metastatic Lung Tumor Study Group of Japan was retrospectively reviewed. Between November 1980 and September 2006, 237 patients underwent resection of pulmonary metastases from primary head and neck cancers. After excluding nonsquamous cell carcinomas, 114 cases were analyzed, and the survival and prognostic factors for pulmonary metastasectomy for metastases from head and neck cancers were determined.

Results. The overall 5-year survival rate after pulmonary metastasectomy was 26.5%, and the median survival time was 26 months. As determined by univariate analysis, poor prognostic factors were oral cavity cancers, lymph node metastasis, a disease-free interval of 24 months or less, and incomplete resection. Multivariate analysis revealed that poor prognostic factors were being male, having oral cavity cancers, lymph node metastasis, and incomplete resection. When patients were divided into males with oral cavity cancers (n = 17) and all others (n = 97), the 5-year survival rates were 0% and 31.6%, respectively. Survival of male patients with oral cavity cancer that metastasized was significantly reduced ($p < 0.001$).

Conclusions. Male sex, oral cavity cancers, lymph node metastasis, and incomplete resection were poor prognostic factors for pulmonary metastases, but there is the potential for a good surgical outcome in carefully selected patients.

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The lung is the major organ of distant metastasis from head and neck cancers [1, 2]. Although surgical resection is an important treatment for pulmonary metastasis [3–5], pulmonary metastasis is commonly considered to reflect systemic disease, and the prognosis for pulmonary metastasis from head and neck cancers remains poor. The incidence of pulmonary metastasis from head and neck cancers is reported to range from 6.0% to 9.1% [6, 7]. With regard to pulmonary metastasectomy, Wedman and colleagues [6] reported a 5-year survival rate of 59% in a pulmonary metastasectomy group, but only 4% in a non-metastasectomy group. Although Yamagata and associates [7] reported that docetaxel-based chemotherapy had a better response rate for pulmonary metastases than non–docetaxel-based chemotherapy, the role of chemotherapy seems to be limited.

The survival rate after surgery for pulmonary metastases from head and neck cancers is reported to range from 29.0% to 59.4% [6, 8–10]. However, owing to the small number of previous studies, prognosis after surgery for pulmonary metastases from head and neck cancers has not been thoroughly examined. The 2006 annual report by the Japanese Association for Thoracic Surgery documents 4,912 patients who underwent pulmonary metastasectomy, among whom were 260 patients (5.3%) with head and neck cancers [11]. The number of cases of pulmonary metastasectomy for head and neck cancers in a single institution is limited; therefore, we retrospectively reviewed cases registered in the database of the Metastatic Lung Tumor Study Group of Japan of patients who underwent surgical treatment for pulmonary metastases from head and neck squamous cell carcinomas. From this, we identified prognostic

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factors for patients who underwent pulmonary metastasectomy. Furthermore, we identified the surgical indications for pulmonary metastasis from head and neck squamous cell carcinomas.

Patients and Methods

The Metastatic Lung Tumor Study Group of Japan developed a database for registration of lung metastasis cases of patients undergoing surgical resection. It documents the following: sex, age, primary tumor histopathology, stage of the primary tumor, treatment for the primary tumor, date of primary surgery, type of surgery, curability, date of metastasis, disease-free interval (DFI), side, size, number of resected metastases, date of metastasectomy, relapsed sites, and follow-up. The DFI was calculated from the date of the initial treatment for the primary cancer to the date of diagnosis of pulmonary metastasis. Between March 1984 and September 2006, 237 patients who underwent resection of pulmonary metastases from head and neck cancers were enrolled in the database. The endpoint of our retrospective study was survival after pulmonary metastasectomy. The Ethics Committees of the affiliated institutions approved this database study and waived the need for informed consent from patients as long as patient data remained anonymous.

Of the 237 patients with pulmonary metastases, 98 patients had nonsquamous cell carcinoma. Because nonsquamous cell carcinomas have various histological types and better survival, cases with lung metastasis from nonsquamous cell carcinoma were excluded from analysis [6, 10]. In addition, because pulmonary metastases from oral cavity cancers are reported to have a poor prognosis [8], special emphasis was placed on investigating the differences between oral cavity and non–oral cavity cancers. Twenty-five cases were excluded because of incomplete data, including the number of resected metastases, age, DFI, or prognosis. A central review of the pathologic specimens was not carried out by our study group. The data from the remaining 114 patients were retrospectively reviewed. Preoperative examination, surgical indication, operative procedure, and postoperative treatment were at the discretion of the institution where the patient was treated. The surgical procedure depended on the location, size, or number of metastases. Lymph node dissection was not routinely performed and depended on the institution. As previously reported [12], general indications for surgical resection of pulmonary metastasis followed the criteria of Thomford and coworkers [13]: the primary lesion was under control or was planned to be under control; there were no metastases to other organs; and the patient's general condition was good enough to withstand surgery.

Statistical Analysis

Overall survival was analyzed by the Kaplan-Meier method, and differences in variables were calculated by the log-rank test. The date of pulmonary resection was defined as the starting point. The Cox proportional hazards regression analysis was used for multivariable analysis. The data were calculated using version 5.0 of the StatView software package (SAS Institute, Cary, NC). Values of p less than 0.05 were considered statistically significant.

Table 1. Characteristics of Patients Undergoing Surgery for Pulmonary Metastases From Head and Neck Squamous Cell Carcinomas

Characteristic	Number (%)
Male	93 (82)
Female	21 (18)
Location of primary site	
Larynx	32 (28)
Oral cavity	27 (24)
Hypopharynx	24 (21)
Nasal cavity and paranasal sinuses	9 (8)
Mesopharynx	7 (6)
Salivary gland	4 (4)
Epipharynx	3 (3)
Other sites	8 (7)
Treatment for the primary tumor	
Surgery and radiotherapy	40 (35)
Surgery	30 (27)
Surgery and chemoradiotherapy	26 (23)
Surgery and chemotherapy	7 (6)
Radiotherapy	5 (4)
Chemoradiotherapy	4 (4)
Unknown	2 (2)
Surgical procedure	
Lobectomy	62 (54)
Wedge resection	37 (33)
Segmentectomy	10 (9)
Pneumonectomy	5 (4)
Lymph node dissection	
Positive	55 (48)
Negative	40 (35)
Unknown	19 (17)

Results

Patient characteristics and the primary site locations of head and neck squamous cell carcinomas are listed in Table 1. The TNM stages for head and neck primary cancers were not recorded in detail. The patients' mean age at the time of pulmonary metastasectomy was 63 years (range, 26 to 81). The mean tumor size was 3.3 cm (range, 0.7 to 11 cm). The median number of resected metastatic lesions per patient was 1 lesion (range, 1 to 6). The median DFI was 16 months (range, 0 to 87). The median follow-up time from the time of metastasectomy was 120 months.

One patient died of pneumonia during the perioperative period. The operative mortality rate was 0.9%. The overall 5-year survival rate after pulmonary metastasectomy was 26.5% (Fig 1). The median survival time was 26 months. Survival according to each primary site is shown in Table 2. The patients with oral cavity cancer showed

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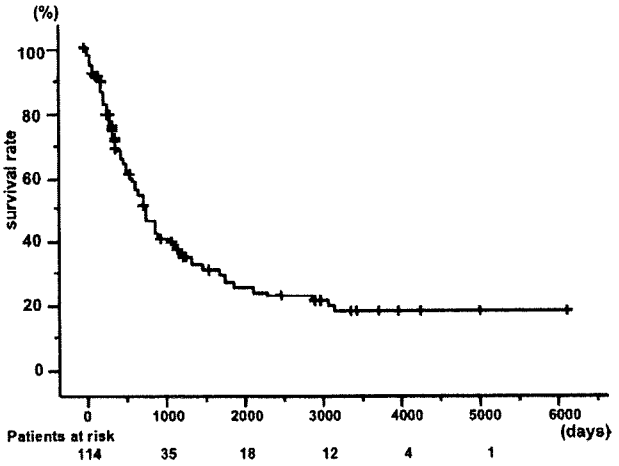

Fig 1. Overall survival of 114 patients after pulmonary metastasectomy for head and neck squamous cell carcinoma. The 5-year survival rate was 26.5%.

significantly worse prognosis than the other cancer patients ($p = 0.03$). Postoperative therapy after pulmonary metastasectomy was as follows: chemotherapy in 17 patients, chemoradiotherapy in 6 patients, and radiation in 5 patients. There was no significant difference between the no adjuvant therapy group and the adjuvant groups. We assessed the relationship between survival and the following clinical factors: sex, age (≥ 75 or < 75 years), tumor size (> 3 or ≤ 3 cm), number of resected metastases (solitary or multiple), resected side (unilateral or bilateral), location of primary sites (oral cavity or non–oral cavity), primary lymph node metastasis, DFI (> 24 or ≤ 24 months), and curability (complete or incomplete). Table 3 shows the results of univariate analyses of survival and clinical factors. Oral cavity cancers, lymph node metastasis, DFI 24 months or less, and incomplete resection are poor prognostic factors. Table 4 shows the results of multivariate analyses of survival and clinical factors. Male sex, oral cavity cancers, lymph node metastasis, and incomplete resection were poor prognostic factors.

Because being male and having oral cavity cancers could be considered preoperative prognostic factors, the patients were divided into two groups for further analysis: males with oral cavity cancers (n = 17), and others (n = 97). Survival of the male patients with metastatic oral cavity cancer was significantly worse ($p < 0.001$; Fig 2). No male patients with metastatic oral cavity cancer remained alive 5 years after undergoing pulmonary metastasectomy.

Table 2. Five-Year Survival of Patients With Pulmonary Metastasis According to Primary Site

Primary Site	Number	5-Year Survival (%)
Larynx	32	40.9
Oral cavity	27	9.2
Hypopharynx	24	19.8
Nasal cavity and paranasal sinuses	9	47.6
Mesopharynx	7	28.6
Salivary gland	4	33.3
Epipharynx	3	0
Other sites	8	42.9

Table 3. Survival of 114 Patients According to Clinical Variables of Pulmonary Metastases

Variables	n (%)	5-Year Survival (%)	p Value
Male	93 (82)	22.9	0.355
Female	21 (18)	41.3	
Age, years			
≥75	17 (15)	43.7	0.234
<75	97 (85)	24.4	
Size			
≤ 3 cm	70 (61)	31.8	0.820
> 3 cm	44 (39)	23.3	
Number			
Solitary	84 (74)	26.0	0.646
Multiple	30 (26)	27.3	
Resected side			
Unilateral	104 (91)	25.8	0.240
Bilateral	10 (9)	34.3	
Primary site			
Non-oral cavity	87 (76)	32.4	< 0.001
Oral cavity	27 (24)	9.2	
Lymph node metastasis			
Positive	30 (26)	13.8	0.010
Negative	84 (74)	32.0	
Disease-free interval			
> 24 months	31 (27)	40.0	0.044
≤ 24 months	83 (73)	21.0	
Curability			
Complete	102 (89)	26.7	0.037
Incomplete	12 (11)	25.0	

Table 4. Relationship of Individual Variables to Outcome, Cox's Proportional Hazards Regression Analysis

Variable	Risk Ratio	95% CI	p Value
Male	2.93	1.41–6.09	0.004
Age, < 75 years	0.99	0.44–2.23	0.986
Size > 3 cm	0.91	0.55–1.51	0.715
Multiple lung metastasis	1.70	0.97–3.00	0.066
Bilateral lung metastasis	0.49	0.14–1.65	0.248
Oral cavity origin	3.67	1.99–6.76	< 0.001
Lymph node metastasis	2.09	1.20–3.61	0.009
Disease-free interval ≤ 24 months	1.70	0.94–3.07	0.081
Incomplete resection	2.47	1.25–4.87	0.009

CI = confidence interval.

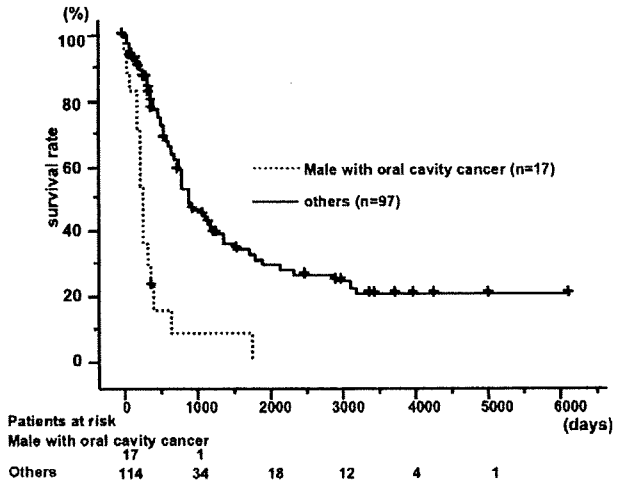

Fig 2. Survival curves based on two groups, males with oral cavity cancer (dotted line [n = 17]) and others (solid line [n = 97]), obtained by dividing by prognostic factors.

Comment

This study revealed a 5-year survival rate after pulmonary metastases from head and neck squamous cell carcinoma of 26.5%, and showed that male sex, oral cavity cancer, lymph node metastasis, and curability are prognostic factors. Nibu and colleagues [8] investigated 32 patients with squamous cell carcinoma of the head and neck who underwent thoracotomy for pulmonary metastases, and reported an overall 5-year survival rate of 32%. Finley and coworkers [9] reported a 29% 5-year survival rate for 18 patients who underwent complete resection of pulmonary metastases from head and neck squamous cell carcinomas, and showed that the survival of patients who underwent metastasectomy was significantly better than that of patients who did not. Wedman and colleagues [6] investigated 138 patients with pulmonary metastases from head and neck cancers, and reported that the 5-year survival rate for 21 patients who underwent pulmonary metastasectomy was 59%, compared with 4% in the nonmetastasectomy group.

Compared with other treatments, we believe that the role of pulmonary resection for metastasis from head and neck squamous cell carcinomas has been established. However, previous studies have been small and of limited sample size. We analyzed data from a large number of patients enrolled in a multi-institutional registry and assessed prognostic factors for pulmonary metastasectomy by multivariate analysis. With regard to prognostic factors after pulmonary metastasectomy that were determined by other investigators, oral cavity cancers, mediastinal lymph node metastasis, and pleural invasion were all negative factors [8]. As determined by multivariate analysis, incomplete resection, complications associated with surgery, and adjuvant therapy of the primary tumor were unfavorable prognostic factors [14]. Liu and associates [15] reported 83 cases of pulmonary metastasis from head and neck cancers, and demonstrated that incomplete resection, age less than 50 years, and DFI of 2 years or less were poor prognostic factors. Disease-free interval is commonly recognized as a significant prognostic factor in various types of pulmonary metastasis [4]. In other reports, DFI longer than 12 months and solitary lung metastasis were favorable prognostic factors for metastatic head and neck cancers [9, 10]. However, the multivariate analysis of this study determined that DFI was not significantly associated with surgical outcome. Disease-free interval depends on the follow-up period or accuracy of diagnostic procedure. Although a short DFI might reflect aggressive and invasive biological behavior, the evaluation of DFI remains difficult as a prognostic factor.

In accordance with a report by Nibu and colleagues [8], our analysis demonstrated that metastatic oral cavity cancer was a significantly worse prognostic factor. On the other hand, Leon and associates [16] showed that the primary site of head and neck cancers is a significant prognostic factor for distant metastasis, and that cancer in the oral cavity had the lowest risk for distant metastasis. Distant metastases originating from oral cavity cancer depend on disease stage [17]. In our multi-institutional study, we could not determine in detail the stage of primary cancer, and oral cavity cancer patients with pulmonary metastasis might have included many advanced stage cases. We investigated the basis behind why metastasized oral cavity cancer has a worse prognosis than other types of head and neck cancer, but unfortunately, could not find the reason. The biological behavior of oral cavity cancers might be different from that of other head and neck cancers. Further study is needed to clarify this issue.

Male sex has been reported to be a prognostic factor in resected pulmonary metastasis from head and neck squamous cell carcinoma [10]. We did not investigate smoking behavior or comorbidities in this study, and that is an important weakness in the study. Smoking status is a well-known unfavorable prognostic factor and might be related to the results of this study with regard to male sex.

Head and neck cancers have varying histology and occur at various primary sites. Some investigators have studied pulmonary metastasis from nonsquamous cell head and neck cancers [6, 10, 15]. The benefits of metastasectomy for nonsquamous cell carcinoma have not been clarified [15]. Because the biological character of nonsquamous cell carcinoma in head and neck cancers is very different from that of squamous cell carcinoma, we focused our study on squamous cell carcinoma. There were 98 nonsquamous cell carcinoma cases registered in our database. The origins of the metastatic cancers were as follows: thyroid 35, salivary gland 34, oral cavity 3, epipharynx 2, mesopharynx 2, and other sites 22. The overall survival of patients with nonsquamous cell carcinomas was significantly better than that of patients with squamous cell carcinoma, and the 5-year survival rate of nonsquamous cell carcinoma patients was 68.4% (data not shown).

In this study, the percentage of lobectomy was relatively high at 54%. We consider the reason for the high rate of lobectomy to be the difficulty in the preoperative diagnosis of pulmonary metastasis from primary lung cancer. In

addition, as the mean tumor size was 3.3 cm, lobectomy was a suitable indication for the large tumor size.

Rush [3] has stated that the most important factors for selecting patients for surgery are control of the primary tumor, ability to resect all metastatic disease, absence of extrathoracic disease, and lack of better alternative systemic therapy. Regarding the surgical indications for pulmonary metastasis from head and neck cancers, Liu and colleagues [15] gave the following criteria: the metastasis is limited to the lungs, the lesions are all resectable, and locoregional control of the head and neck primary cancer is obtained or obtainable. Complete resection and locoregional control of primary sites are obviously essential. Our data also suggest that metastatic oral cavitary cancers, especially in male patients, have a poor prognosis. Therefore, indications for surgery in metastatic head and neck squamous cell carcinomas should include consideration of the types of primary sites.

There are some limitations to our study. First, we should examine each primary site, but the numbers of cancers at each site are quite small. Large-scale databases are needed to clarify each head and neck cancer. Second, a histopathologic differential diagnosis between head and neck squamous cell carcinomas and lung squamous cell carcinomas is often difficult. In general, the patient's clinical course and stage of primary sites were used to help diagnose whether the patient had pulmonary metastasis or primary lung squamous cell carcinoma. Because the present study was a retrospective analysis, and the differential diagnosis was made at the discretion of each institution, our data might include primary lung cancer. A report on a loss of heterozygosity analysis revealed that 18 of 44 cases of squamous cell lung lesions, which had been clinically interpreted as metastases from head and neck cancers, were considered to be second primary lung cancers [18]. Additional advances in molecular techniques and analysis are needed to further elucidate this complicated problem. Third, there is a selection bias affecting pulmonary metastasectomy outcome results. Most of the reports of the results of metastasectomy come from thoracic surgery centers [6], and candidates for pulmonary metastasectomy are strictly selected based on the aforementioned criteria. Therefore, the present study may be presenting relatively good survival rates. However, selection bias is unavoidable and is a difficult problem to resolve. A prospective randomized trial for pulmonary metastasis treatment is ethically difficult, and is not practical.

In conclusion, we identified prognostic factors for pulmonary metastases from head and neck squamous cell carcinomas. According to multivariate analyses, male sex, oral cavity cancer, lymph node metastasis, and incomplete resection are poor prognostic factors. Male patients with pulmonary metastasis from oral cavity cancers have a poor prognosis, and pulmonary metastasectomy for these patients may not be beneficial. Since there are adequate selection criteria for patients, a good outcome after surgery is promising, with careful preoperative examination, including positron emission tomography [17, 19], and selection.

References

1. Shingaki S, Suzuki I, Kobayashi T, Nakajima T. Predicting factors for distant metastases in head and neck carcinomas: an analysis of 103 patients with locoregional control. J Oral Maxillofac Surg 1996;54:853–7.
2. Ferlito A, Shaha AR, Silver CE, Rinaldo A, Mondin V. Incidence and sites of distant metastases from head and neck cancer. ORL J Otorhinolaryngol Relat Spec 2001;63: 202–7.
3. Rusch VW. Pulmonary metastasectomy. Current indications. Chest 1995;107:322–31.
4. Pastorino U, Buyse M, Friedel G, et al. Long-term results of lung metastasectomy: prognostic analyses bases on 5206 cases. J Thorac Cardiovasc Surg 1997;113:37–49.
5. Davidson RS, Nwogu CE, Brentjens MJ, Anderson TM. The surgical management of pulmonary metastasis: current concepts. Surg Oncol 2001;10:35–42.
6. Wedman J, Balm AJ, Hart AA, et al. Value of resection of pulmonary metastases in head and neck cancer patients. Head Neck 1996;18:311–6.
7. Yamagata K, Onizawa K, Otsuka Y, Yoshida H. Treatment for lung metastasis from head and neck squamous cell carcinoma: a preliminary study of docetaxel. Oral Maxillofac Surg 2008;12:13–8.
8. Nibu K, Nakagawa K, Kamata S, et al. Surgical treatment for pulmonary metastases of squamous cell carcinoma of the head and neck. Am J Otolaryngol 1997;18:391–5.
9. Finley RK, Verazin GT, Driscoll DL, et al. Results of surgical resection of pulmonary metastases of squamous cell carcinoma of the head and neck. Am J Surg 1992;164:594–8.
10. Chen F, Sonobe M, Sato K, et al. Pulmonary resection for metastatic head and neck cancer. World J Surg 2008;32:1657–62.
11. Ueda Y, Fujii Y, Udagawa H. Thoracic and cardiovascular surgery in Japan during 2006. Gen Thorac Cardiovasc Surg 2008;56:365–88.
12. Kawamura M, Nakajima J, Matsuguma H, et al. Surgical outcomes for pulmonary metastases from hepatocellular carcinoma. Eur J Cardiothorac Surg 2008;34:196–9.
13. Thomford NR, Woolner LB, Clagett OT. The surgical treatment of metastatic tumors in the lungs. J Thorac Cardiovasc Surg 1965;49:357–63.
14. Winter H, Meimarakis G, Hoffmann G, et al. Dose surgical resection of pulmonary metastases of head and neck cancer improve survival? Ann Surg Oncol 2008;15:2915–26.
15. Liu D, Labow DM, Dang N, et al. Pulmonary metastasectomy for head and neck cancers. Ann Surg Oncol 1999;6: 572–8.
16. Leon X, Quer M, Orus C, del Prado Venegas M, Lopez M. Distant metastases in head and neck cancer patients who achieved loco-regional control. Head Neck 2000;22:680–6.
17. Betka J. Distant metastases from lip and oral cavity cancer. ORL J Otorhinolaryngol Relat Spec 2001;63:217–21.
18. Geurts TW, Nederlof PM, van den Brekel MW, et al. Pulmonary squamous cell carcinoma following head and neck squamous cell carcinoma: metastasis or second primary? Clin Cancer Res 2005;11:6608–14.
19. Wong RJ. Current status of FDG-PET for head and neck cancer. J Surg Oncol 2008;97:649–52.

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Breast cancer resistant protein (BCRP) is a molecular determinant of the outcome of photodynamic therapy (PDT) for centrally located early lung cancer

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ABSTRACT

The ATP-binding cassette (ABC) transporter protein, BCRP (breast cancer resistance protein)/ABCG2 pumps out some types of photosensitizers used in photodynamic therapy (PDT) and causes resistance to the antitumor effect of PDT. The purpose of this study was to investigate the association between the expression of BCRP and the efficacy of PDT using Photofrin, or the second-generation photosensitizer, NPe6, for centrally located early lung cancers.

Using human epidermoid carcinoma cells, A431 cells and the BCRP-overexpressing A431/BCRP cells, we examined the effects of BCRP expression on the effect of PDT by cell viability assay *in vitro*, and investigated the expression of BCRP by immunohistochemical analysis in 81 tumor samples obtained from patients with centrally located early lung cancers.

The A431/BCRP cells were more resistant to Photofrin-PDT than A431 cells *in vitro*, and Fumitremorgin C, a specific inhibitor of BCRP, reversed the resistance.

However, there was no significant difference in the antitumor effect of NPe6-PDT between these cells. All of the 81 centrally located early lung cancer lesions were BCRP-positive (2+, 45 lesions; 1+, 30 lesions) and all the patients were male and heavy smokers (>30 pack-years). The expression of BCRP significantly affected the efficacy of Photofrin-PDT in cancer lesions $\geq$10 mm in diameter ($P = 0.04$). On the other hand, NPe6-PDT exhibited a strong antitumor effect, regardless of the expression status of BCRP.

Photofrin may be a substrate of BCRP and be pumped out from the cells, therefore, BCRP may be a molecular determinant of the outcome of Photofrin-PDT.

1. Introduction

Photodynamic therapy (PDT), used as a treatment modality for many cancers, uses a tumor-specific photosensitizer and laser irradiation to induce the production of reactive oxygen species in cancer cells [1,2]. PDT is widely used as a treatment option for solid cancers and also for some non-cancerous diseases [3]. The first health agency approval for PDT using Photofrin®, the most commonly employed photosensitizer, was obtained in Canada in 1993, and the substance was then approved by the United States Food and Drug Administration (FDA) for the treatment of early stage lung cancer as well as advanced esophageal and lung cancers [1,3].

In Japan, PDT is recommended as a treatment option for centrally located early lung cancers, which are roentgenograhically occult squamous cell carcinomas located no distal to the segmental bronchi, that are histologically determined to be carcinoma *in situ* or carcinoma showing only limited invasion, with no evidence of invasion beyond the bronchial cartilage, as defined in the therapeutic guidelines for lung cancer established by the Japanese Ministry of Health, Labour and Welfare based on the principles of evidence-based medicine [4,5]. Centrally located early lung cancers can be detected in patients at high risk by either sputum cytology or bronchoscopic evaluation [6]. One to 4% of these patients have a synchronous lung cancer, and the risk of a second lung cancer ranges from 1% to 25% per year [7]. For the patients with centrally located early lung cancer, PDT allows preservation of lung function and effective treatment, and is recommended for treatment in the American College of Chest Physician (ACCP) evidence-based clinical practice guidelines [8]. The second-generation photosensitizer,

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talaporfin sodium (NPe6, laserphyrin), which has a major absorption band at 664 nm, was approved by the Japanese government for use in the diagnosis/treatment of centrally located early lung cancer [4,5]. A phase II clinical study using NPe6 and a diode laser for early stage lung cancer demonstrated excellent antitumor effects and safety, including a significantly lower skin incidence of photosensitivity as compared to that observed with photofrin [9]. The Japanese government approved the use of NPe6 for PDT in 2003, and the product has been available in the Japanese market since June 2004 [4,5]. Recently, we established the autofluorescence diagnosis system integrated into a videoendoscope (SAFE-3000) as a very useful technique for the early diagnosis of lung cancer [10]. The novel photodynamic diagnosis (PDD) system using SAFE-3000 and NPe6 improved the quality and efficacy of PDT and avoided misjudgment of the dose of the photosensitizer or laser irradiation in PDT [11]. It has been reported that PDT induces direct tumor cell kill, as well as indirect effects on the tumor microenvironment [12]. PDT rapidly induces apoptosis, inflammatory reactions, tumor-specific and/or—non-specific immune reactions and damage to the microvasculature of the tumor bed [13–15]. Sitnik et al. reported that the microvasculature damage induced by PDT is readily observable histologically and is associated with a significant decrease of the blood flow and severe hypoxia in the tumor [16]. Recently, we examined the role of immunological reactions in the antitumor effects of PDT using cytokine-overexpressing cells [17], and we demonstrated that the extent of photodamage of the anti-apoptotic protein, Bcl-2, caused by PDT determined the sensitivity of cancer cells to apoptosis and the overall cell killing by PDT [18–20]. However, the relationship between the anticancer potency of PDT and the immunological reactions induced by it, is still controversial, and the precise mechanism of the antitumor effect of PDT remains unclear.

Recently, it was reported that the expression of the ATP-binding cassette (ABC) transport proteins renders tumor cells resistant to chemotherapeutic drugs that are substrates of these proteins [21–23], and the effect of these transporters on the intracellular accumulation of photosensitizer has been examined as a potential cause of resistance to PDT [24]. Several members of the ABC transporter protein family may be involved in MDR (multi-drug resistance) in human tumor cells, including P-glycoprotein (Pgp), MDR protein (MRP)1, MRP2, and MRP3 [20]. Elevated expression of breast cancer resistant protein (BCRP) in particular, also known as ABCG2, has been shown to cause resistance to anticancer drugs *in vitro*, including to topotecan, irinotecan, mitoxantrone and doxorubicin [21,22]. Robey et al. reported that BCRP transported some photosensitizers out of cells to decrease intracellular photosensitizer accumulation, suggesting that the presence of BCRP might be a possible cause for cellular resistance to PDT [24]. Jonker et al. also showed that BCRP-knockout mice were photosensitive because of increased intracellular protoporphyrin IX (PpIX) levels [25].

In this study, we examined the association between the expression of BCRP and the efficacy of PDT by retrospectively examining the expression levels of BCRP in clinical samples of centrally located early lung cancers, and investigated whether BCRP expression might be a determinant of the outcome of PDT in lung cancers.

2. Material and methods

2.1. Cell culture

A431 human epidermoid carcinoma cells were cultured in Dulbecco's modifided Eagle's medium (DMEM) supplemented with 10% fetal bovine serum at 37 °C in 5% CO_2 [22]. A431/BCRP cells were established by the transduction of A431 cells with a HaBCRP retrovirus vector composed of Myc-tagged human BCRP cDNA in the Ha retrovirus vector [22,26]. The stably transfected cell line was maintained in the drug-free medium for up to 3 months.

2.2. Photosensitizer

Photofrin (Wyeth Japan K.K., Tokyo, Japan), a hydrophobic hematoporphyrin derivative, remains in a complex mixture with inherent variability, and has been shown to exhibit strong tumor affinity [5,11,17,20]. It is activated by a highly transmissive red light having a wavelength of 630 nm, to produces a photochemical reaction [4,5,20]. NPe6 (Meiji Seika, Tokyo, Japan) is a second-generation water-soluble photosensitizer with a molecular weight of 799.69 and a chlorine annulus, and has its highest absorption peak at the wavelengths of 407 nm and a second peak at the wavelength of 664 nm. NPe6 exhibits superior in tumor affinity as compared to Photofrin, and is excited by visible red light with a longer wavelength of 664 nm, which allows deeper and better penetration into living tissues [4,5,11,20].

2.3. Laser unit

An excimer dye laser (Hamamatsu Photonics K.K., Hamamatsu, Japan) emitting pulse-wave laser light at a wavelength of 630 nm was used as the light source for the excitation of Photofrin [4,5]. A diode laser (Matsushita Electric Industrial Co., Osaka, Japan) emitting continuous-wave laser light at a wavelength of 664 nm was used as the light source for the excitation of NPe6 [4,5,11].

2.4. Measurement of the fluorescence intensity of Photofrin and NPe6 in the cells

Cells were exposed to Photofrin (2.5 μg/ml) or NPe6 (15 μg/ml) for 4 h and washed with phosphate-buffered saline (PBS). The photosensitizers were used at the IC_{50} dose. The photosensitizer in the cells was excited at 405 nm, and the fluorescence was detected with a charge-coupled device (CCD) camera system (Argus/Hisca, Hamamatsu Photonics Co. Ltd., Hamamatsu, Japan) through a multilaminate interference filter that can select the fluorescence wavelength at 630 as previous report [27].

2.5. Determination of the cell viability

Cells were seeded into 96-well microculture plates at 1×10^4 cells/well and allowed to adhere to the dish overnight. The medium was removed and replaced with that containing or not containing a specific inhibitor of BCRP inhibitor, Fumitremorgin C (FTC) (Alex biochemical Inc., CA, USA) [24,26,28]. Fifteen minutes later, the photosensitizer (Photofrin or NPe6) was added to the cells in increasing concentrations, followed by incubation at 37 °C in the dark for 4 h. The cells were washed with PBS and incubated with 10% FBS-DMEM for 1 h, and then washed again with PBS and irradiated with laser (33 mW/cm^2, total energy 10 J/cm^2) [27], followed by incubation for an additional 24 h. Cell viability was measured using the tetrazolium salt WST-1 assay, in accordance with the manufacturer's instructions [27,29]. Independent experiments were repeated at least three times to confirm the data.

2.6. Criteria for the diagnosis of centrally located early lung cancer

Lung cancers located no distal to segmental bronchi, diagnosed histologically as squamous cell carcinoma and determined to be carcinoma *in situ* or carcinoma showing only limited invasion with no evidence of invasion beyond the bronchial cartilage were defined as centrally located early lung cancers, which are roentgenographically occult [4,5,11]. We routinely determined the tumor depth

by EBUS (endobronchial ultrasonography), and it was confirmed that the tumors did not invade the bronchial wall beyond the level of the carlitage and that they were confined to the basal membrane of the mucosa, submucosa or intracarlitagious layers of the bronchial wall [4,5,11]. In 2003, the Japan Photodynamic Association and Japanese Society of Laser Surgery and Medicine established the following therapeutic criteria for PDT in cases with centrally located early lung cancers [4,5,11]: patients with [1] endoscopically assessable early lung cancer [2], normal chest X-ray and CT (roentgenographically occult) [3], no metastasis to lymph nodes or distant metastasis as revealed by routine clinical diagnostic methods, including fluorodeoxyglucose-position emission tomography (FDG-PET) for staging.

2.7. Procedures of PDT and follow-up

PDT was performed using Photofrin or NPe6. Laser irradiation (630 nm) for Photofrin-PDT was transmitted via quartz fibers inserted through the biopsy channel of the endoscope, 48 h after the administration of the photosensitizer, Photofrin (2 mg/kg). On the other hand, for NPe6-PDT, laser irradiation was accomplished 4 h after the administration of NPe6 using a diode laser, the PD laser (40 mg/m^2). The total energy of the laser irradiation was: 100 J/cm^2, 150 mW/cm^2 [4,5,9,11].

The Japanese government approved the use of NPe6 for PDT against centrally located early lung cancers in 2003, and the product became available in the Japanese market in June 2004 [4,5,11]. Ever since, we have used NPe6 for PDT. Fiberoptic bronchoscopy with cytological and histological examination was performed at 1, 2 and 3 months after the PDT, and thereafter, at 3-month intervals during the first year and 6-month intervals during the second year after PDT. The antitumor effect of the initial treatment was rated based on endoscopic measurement of the tumor size using forceps, the morphologic appearance, and the pathological findings of the biopsy specimens, in accordance with the general rules of the Japan Lung Cancer Society and the Japan Society of Clinical Oncology [4,5,11]. The antitumor effect was again evaluated at 3 months after the PDT. The tumors were then classified as showing complete response (CR) (no microscopically demonstrable tumor in the brushings and or biopsy specimens over a period of 4 weeks) [5,9,11].

2.8. Patient selection

A total of 110 patients (128 lesions) with centrally located early lung cancer received PDT at the Tokyo Medical University Hospital between January 1998 and December 2006. Adequate tumor biopsy specimens were obtained from 81 of these lesions (57 from the Photfrin-PDT group and 24 from the NPe6-PDT group, and the specimens were analyzed retrospectively in this study. The clinicopathological characteristics of the patients are listed in Table 1. Their median age at diagnosis was 71 years (range, 56–84). All the patients were male and heavy smokers with a smoking history of >30 pack-years. All of the lesions were diagnosed as squamous cell carcinoma. PDT and tumor biopsy were undertaken in the patients after obtaining their informed consent in accordance with the institutional guidelines, on the basis of the criteria for PDT criteria, all of patients underwent tumor biopsy and PDT.

2.9. Immunohistochemical analysis

Immunohistochemical staining was performed on 4 μM formalin-fixed, paraffin-embedded tissue sections [31]. The slides were deparaffinized in xylene and dehydrated in a graded ethanol series. Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol for 10 min. All of the slides were heated to 95 °C by exposure to microwave irradiation for 20 min. The slides were then cooled for 1 h at room temperature and washed in PBS. Non-specific binding was blocked by preincubation with 1% BSA for 30 min. After washing with PBS, the slides were incubated for 1 h at room temperature with anti-BCRP antibody (Bxp-21; Chemicon, Temecula, CA, USA) [22,26,30–34]. Staining with the antibodies was considered to be positive if ≥10% of the tumor cells were stained, based on the use of the 10% cutoff level in several previous studies [31,33,34]. All of the slides were examined and scored independently by two observes without knowledge of the patient clinical data.

The immunohistochemical staining was scored based on the estimated average staining intensity of the tumor cells: (−), negative; (+), intermediate; and (2+), strongly positive [35,36]. This study was conducted with the approval of the Ethical Committee of Tokyo Medical University.

Table 1
Characteristics of centrally located early lung cancer (January 1998–December 2006).

Characteristics	Number of lesions
Patients (lesions)	79 (81)
Age	67–83
Gender	Male: 79 Female: 0
Histology	Sq. cell ca.: 81 lesions
Smoking history	Positive: 79 (>30 pack-years) Negative: 0
PDT	
Photofrin:	57 lesions
NPe6:	24 lesions

2.10. Statistical analysis

The correlations between immunohistochemical expression and the clinical variables and response to Photofrin-PDT were evaluated by χ^2-test or Fisher's exact test when required; p-values of less than 0.05 were considered to be significant [31,37].

3. Results

3.1. Cellular accumulation of the photosensitizers Photofrin and NPe6 in the A431 cells and A431/BCRP cells

We examined the cellular accumulation of Photofrin and NPe6 in the A431 cells and A431/BCRP cells based on the fluorescence intensities, because photosensitizer accumulation has been considered as a factor influencing the cellular sensitivity to PDT [2,19,20]. As reported previously, Photofrin localized not only to the mitochondria, but also to the endoplasmic reticulum (ER), Golgi complexes and possibly other intracellular organelles (Fig. 1A). NPe6 localized not only to the lysosomes, but also to the ER (Fig. 1A) [20]. The photosensitizers did not localize to the plasma membrane or the nucleus, which was consistent with previous reports [18–20]. We analyzed the fluorescence intensity of the red fluorescence of Photofrin or NPe6. The fluorescence intensity of Photofrin was significantly higher in the A431 cells than in the A431/BCRP cells (Fig. 1B). The fluorescence intensity of Photofrin decreased in A431/BCRP cells in the presence of a specific inhibitor of BCRP, Fumitremorgin C (data not shown). However, there was no difference of the fluorescence intensity of NPe6 between the A431 cells and A431/BCRP cells (Fig. 1B). These results suggest that while BCRP was able to pump out Photofrin from the cells, but not NPe6, and that Photofrin may thus be a substrate of BCRP.

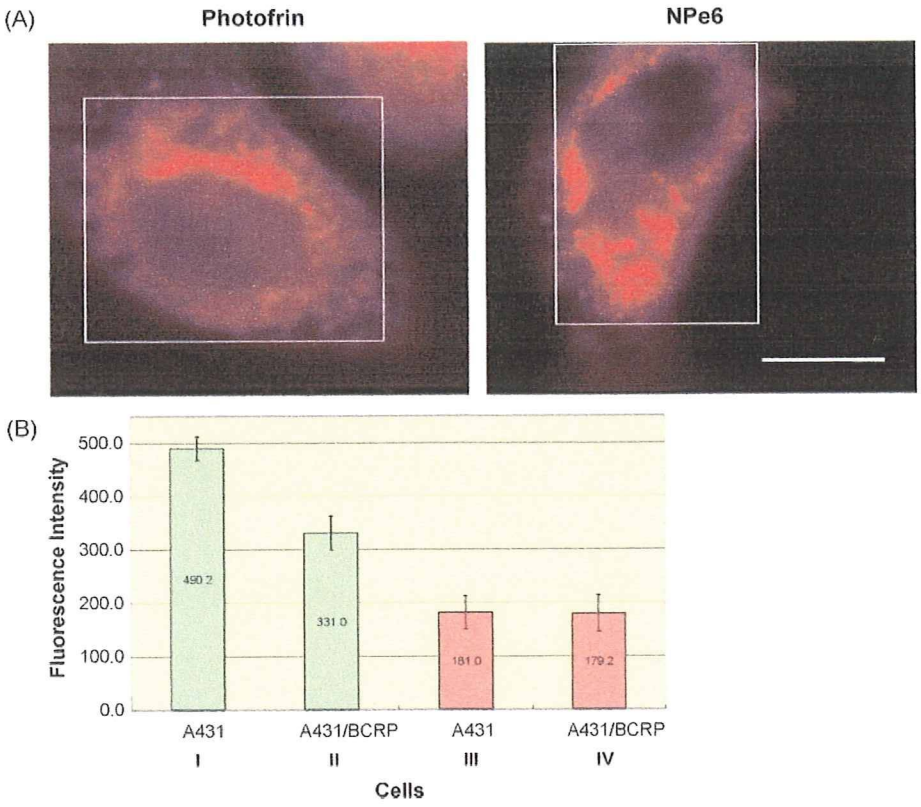

Fig. 1. (A) Localization of Photofrin and NPe6 in A431 cells. Cells were exposed to 2.5 μg/ml Photofrin (A) and 15 μg/ml NPe6 (B) for 4 h and then washed. The photosensitizers were used at the IC_{50} dose. The Photofrin or NPe6 in the cells was excited at 405 nm and the fluorescence was detected using a CCD camera system. *Scale bar*, 5 μm. (B) The fluorescence intensity per cell. We counted the fluorescence intensity of 10 cells and showed the average intensity per cell. The fluorescence of Photofrin in the A431 cells (i) and A431/BCRP cells (ii), and the fluorescence of NPe6 in the A431 cells (iii) and A431/BCRP cells (iv). There was a significant difference in the intensity of Photofrin between the A431 and A431/BCRP cells ($P < 0.05$).

3.2. Growth-inhibitory effect of Photofrin-PDT and NPe6-PDT on BCRP-overexpressing cells

We evaluated the antitumor effect of Photofrin-PDT and NPe6-PDT on the BCRP-overexpressing A431/BCRP cells by the WST assay [28,29]. The survival curves indicate that the A431/BCRP cells were comparatively resistant as compared to the parental A431 cells to Photofrin-PDT (Fig. 2A). At the 50% survival level, the presence of BCRP provided a dose-modifying factor of 1.86. Moreover, Fumitremorgin C, a specific inhibitor of BCRP, reversed the resistance of the A431/BCRP cells to Photofrin-PDT (Fig. 2A). These results suggest that Photofrin, a photosensitizer for PDT, may be transported out of the cells by BCRP and that BCRP expression may cause resistance to Photofrin-PDT. The survival curves indicate that, on the other hand, there was no significant difference in the antitumor effect of NPe6-PDT between the A431/BCRP cells and the parent A431 cells (Fig. 2B). This result suggests that NPe6 is not a substrate of BCRP and that BCRP expression does not exert any significant regulatory effect on the cell survival in NPe6-PDT. These results indicate that BCRP is a molecular determinant of resistance to Photofrin-PDT, but not to NPe6-PDT.

3.3. Expression of BCRP in centrally located early lung cancers

Previously we examined the immunohistochemical analysis of BCRP expression in A431 and A431/BCRP cells. We observed the negative expression of BCRP on A431 cells using anti-BCRP antibody, Bxp-21 [30]. Representative immunohistochemical BCRP staining is shown in Fig. 3A–C. It has been reported that BCRP is expressed in the normal small intestine, colon, liver, and mammary gland of the breast but is quite a low level in the lung [31,32]. In Fig. 3, the immunostaining of BCRP was both membranous and cytoplasmic as previous reports (Fig. 3) [33,34]. All of the 81 cancer lesions were BCRP-positive and were examined and scored according to the intensity of staining as compared with that in the negative control (+, positive; 2+, strong positive) independently by two observers [35,36]. In Fig. 3A and B, carcinoma cells showed strong positive reaction (2+) to anti-BCRP antibody, whereas in Fig. 3C and D, they showed positive reaction (1+).

3.4. Relationship between the expression of BCRP and the efficacy of PDT

Evaluation of the efficacy of PDT is shown in Table 2. The complete response rate of the centrally located early lung cancer lesions to Photofrin-PDT was 73.6% (42/57 lesions, BCRP(+); 24 lesions, BCRP(2+); 18 lesions). Of the 57 lesions, the remaining 15 showed PR or recurrence after CR (BCRP(+); 6 lesions, BCRP(2+); 9 lesions). As shown in Tables 3 and 4, 25 lesions were <1.0 cm in diameter and

Table 2
Relationship between expression of BCRP and response to Photofrin-PDT ($n = 57$).

PF-PDT	Lesions	BCRP		
		(−)	(1+)	(2+)
CR	42	0	24	18
[a]Rec, PR	15	0	6	9
CR rate	736.6%		80.0%	66.7%

[a] Rec, recurrence.

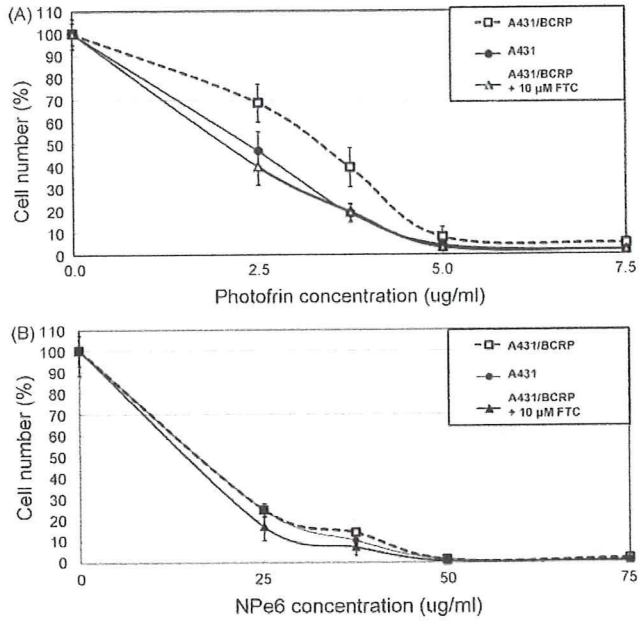

Fig. 2. (A) Growth-inhibitory effect of Photofrin-PDT in A431 cells (●), A431/BCRP cells (□) and A431/BCRP cells in the presence of Fumitremorgin C (△). Cells were exposed to Photofrin (0, 2.5, 3.75, 5, 10 μg/ml) for 4 h and then washed twice and incubated in fresh medium containing or not containing 10 μM FTC for 1 h, followed by laser-irradiation (664 nm) at 10 J/cm^2. The growth-inhibitory effect was measured 24 h after the PDT by WST assay. (B) Growth-inhibitory effect of NPe6-PDT in A431 cells (●), A431/BCRP cells (□) and A431/BCRP cells in the presence of Fumitremorgin C (▲). Cells were exposed to Photofrin (0, 25, 37.5, 50, 75 μg/ml) for 4 h and then washed twice and incubated in fresh medium containing or not containing 10 μM FTC for 1 h, followed by laser-irradiation (664 nm) at 10 J/cm^2. The growth-inhibitory effect was measured 24 h after the PDT by WST assay.

32 were ≥1.0 cm in diameter prior to the PDT, showing CR rates of 92% (23/25) and 59% (19/32), respectively (significant difference). As shown in Table 3, there was no difference of CR rate between BCRP(1+) and BCRP(2+) in tumor lesions <1.0 cm. Especially, among

Table 3
Relationship between expression of BCRP and response to Photofrin-PDT in tumor lesion (<1.0 cm) (n = 25).

Size <1.0 cm	Lesions	BCRP		
		(−)	(1+)	(2+)
CR	23	0	10	13
[a]Rec, PR	2	0	1	1
CR rate	92.0%		90.9%	92.9%

[a] Rec, recurrence.

Table 4
Relationship between expression of BCRP and response to Photofrin-PDT in tumor lesion (≥1.0 cm) (n = 32).

Size ≥1.0 cm	Lesions	BCRP		
		(−)	(1+)	(2+)
CR	19	0	14	5
[a]Rec, PR	13	0	5	8
CR rate	59.3%		73.7%[b]	38.5%[c]

[a] Rec, recurrence.
[b,c]Statistically significant difference of CR rate between BCRP (1+) and BCRP (2+) (P < 0.05).

lesions ≥1.0 cm in diameter, eight lesions that showed recurrence or only PR were BCRP(2+) and 5 lesions were BCRP(+). The efficacy with a significant difference of CR rate was seen in lesions with BCRP(2+) (38.5%) compared to lesions with BCRP(1+) (73.7%) (Table 4). These results, in particular, indicate that the expression of BCRP can significantly affect the efficacy of Photofrin-PDT for lesions ≥1.0 cm (Fisher's exact test; P = 0.04). On the other hand, as shown in Table 5 the CR rate of the lesions to NPe6-PDT was 91.6% (22/24 lesions), and much higher as compared with that to Photofrin-PDT (73.6%). Of these 24 lesions for which NPe6-PDT was undertaken, there were 2 BCRP(+) cases that showed PR. All the remaining 22 lesions were BCRP(1+) or strongly positive for BCRP(2+). NPe6-PDT achieved CR in all 18 lesions with BCRP(2+), the CR rate was 100%. These data suggest that NPe6-PDT exerted strong

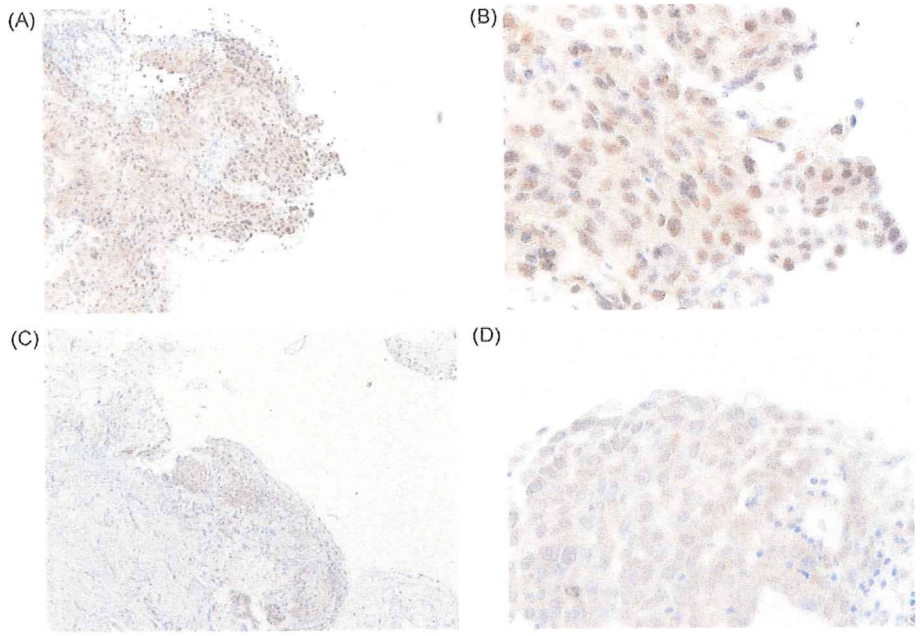

Fig. 3. Immunohistochemical staining of centrally located early lung cancers with anti-BCRP antibody (Bxp-21). The immunohistochemical staining was scored based on the estimated average staining intensity as strongly positive (2±) (A, ×40; B, ×400) and intermediate (1±) (C, ×40; D, ×400).