Table 5 The average number of cancer patients treated with radiation and radiation oncology personnel in institutions according to patient load/FTE radiation oncologists or number of new patients

	all facilities (n=721)	heavy load/FTE R.O.* institution in group B (n=35)	heavy load/FTE R.O.* institution in group A (n=83)	new patients ≧800 institution in all facilities (n=21)
平均年間新規患者数	236.1	323.2	421.0	1033.2
平均年間実患者数	284.4	394.7	542.0	1288.9
平均放射線治療担当医FTE数	1.15	0.58	1.35	6.49
平均放射線治療担当技師FTE数	2.27	2.27	3.18	7.39
平均医学物理士FTE数	0.09	0.06	0.08	0.70
平均放射線治療品質管理士FTE数	0.15	0.09	0.20	0.62

annual no. patients/FTE R.O. ≧300, B施設層はFTE=1として計算.

Table 6 Region and number of radiation oncology facilities according to patient load/FTE radiation oncologists or number of new patients

地域 (都道府県数)	解析施設数 (%)	heavy load/FTE R.O. (%) institution in group B (n=35)	heavy load/FTE R.O. (%) institution in group A (n=83)	new patients ≧800 (%) institution in all facilities (n=21)
北海道(1)	30 (4.2)	1 (2.9)	9 (10.8)	2 (9.5)
東北(6)	59 (8.2)	2 (5.7)	2 (2.4)	1 (4.8)
関東(8)	198 (27.5)	14 (40.0)	30 (36.1)	12 (57.1)
信越・北陸(5)	50 (6.9)	2 (5.7)	7 (8.4)	1 (4.8)
東海(4)	87 (12.1)	3 (8.6)	12 (14.5)	2 (9.5)
近畿(6)	127 (17.6)	7 (20.0)	9 (10.8)	2 (9.5)
中国(5)	54 (7.5)	2 (5.7)	3 (3.6)	0 (0.0)
四国(4)	27 (3.7)	1 (2.9)	1 (1.2)	0 (0.0)
九州・沖縄(8)	89 (12.3)	3 (8.6)	10 (12.0)	1. (4.8)
全国(47)	721*(100)	35(100)	83(100)	21(100)

* 2007年放射線治療実施施設数は765施設と推測され, 721施設は94.2%に相当.

Table 7 Number of facilities (%) by their category according to patient load/FTE radiation oncologists or number of new patients

	施設組織区分*						Total
	U	G	N	P	O	H	
all facilities(%)	114(15.8)	29 (4.0)	65(9.0)	208(28.8)	174(24.1)	131(18.2)	721(100)
heavy load/FTE R.O. institution in group B(%)	6(17.1)	4(11.4)	1(2.9)	7(20.0)	8(22.9)	9(25.7)	35(100)
heavy load/FTE R.O. institution in group A(%)	12(14.5)	8 (9.6)	5(6.0)	17(20.5)	23(27.7)	18(21.7)	83(100)
new patients ≧800 institution in all facilities(%)	9(42.9)	8(38.1)	0(0.0)	1 (4.8)	1 (4.8)	2 (9.5)	21(100)

* 施設組織区分
U: 大学附属病院
G: 国立がんセンター・成人病センター・地方がんセンター
N: 独立行政法人国立病院機構(がんセンター等を除く)
P: 公立(都道府県市町村立)病院(がんセンター等を除く)
O: 赤十字病院, 済生会病院, 企業/公社病院, 国保/社保/共済/労災/組合/厚生連病院等
H: 医療法人, 医師会病院, 個人病院, その他

で施行していた. 全身照射は, 22.9%, 49.4%, 71.4%の施設で施行していた. 定位(脳)照射は全体では25.8%, それぞれ45.7%, 43.4%, 66.7%の施設で施行していた. 定位(体幹部)照射は全体では17.1%, それぞれ17.1%, 30.1%, 81%の施設で施行していた. IMRTは全体で 8.0%, それぞれ5.7%, 13.3%, 71.4%の施設で施行していた. Table 11に, 脳転移, 骨転移の全放射線治療実患者数に対する施行割合を示している. 高負荷施設(B施設層)で脳転移が全国平均より1.5倍以上と高くなっていた. 骨転移は低くなっていた. 同(A施設層)でも同様に, 脳転移は全国平均値より高く, 骨転移は低くなっていた. 大規模施設では, 脳転移, 骨転移ともに相対的に減少していた.

考 察

今回の第 9 次JASTRO定期構造調査結果の全体像につい

Table 8 Number of equipments and their function in radiation oncology facilities according to patient load/FTE radiation oncologists or number of new patients

治療機器(機能)と周辺機器	all facilities (n=721)		heavy load/FTE R.O. institution in group B (n=35)		heavy load/FTE R.O. institution in group A (n=83)		new patients ≧800 institution in all facilities (n=21)	
linac	807		34		103		58	
with dual energy function	539	66.8%*	25	73.5%*	71	68.9%*	45	77.6%*
with 3DCRT function (MLC width=<1.0 cm)	555	68.8%*	22	64.7%*	77	74.8%*	53	91.4%*
with IMRT function	235	29.1%*	11	32.4%*	38	36.9%*	38	65.5%*
with IGRT function	108	13.4%*	4	11.8%*	17	16.5%*	22	37.9%*
with CT on rail	47	5.8%*	0	0%*	7	6.8%*	4	6.9%*
with treatment position verification system	110	13.6%*	4	11.8%*	18	17.5%*	22	37.9%*
annual no. patients/linac	243.2**		360.4**		406.2**		466.7	
betatron	0		0		0		0	
telecobalt(actual use)	28(15)		0(0)		3(3)		1(1)	
Gamma Knife®	46		7		11		2	
other accelerator	15		0		0		3	
new type Co-60 RALS (actual use)	16(16)	2.2%***(2.2%)	1(1)	2.9%***(2.9%)	3(3)	3.6%***(3.6%)	0(0)	
old type Co-60 RALS (actual use)	39(29)	5.4%***(4.0%)	3(3)	8.6%***(8.6%)	8(7)	9.6%***(8.4%)	1(1)	4.8%***(4.8%)
Ir-192 RALS(actual use)	127(123)	17.6%***(17.1%)	4(4)	11.4%***(11.4%)	27(27)	32.5%***(32.5%)	18(18)	85.7%***(85.7%)
X-ray simulator	445	60.9%***	18	45.7%***	52	62.7%***	19	90.5%***
CT simulator	497	65.6%***	26	71.4%***	63	71.1%***	25	95.2%***
RTP computer(2 or more)	1,070(168)		43(8)		144(33)		121(20)	

* linacの台数に対する機能の割合.
** linacが設置されていない施設を除いたデータから算出(n=680, 77, 31).
*** 機器を保有している施設の割合(機器台数には1施設2台以上保有しているものも含まれる).

Table 9 Number of reimbursement request on radiation treatment planning by complexity and patient load/FTE radiation oncologists or number of new patients

管理料種類	放射線治療管理料数(放射線治療管理料総数に対する割合) all facilities(%) (n=548*)	heavy load/FTE R.O.(%) institution in group B (n=18*)	heavy load/FTE R.O.(%) institution in group A (n=61*)	new patients ≧800(%) institution in all facilities (n=21)
単純 (1門照射, 対向2門照射)	67,174(46.8)	2,400(40.7)	13,396(45.1)	8,393(38.9)
中間 (非対向2門照射, 3門照射)	41,189(28.7)	1,629(27.6)	8,447(28.4)	6,303(29.2)
複雑 (4門以上の照射, 運動照射, 原体照射)	35,239(24.5)	1,871(31.7)	7,857(26.5)	6,896(31.9)
合計	143,602	5,900	29,700	21,592

* 放射線治療管理料請求数が未記入であった施設を除いたデータから算出.

ては, 第1報にて詳細を報告した[12]. その分析で, 放射線治療患者数の伸びが当初のPCSでの予想より少し頭打ちになっていたが, 全がん患者への放射線治療適用率は26.1%であり[17], 2005年の24.5%より1.6%増加していた. linacの各機能やCT simulatorに代表されるように, 装備はより良いものに改善されていた. しかし, 放射線治療担当医数の伸びは十分でなかった. 1 FTE放射線治療担当医が扱う年間がん患者実数(新患+再患)は248.2人であり, 前回より1.4人増加し, 米国および日本のブルーブックの基準[14), 15)]200人をかなり凌駕していた. この放射線治療担当医数の不足が放射線治療技術の複雑化, 高度化に加えて, 支援スタッフ寡少のわが国の治療現場を疲弊させる原因になっていないか, 2005年同様危惧された. 今後の放射線腫瘍学分野の発展のためには放射線腫瘍医ならびに支援スタッフを増やすことが優先課題である. 本報告では, わが国の現状を構造調査結果にもとづいて正しく把握し, 各施設が人員増に向

Table 10 Special radiation therapy other than external irradiation according to patient load/FTE radiation oncologists or number of new patients

特殊照射	all facilities(%) (n=721)	heavy load/FTE R.O.(%) institution in group B (n=35)	heavy load/FTE R.O.(%) institution in group A (n=83)	new patients ≧800(%) institution in all facilities (n=21)
腔内照射				
施行施設数	172(23.9)	8(22.9)	39(47.0)	19(90.5)
治療症例数	3,235	124	668	578
組織内照射				
施行施設数	97(13.5)	1(2.9)	20(24.1)	16(76.2)
治療症例数	3,301	29	762	578
前立腺ヨード治療				
施行施設数	78(10.8)	1(2.9)	15(18.1)	13(61.9)
治療症例数	2,690	29	651	444
全身照射				
施行施設数	185(25.7)	8(22.9)	41(49.4)	15(71.4)
治療症例数	1,707	45	412	231
術中照射				
施行施設数	41(5.7)	1(2.9)	4(4.8)	6(28.6)
治療症例数	251	3	15	79
定位(脳)照射				
施行施設数	186(25.8)	16(45.7)	36(43.4)	14(66.7)
治療症例数	12,554	2,101	3,919	853
定位(体幹部)照射				
施行施設数	123(17.1)	6(17.1)	25(30.1)	17(81.0)
治療症例数	2,490	338	711	502
IMRT				
施行施設数	58(8.0)	2(5.7)	11(13.3)	15(71.4)
治療症例数	2,799	249	796	792
温熱併用照射				
施行施設数	23(3.2)	0(0.0)	1(1.2)	3(14.3)
治療症例数	340	0	2	96
Sr-90翼状片治療				
施行施設数	4(0.6)	0(0.0)	0(0.0)	0(0.0)
治療症例数	149	0	0	0

けて，病院事務や行政との交渉に利用可能な数値データを提供することを目的としている．

国全体で56%の放射線治療施設(B施設)において，FTE ≧ 1 人の放射線治療担当医が確保されていない．これは2005年より 6%改善はしてきている[9), 10)]．これらの施設では，2005年で年間平均157人の患者数を治療しているので，ブルーブックの基準(150～200人)からは，B施設の患者数の多い方の半数である約200施設では，1 人の放射線治療担当医の配置はそろそろ必要な状態といえる．今後の急速な患者数の増加を吸収するために重要な役割を担うのは，この規模の施設でもあろう．したがって，これらの施設にFTE ≧ 1 人の常勤放射線治療医を配置することは重要である．この規模における放射線治療の適用率が長らく常勤放射線治療担当医不在のために低く，国全体のがんに対する放射線治療適用率を現在の26.1%に留めている可能性がある[12)]．一方．残り44%のA施設の上位25%の施設は 1 FTE放射線腫瘍医当たり改善警告値[14)]300人を超えた患者を治療しており，過剰労働状況にあった．現状のインフラのままでは患者数増加の吸収が困難となりつつある．この施設への放射線治療専門医の配置も優先的に進めなければならない．がん対策基本法の追い風を得て，国全体で早急な人材育成を計るべきで，現在は過渡期と理解される．B施設の上位10%も改善警告値である年間300人を超えて治療しており，人員確保の標的となりうるが，内容を分析してみると，そのうち44%の施設が定位(脳)照射に特化した施設であることが推定された．高負荷施設への放射線腫瘍医の重点的供給とともに大学勤務医師のB施設での非常勤医師としての兼務実態について，大学の給与体系の低さとの比較を含めて総合的に検討すべきである．今回，兼務の情報も追跡・分析すべく調査しており，現在，詳細を分析中である．一方，診療放射線技師の場合は，放射線治療担当技師 1 FTE当たりの実患者数は125.5人であり，2005年より8.5人増加した．患者数に応じて負荷が増えているようにも見えるが，今回は医学物理士，品質管理士業務を除いた実質的FTE値を詳細に集積したので，前回の方が診療放射線技師に関する国全体のマンパワーとしては少しだけ過大評価し，それに今回は患者数増加が加わっているために前回より負荷が大きく増えているように算定されたと想定される．全体と

Table 11 Annual number of total cancer patients (new+repeat) treated for brain metastasis and bone metastasis by patient load/FTE radiation oncologists or number of new patients

転移	実患者数（放射線治療実患者総数に対する割合）			
	all facilities(%) (n=721)	heavy load/FTE R.O.(%) institution in group B (n=35)	heavy load/FTE R.O.(%) institution in group A (n=83)	new patients ≥800(%) institution in all facilities (n=21)
脳転移	21,237(10.4)	2,335(16.9)	6,796(15.1)	2,401(8.9)
骨転移	27,970(13.6)	1,716(12.4)	5,898(13.1)	3,603(13.3)

して，患者数負荷に対して平均的には人員をガイドライン内で配置できている．医学物理士FTE数，品質管理士FTE数はどちらも寡少であったが，A施設層はB施設層の2倍の人員を擁していた．日米ブルーブック[14), 15)]では，医学物理士は400～500人の患者に1人の配置が必要で，現状の負荷は3,000人を超えている．早急な人材育成が必要である．

がん診療連携拠点病院は全国平均よりも装備の機能は約10%ずつ充実しており，患者負荷も約35%，約100人多かった．2005年と比較して，全体でも改善してきている．しかし，今回指定された拠点病院の37%はB施設層であり，1 FTE以上の放射線治療担当医が確保されていなかった．この割合は低下し，改善してきている．拠点病院のB施設の平均患者数負荷は約186.4人で，常勤放射線治療担当医を確保すべき状況に近い．以上のように，放射線治療担当医は不足しているので，優先順位をつけて配置していくと同時に，当面は現状のスタッフ数で患者サービスを提供するために地域施設間の医療連携が重要である[14)]．よくいわれているように，欧米のようながん患者の施設集中化をわが国で定着させるべきか否かは，医療従事者の待遇を含めた医療体制の根本にかかわる現実的な施策の中から考案すべきである．現状は放射線治療施設の地域分布について，わが国はよく実現できている．一方，国全体での放射線治療を要する患者数が増加しており，センター，成人病センターや大学病院での患者数急増も，これらの施設の大型化とそこへの患者集中化が促されていることを反映しているのかもしれない．地域別の患者数負荷は各地域の患者数と担当のマンパワーに依存し，放射線治療担当医で3倍，放射線治療担当技師で2.96倍の地域差が観察された．特に負荷の多い地域では人員補充と周辺地域との連携が必要である．現在，基準値の範囲にある施設も，今後の高齢化および放射線治療適用率上昇に伴う患者数の増加に備えて人員補充を怠らないことが肝要である．医学物理士，放射線治療品質管理士は寡少で，分析は困難であるが，大都市圏に集中する傾向がみられる．本データが有効に利用されることを望む．

放射線治療担当医について，人員補充の標的と考えられる日本版ブルーブックの改善警告値[14)]を超える高負荷施設(300人/FTE放射線治療担当医以上)と大規模施設(新患800人以上)について，全体データと比較して分析した．地域的にはB施設層は，関東，近畿により多く，同(A施設層)と大規模施設は，関東，北海道により多かった．施設区分では，全体に比し高負荷施設(B施設層)はG，Hがより多く，同(A施設層)はG，Oがより多く，大規模施設はほとんどUとGであった．2005年と比べ，B施設層を除いてほぼ同様の傾向であった．これらの施設区分の病院を管轄する国・自治体において，患者数負荷増加の実態が理解され，人員補充が重点的に行われることを望む．ただ，前回同様に同(B施設層)は，Tables 10，11から分かるように，半数にγナイフあるいは脳定位照射を行う施設が含まれている．これらは分割回数が少ないため，一般外部照射の人員負荷の分析とは区別する必要があるが，今回も個々の症例の診療内容までの調査はしていないので，厳密には区別できていない．装備は，同(B施設層)でbrachytherapyが普及していないことを除いて，同(A施設層)，大規模施設になるにしたがって，全体平均より充実していた．linac 1台当たりの年間患者数負荷は，いずれも日本版ブルーブックガイドライン[14)]の300人/装置を超えており，同(A施設層)と大規模施設では，さらに同改善警告値400人[14)]を凌駕していた．したがって2007年時点でも，これらの施設104施設(83+21)にはlinac 1台の追加設置が必要と考えられた．これらの施設は2005年調査時より13施設増加していた．放射線治療計画の請求の種類は負荷が大きく，大規模施設では，単純が7.9%減少して，複雑が7.4%増加していた．2005年より，全体の施設でも単純が6.5%減少，複雑が3.9%増加していたが，大規模施設ではこの傾向がさらに顕著になっていた．しかし，高負荷施設(A施設層)の単純，複雑の比率は，全体の施設とほぼ同様であった．これらの施設層では患者数の負荷が，治療計画の複雑化，高精度化を阻害しているのかもしれない．特殊治療の施行数も患者数負荷が大きい程，大規模施設程，増加傾向にあった．大規模施設では1 FTE当たりの患者数負荷は，ブルーブックのガイドラインの基準値200人/FTE放射線治療担当医[14), 15)]の範囲にあるが，これらの施設区分はTable 7にあるように，42.9%はU：大学附属病院，38.1%はG：国立がんセンター・成人病センター・地方がんセンターであり，教育，研究の責任が他の施設区分よりかなり高く，肝心の人材供給源であることも考慮すると，さらに多くの人員配置が必要であろう．

国全体で今後の患者数増加をどこで吸収するかという視点が重要となる．既述のように，欧米のような集中化，大型化は将来の1つの方向性ではあるが，理想的過ぎるかもしれない．本調査で明らかとなったわが国の現状から，まずは，がん診療連携拠点病院での装備や人員の重点配備は

現実的な選択肢である．ただ，この指定とはかかわりなく，地域の放射線治療に重要な貢献をしている施設は多数あることも明らかである．本調査では人員を早急に補充すべき施設をデータとしてある程度特定できた．いずれにしても，人材育成と供給が最重要で，U：大学やG：がんセンターの果たす役割は大きい．これらの施設に所属する常勤スタッフの給与水準は非常に低く，非常勤ポストとの兼任の実態を本調査より詳細に分析し，報告する予定である．並行して，将来のスタッフとしての活躍の場として，常勤ポストを医学物理士ポストともに各医療機関に確保し，装備整備も着実に進めていくことも重要である．各地域において，本調査のデータが有効利用されることを希望する．地域の詳細な分析依頼にも常時応ずるものである．

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要旨：JASTROの2007年放射線治療施設構造調査を2008年 3 月から2009年 1 月までに調査票を送付して行った．回答率は94.2%(721/765)であった．1 FTE(full time equivalent)放射線治療担当医当たりが治療する年間実患者数(=患者負荷)は248.2人であった．施設層別の同様の値は≧1 FTE放射線治療担当医を有するA施設層で212.9人，<1 FTEのB施設層で157人であった(B施設層では過大評価を避けるため，本計算ではFTE=1 として算出した．その施設の年間総患数と同一)．A施設では全体の25%で，B施設の10%で300人以上(診療の質低下が懸念される改善警告値)を治療していた．1 FTE放射線治療担当技師当たりの年間総患数は125.5人であった．がん診療拠点病院では，全国平均より優れた機能を装備したlinacならびにCT simulatorを使用していた．地域的に 1 FTE放射線治療担当医当たりの年間患者総数は130.7～391.6人まで，また 1 FTE放射線治療担当技師当たりの年間患者数は87.3～258.6人までの顕著なバリエーションが観察された．1 FTE放射線治療担当医が年間300人以上(改善警告値)治療する高負荷施設(A施設層)と年間新規患者数が800人以上の大規模施設(計104施設)では，linac 1 台当たりの患者数が400人(改善警告値)を超過していた．

Original Article

Prognostic Impact of Mitotic Index of Proliferating Cell Populations in Cervical Cancer Patients Treated With Carbon Ion Beam

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BACKGROUND: The authors previously reported that the mitotic index of a proliferating cell population (pMI) was a potent prognostic factor in cervical cancer patients treated with photon beam therapy. In this study, they investigated whether the pMI accurately predicted prognosis in cervical cancer patients treated with carbon ion beam. **METHODS:** Tissue sections were obtained from 27 consecutively treated patients with stage IIIB bulky (19 patients) and stage IVA (8 patients) squamous cell carcinomas of the cervix treated with carbon ion beam at the National Institute of Radiological Sciences, Japan, as a phase I/II study with dose escalation methodology (52.8-72 grays equivalent radiation dose/24 fractions). The mitotic index (MI) and Ki-67 labeling index (Ki-67-LI) were determined by hematoxylin and eosin staining and immunohistochemical staining, respectively. The pMI was calculated using the following formula: pMI = MI/Ki-67-LI. **RESULTS:** The pMI ranged from 0.6 to 8.9 (mean, 3.9 ± 2.6; median, 3.2). Twelve of the 27 specimens had a pMI >3.5. The local control rate in tumors with a pMI >3.5 was 17%, significantly lower than the 73% in the tumors with a pMI <3.5 ($P = .005$). Multivariate analysis indicated that the pMI had the strongest impact on local control (standard regression coefficient = 0.48, $P = .002$) among the variables, including clinical stage, irradiated dose, age, and tumor volume. **CONCLUSIONS:** These results suggest that a high pMI is an indication of a poorer prognosis, and is a powerful prognostic factor in patients with squamous cell carcinomas of the cervix treated with carbon ion beam therapy. **Cancer 2009;115:1875–82. © 2009 American Cancer Society.**

KEY WORDS: carbon ion beam, Ki-67, mitotic index, cervix, squamous cell carcinoma.

Heavy charged particle radiation therapy for cancer treatment began at the National Institute of Radiological Sciences (NIRS, Chiba, Japan) in June 1994 using carbon ions generated by the heavy-ion medical accelerator in Chiba, and more than 3000 patients have been treated to date.[1] Several reports have demonstrated the favorable results of carbon ion beam radiotherapy (CIRT) in the treatment of malignant

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tumors, including head and neck cancer,[2] stage I nonsmall cell lung cancer,[3,4] hepatocellular carcinoma,[5] choroidal melanoma,[6] prostate cancer,[7,8] bone and soft tissue sarcomas,[9] and sacral chordomas.[10]

In terms of cervical cancer, a phase I/II clinical trial for advanced cancers of the uterine cervix (protocol 9403) began in June 1995 and ended in February 1998. The preliminary results of those clinical trials have been reported by Nakano et al[11] and Kato et al.[12] These reports have shown that carbon ion beam therapy for cervical cancer is feasible,[11] and the local control rate of patients treated with ≥62.4 grays equivalent radiation dose (GyE) was favorable even for the patients with stage IVA disease, or for those with tumors ≥6.0 cm.[12] Furthermore, we have also reported similar disease-free survival and local control rates between hypoxic and oxygenated tumors before and during treatment, indicating that the tumor oxygenation status is relatively unimportant in local control in carbon beam therapy.[13]

The proliferative activity of tumor cells has been determined immunohistochemically by Ki-67 antigen, as well as other markers. The Ki-67 labeling index (Ki-67-LI) has been reported to be a significant prognostic factor in many malignant tumors.[14-22] The mitotic index (MI; percentage of mitotic cell) of tumor cells also correlates to the cell proliferation speed, and it too has been reported as a significant prognostic factor.[21-29] We have reported that Ki-67-LI and MI correlate with prognosis in patients with cervical cancer treated with photon radiation therapy.[30] However, the mitotic index in vivo is sometimes biased by the presence of quiescent cells whose population is larger than the cycling cell population in human cancers.[30] We have proposed that the MI of a proliferating cell population (pMI) can express the relative cell cycle speed and can be estimated by the counting of the MI and Ki-67-LI.[30] Furthermore, we have reported that the pMI correlates well with the prognosis of patients with cervical cancer who received photon beam therapy.[30-32] The pMI was found to be a significantly stronger prognostic factor than Ki-67-LI.[31]

Carbon ion beam therapy has various biologic advantages in terms of high linear energy transfer (LET) radiation. Potentially lethal and sublethal damage repair is attenuated by high LET radiation.[33] Blakely et al reported that high LET beam diminished cell cycle–dependent radiosensitivity compared with those observed with low LET radiation.[34] Barendsen et al reported that high LET radiation is apparently most effective on tumors with a large G0 fraction.[35] As for tumor oxygen status, which is an important issue in radiation therapy, high LET radiation induces cell death independently of the tissue oxygen status, as we previously demonstrated.[14] Furthermore, Takahashi et al[36] reported that the carbon ion beam can induce apoptosis regardless of p53 status. Hence, the prognostic factor for high LET radiation therapy may be completely different from that of low LET radiotherapy. In this study, we investigated whether Ki-67-LI, MI and/or pMI predicted prognosis in cervical cancer patients treated with a carbon ion beam.

MATERIALS AND METHODS

Patient Characteristics and Specimens

Twenty-seven patients with stage IIIB bulky and stage IVA squamous cell carcinomas of the cervix were treated with carbon ion beam at the NIRS between 1995 and 1998 as a phase I/II study in a dose escalation manner (protocol: 9403). Their ages ranged from 36 to 72 years (mean ± standard deviation [SD], 56.2 ± 9.3; median, 54 years). All the tumors had a maximum diameter >50 mm. Tumor size was assessed by both pelvic examination and magnetic resonance imaging (MRI), and dimensions of the cervical tumor were measured according to T2-weighted MRI images.[37,38] The mean ± SD and median tumor volume were 153 ± 113 and 109 mm^3, respectively. Of these, 19 patients had stage IIIB disease, and 8 had stage IVA disease, all of which involved bladder invasion, except 1 case with rectal invasion. The clinical staging and histologic classification were based on the criteria of the International Federation of Gynecology and Obstetrics and the World Health Organization.[39,40] All patients were followed for a minimum of 5 years or until death. Tissue sections were obtained from all 27 patients before treatment. They were excised from the cervical tumors and fixed in 10% formaldehyde for approximately 24 hours and embedded in paraffin.

The disease status and treatment methods were explained carefully and precisely to the patients by the treating physicians. In addition, an explanation of the tumor biopsy measurement procedure was provided to all patients who qualified for the study. After these

explanations, written informed consent was obtained from the patients and the family, according to the institutional regulations.

CIRT

CIRT was performed at the National Institute of Radiological Sciences in Chiba, Japan. Details of the treatment protocol have been previously reported by Kato et al[12] and Nakano et al.[13] Briefly, the total number of treatment fractions and the time period were fixed at 24 fractions over 6 weeks with 4 fractions per week. Anteroposterior and posteroanterior ports were used for 16 fractions over 4 weeks to irradiate cervical tumors and pelvic lymph node chains. An additional 8 fractions over 2 weeks were given by lateral opposing ports to boost only cervical tumors. As this represents a phase I/II study with a dose escalation methodology, the treatment was initiated with a fraction dose of 2.2 GyE,[41] and the fraction doses increased by 0.2 GyE per step to 2.4 GyE, 2.6 GyE, 2.8 GyE, and 3.0 GyE. Therefore, the initial dose of 52.8 GyE was increased by 4.8 GyE per step to a total of 72.0 GyE. Each dosing group consisted of at least 5 patients. The energy of the carbon ion beam ranged from 350 MeV to 400 MeV.

Measurements of pMI, MI, and Ki-67-LI

The tissue sections, placed on silane-coated microslides, were deparaffinized and dehydrated.

For measurement of the Ki-67-LI, specimens were immunohistochemically stained (Fig. 1). Specimens were heated to >95°C for 20 minutes in 0.01 M citrate buffer, pH 6, using a Microwave Processor (H2800, Energy Beam Sciences Inc., Agawam, Mass) to unmask the antigens. After the unmasking, the sections were cooled at room temperature for 1 hour. Endogenous peroxidases were blocked with 3% hydrogen peroxide for 10 minutes, and the sections were washed 3 times with phosphate-buffered saline (PBS). They were then incubated overnight at 4°C with anti-Ki-67 (MIB-1, Immunotech International, Marseilles, France). After incubation, they were washed with PBS 3 times. They were incubated with a labeled-polymer–conjugated second antibody, an Envision kit (DAKO, Carpinteria, Calif), for 30 minutes. They were washed with PBS and then developed with 3,3′-diaminobenzidine tetrahydrochloride for 5 minutes at room temperature and then lightly counterstained with hematoxylin, dehydrated, and mounted. As a negative control, a specimen was treated without a primary antibody in the incubation step. The Ki-67-LI was calculated as the percentage of Ki-67–positive cancer cells by counting >500 cancer cells. A cutoff value for the Ki-67-LI was defined as 33%.

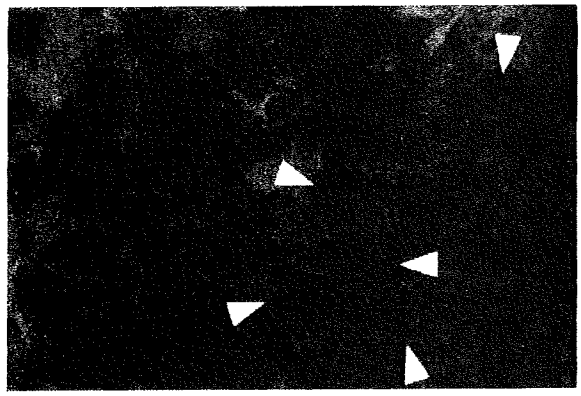

FIGURE 1. Cancer cells were positive for Ki-67 in the intranucleus, exhibiting Ki-67 immunostaining. The white arrow heads indicated mitosis. (Original magnification, ×200.)

For measurement of the MI, specimens were stained with hematoxylin and eosin. The MI was calculated as the percentage of mitotic cancer cells by counting >2000 cancer cells. A cutoff value for the MI was defined as 1.5%.

The pMI was calculated with the following formula:

$$\text{pMI} = \text{M/P} = (\text{M}/[\text{P} + \text{Q}])/(\text{P}/[\text{P} + \text{Q}]) = (\text{MI})/(\text{Ki-67-LI})$$

M indicates mitotic cell population (the number of mitotic cells); P, proliferating cell population (the number of Ki-67–positive cells); Q, quiescent cell population (the number of all cells − the number of Ki-67–positive cells).

A cutoff value for the pMI was defined as 3.5, based on the results of our previous report.[30] A cutoff value for the MI was established as a positive finding.

Statistical Analysis

The Kaplan-Meier method was used for the local control and metastasis-free survival rates, and differences were statistically analyzed with the log-rank test. Mean values of pMI, MI, and Ki-67-LI by irradiated dose group were compared by 1-factor analysis of variance. Univariate

Table 1. Mean Values of pMI, MI, and Ki-67-LI by Dose Group

Dose	Ki-67-LI	MI	pMI
52 GyE	35±10	1.2±1.2	3.0±2.0
57 GyE	36±12	1.9±0.8	5.4±2.0
62 GyE	37±26	1.2±0.7	4.4±2.9
67 GyE	36±12	0.7±0.6	1.7±1.1
72 GyE	33±15	1.3±0.7	4.7±3.2
P	.99	.28	.15

pMI indicates mitotic index of proliferating cell populations; MI, mitotic index; Ki-67-LI, Ki-67 labeling index; GyE, gray equivalent radiation dose.

analysis for local control and metastasis-free survival was applied with Pearson correlation coefficient. Multivariate analysis for local control and metastasis-free survival was applied with the Cox proportional hazard model with a 95.0% confidence interval. The differences were considered statistically significant at $P < .05$. All analyses were performed with StatView (Version 5.0, SAS Institute Inc, Cary, NC).

RESULTS

The pMI ranged from 0.6 to 8.9 (mean ± SD, 3.9 ± 2.6; median, 3.2). A total of 44% (12 of 27) of the tissue specimens had a pMI >3.5. The MI ranged from 0.14% to 3.3% (mean ± SD, 1.23 ± 0.83%; median, 1.02%). A total of 33% (9 of 27) of the tissue specimens had a >1.5% MI. The Ki-67-LI ranged from 15% to 80% (mean ± SD, 35 ± 15%; median, 29%). A total of 44% (12 of 27) of the tissue specimens had a Ki-67-LI >33%. Table 1 shows the means value of pMI, MI, and Ki-67-LI by dose group. There was no significant difference among the dose group. Although there was no significant difference, pMI and MI in the low dose group (≤62 GyE) were bigger than in the high dose group (≥67 GyE).

Nine of 12 patients with a >3.5 pMI had local recurrence, compared with only 4 of 15 patients with a pMI <3.5. The local control rate of the tumors with a pMI >3.5 was 17%, significantly lower than the 73% of the tumors with a pMI <3.5($P = .005$; Fig. 2). The local control rate of the tumors with a >1.5% MI was 17%, significantly lower than the 66% of the tumors with a <1.5% pMI ($P = .02$). The local control rate of the tumors with a Ki-67-LI >33% was 64%, which was higher than the 37% of the tumors with a Ki-67-LI <33%, although the difference was not significant ($P = .13$).

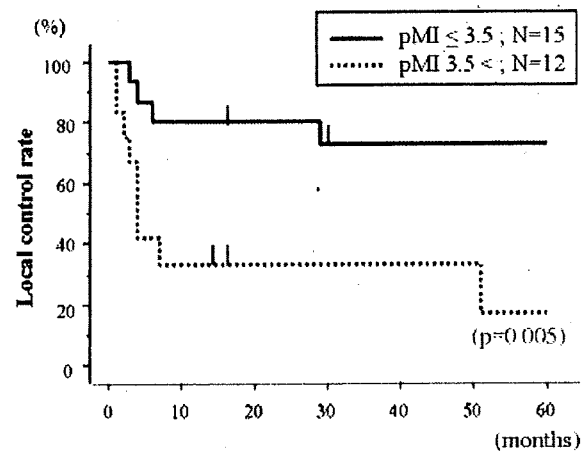

FIGURE 2. The local control rate in 27 patients with cervical squamous cell carcinoma is shown in relation to the mitotic index of a proliferating cell population (pMI). The local control rate of a pMI ≤3.5 was significantly higher than that of a pMI score of >3.5 ($P = .005$).

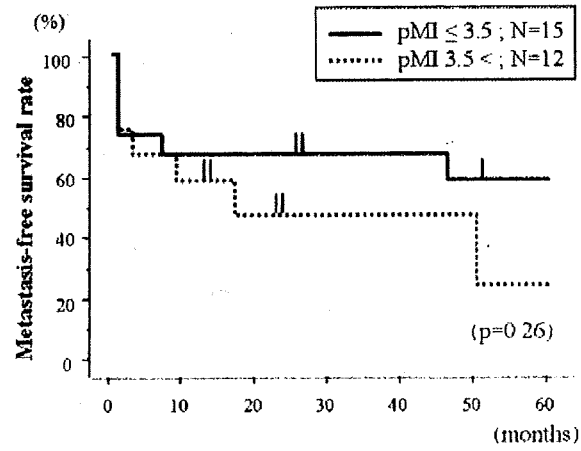

FIGURE 3. The metastasis-free survival rate in 27 patients with cervical squamous cell carcinoma is shown in relation to the mitotic index of a proliferating cell population (pMI). There was no significant difference between the metastasis-free survival rate of a pMI ≤3.5 and that of a pMI >3.5 ($P = .26$).

The metastasis-free survival rate of the tumors with a >3.5 pMI was 23%, lower than the 58% of the tumors with a <3.5 pMI, although the difference was not significant ($P = .26$; Fig. 3). The metastasis-free survival rate of the tumors with an MI >1.5% was 23%, lower than the 54% of the tumors with an MI <1.5%. The difference was not significant ($P = .60$). The metastasis-free survival

Table 2. Correlation Matrix for Local Control and Metastasis-free Survival

Variables	Local Control (*P*)	Metastasis-free Survival (*P*)
Ki-67 labeling index	.265 (.18)	.182 (.37)
Mitotic index	−.419 (.03)	−.052 (.80)
pMI	−.481 (.01)	−.112 (.57)

pMI indicates mitotic index of proliferating cell populations.

rate of the tumors with a >33% Ki-67-LI was 63%, higher than the 34% of the tumors with a <33% Ki-67-LI, although the difference was not significant ($P = .14$).

Table 2 shows the univariate analysis for local control and metastasis-free survival. The pMI and MI significantly correlate to the local control (correlation coefficient, 0.481 and 0.419, respectively). Among Ki-67-LI, MI, and pMI, pMI was the strongest factor for local control. None of these factors significantly correlates to metastasis-free survival. Table 3 shows the multivariate analysis for local control and metastasis-free survival. The pMI had robustly significant impact on local control (standard regression coefficient = 0.484, $P = .021$), but no significant impact on metastasis-free survival (standard regression coefficient = 0.131, $P = .54$) among the variables, including irradiated dose (52.8 GyE vs 57.6 GyE vs 62.4 GyE vs 67.2 GyE vs 72.0 GyE), clinical stage (stage IIIB vs stage IVA), age (≤50 years old vs >50 years old), and tumor volume (≤100 cm^3 vs >100 cm^3).

Table 3. Multivariate Analysis for Local Control and Metastasis-free Survival

Variables	Standard Regression Coefficient	*P*
Local control (recurrence/control)		
Irradiation dose (52.8/57.6/62.4/67.2/72.0 GyE)	−0.033	.87
Clinical stage (IIIB/IVA)	0.064	.78
Age (≤50 y)	−0.013	.95
Tumor volume (≤100 cm^3)	−0.174	.38
pMI (≤3.5)	−0.484	.021
Metastasis-free survival, meta (+)/meta (−)		
Irradiation dose (52.8/57.6/62.4/67.2/72.0 GyE)	−0.320	.16
Clinical stage (IIIB/IVA)	0.069	.78
Age (≤50 y)	−0.115	.65
Tumor volume (≤100 cm^3)	−0.184	.39
pMI (≤3.5)	−0.131	.54

GyE indicates gray equivalent radiation dose; pMI, mitotic index of proliferating cell populations.

DISCUSSION

The MI and Ki-67-LI are well-known cell growth-associated parameters and have been reported to be useful and powerful prognostic factors.[14-29] In general, a high MI and high Ki-67-LI correlate with a worse prognosis. However, in the course of radiation therapy, tumors with higher proliferative activity tend to have higher radiosensitivity and better prognosis.[42] Furthermore, as mentioned earlier, the mitotic index in vivo is sometimes biased by the presence of quiescent cells, the population of which is larger than the cycling cell population in human cancers. Therefore, we have proposed the pMI as a more accurate cell growth–associated parameter and reported that pMI strongly correlates with prognosis in patients with cervical cancer who have received photon beam therapy.[30-32]

In this study, the local control rate of the tumors with a pMI >3.5 was only 17%, although most of the tumors were bulky. To improve the local control rate of radiation therapy, 1) shortening the treatment period (accelerated fractionation),[43,44] and 2) combined use of chemotherapy[45,46] have been investigated. We have reported a phase I/II clinical study of carbon ion radiotherapy for locally advanced carcinoma of the uterine cervix.[12,13] We have fixed the fraction number at 24 fractions in a 6-week period, and an accelerated-fractionated protocol has not yet been planned, because of the relatively high probability of late adverse effects. However, heavy ion beam therapy has the advantage of better dose distribution and biological effect, and the excellent dose distribution of the heavy ion beam enables a reduction in the irradiation volume absorbed by normal tissue. Also, biologically, the therapeutic ratio increases if short-course accelerated-fraction schemes are used in carbon ion beam therapy.[1] Hence, accelerated-fractionated radiotherapy is desirable. In addition, although the "redistribution," "recruitment," and "reoxygenation" of the tumor during radiation therapy are benefits of a fractionated treatment schedule of conventional photon beam therapy, the benefits of fractionation of the high LET beam are believed to be less than the low LET beam, because high LET beam diminishes both the cell cycle–dependent radiosensitivity

and oxygen enhancement ratio compared with low LET radiation.[34,47] At the NIRS in Japan, accelerated-fractionated carbon ion beam therapy has been investigated systematically for a variety of tumor entities, and it has been indicated that a significant reduction of overall treatment time can be accomplished for many tumor entities without enhancing toxicity.[1] Miyamoto et al[4] reported that the treatment regimen of accelerated-fractionated CIRT for stage I lung cancer was 72 GyE/9 fractions in 3 weeks, in which the local control rate was >90%, and the rate of late adverse effects of grade 2 or more severe was only 8%. Furthermore, a dose escalation study on the single-fraction treatment has been initiated.[1] Although cervical cancer has been treated with 24 fractions over 6 weeks and an accelerated-fractionated protocol has not yet been planned because of the relatively high probability of late adverse effects, prostate, which is in close proximity to the rectum and bladder, as in the case of the uterus, has been treated with 66 GyE/20 fractions over 5 weeks, and the rate of late adverse effects was only 7%, and the local control rate was 100%.[7] Hence, although some improvement of treatment is still needed, accelerated-fractionated CIRT for cervical cancer appears feasible.

In the present study, no significant correlations were detected between pMI, MI, and Ki-67-LI and metastasis-free survival rate. However, there was a large difference of approximately 35% in the metastasis-free survival rate between the patients with the low and high pMI tumors. As shown in our recent report,[12] although the local tumor control was relatively good, distant metastases did frequently occur, and the 5-year overall survival rate was still unsatisfactory. To improve the survival rate as well as the local control rate, the use of chemotherapy in combination with CIRT should be further explored, especially for patients with high pMI tumors, although further study is still required to properly assess the significance of pMI for metastasis-free survival with higher patient numbers.

In conclusion, we investigated whether Ki-67-LI, MI, and/or pMI predicted prognosis in cervical cancer patients treated with a carbon ion beam. The results of this study suggest that a high pMI is indicative of a poor prognosis in patients with squamous cell carcinomas of the cervix treated with carbon ion beam therapy. Although further studies with larger data sets is warranted, to improve the local control rate of CIRT for cervical squamous cell carcinoma, especially for those tumors having a high pMI, an accelerated-fractionated regimen together with the use of chemotherapy should be further explored as explored in photon beam therapy.[43-46]

Conflict of Interest Disclosures

The authors made no disclosures.

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CLINICAL STUDY - PATIENT STUDY

Nuclear survivin expression predicts poorer prognosis in glioblastoma

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Abstract Survivin is a member of the inhibitor of apoptosis family, and is expressed in various malignant tumors. Survivin overexpression has been reported to be a poorer prognostic factor in various malignancies. However, the prognostic value of survivin expression in patients with glioblastoma is still controversial. Therefore, in this study the role of survivin as a predictor for survival was investigated in patients with glioblastoma. Tissue specimens were obtained from 66 patients with glioblastoma treated with radiotherapy. Survivin expression was detected by an immunohistochemical method. Nuclear and cytoplasm survivin scores were defined by using the cell positivity and staining intensity. The scores were defined as follows, 0 (no staining), 1 (less than 50% of cell positivity and any staining), 2 (more than 50% of cell positivity and weak to moderate intensity) and 3 (more than 50% of cell positivity and strong intensity). The correlation between survivin scores and the overall survival rate was evaluated. Nuclear and cytoplasm survivin staining were noted in 47 and 58 patients, respectively. The number of patients with nuclear survivin score of 0, 1, 2 and 3, were 19 (28.8%), 26 (39.4%), 9 (13.6%) and 12 (18.2%), respectively. The 3-year overall survival rate of the nuclear survivin score 3 was 0%, significantly lower than the 11.6% of the nuclear survivin score ≤ 2 ($P = 0.0003$). Cytoplasm survivin score did not correlate with the prognosis. Nuclear survivin expression may be a useful biomarker for predicting prognosis in patients with glioblastoma.

Keywords Survivin · Glioblastoma · Radiotherapy · Nuclear · Cytoplasm

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Introduction

Glioblastoma, the most malignant brain tumor in adults, is highly resistant to radiotherapy n and chemotherapy. The recent standard treatments of this disease are considered to be surgical resection to the extent feasible, followed by radiotherapy and chemotherapy including temozolomide. The median survival time of the patients with glioblastoma has remained approximately 12 months over the last few decades, even though multimodal therapies have been applied.

Survivin is 16.5-kDa protein and a member of the inhibitors of apoptosis family. It is expressed in fetal tissues

and tumor cells but not in normal adult differentiated tissues [1, 2]. Survivin is thought to inhibit the processing of procaspase-3 and procaspase-7 by direct binding [3]. Survivin overexpression is reported to be an unfavorable prognostic factor in various malignancies, including prostate cancer [4], colorectal [5], pancreatic [6] and non-small cell lung carcinomas [7, 8]. However, several reports have shown survivin expression to be associated with a favorable prognosis in gastric cancer [9], transitional cell carcinoma of bladder [10], breast cancer [11] and osteosarcoma [12]. Chakravarti et al. [13] reported that survivin expression in glioblastoma patients was an adverse prognostic factor, by using semiquantitative western blot analysis. However, their study did not distinguish between nuclear and cytoplasm survivin expression and the prognostic value of survivin expression by immunohistochemistry is still controversial. Therefore, we assessed the prognostic value of nuclear and cytoplasm survivin expression by immunohistochemistry in patients with glioblastoma.

Materials and methods

Patients and specimens

Available tissue specimens were obtained from all of the 66 patients with pathologically confirmed glioblastoma treated with radiotherapy at the Gunma University Hospital (Maebashi Japan), Takasaki National Hospital (Takasaki Japan) and Maebashi Red Cross Hospital (Maebashi Japan) between 1982 and 2005. The median follow-up period was 12 months. Sixty-one patients died due to tumor progression and 5 patients were alive with follow-up period ranging from 15 to 60 months. The mean and median of age were 55.1 ± 16.2 and 59 years old, respectively. There were 40 male and 26 female patients. Gross total resection was performed in 18 patients, partial resection in 43 patients and biopsy in 5 patients. The mean and median radiation doses were 56.9 ± 9.3 Gy and 60 Gy, respectively. In general, radiotherapy was performed by the conventional therapy (5 days × 2 Gy/week). However, some patients were performed by hyper- and hypo-fractionated radiotherapy. Therefore, we used the biological effective dose (BED) for analysis and set the α/β ratio as 10.0 Gy. The mean and median BED were 68.7 ± 10.9 and 72.0, respectively. Chemotherapy was performed in 45 patients as an initial treatment before and/or after irradiation. There were 64 patients with glioblastoma multiforme in 64 patients and 2 with giant cell glioblastoma, and the pathological diagnosis was based on the WHO Classification of Tumors of the Nervous System (2000) [14]. The pathological features, including the extent of necrosis, vascularity, cell density and existence of giant cells were evaluated.

Immunohistochemistry

The tissue specimens were cut into 4 μm sections on cylan-coated slides, then were deparaffinized and hydrated. For antigen retrieval, they were heated by microwave for 75 min in Dako Target Retrieval Solution (Dako, CA, U.S.A.) at 93.0°C and were cooled for 20 min. After washing with Phosphate Buffered Saline (PBS), nonspecific binding was blocked with peroxidase-blocking solution (Dako) for 15 min. The specimens were incubated overnight at 4.0°C with anti-survivin antibody (Novus Biologicals, CO, U.S.A.) diluted with antibody diluent (Dako) at 1:300. Then, they were washed with PBS. They were incubated with a labeled-polymer conjugated second antibody EnVision + kit (Dako) for 30 min. After washing in PBS, the specimens were developed with 3,3′-diaminobenzidine tetrahydrochloride (Dako) for 2 min. Specimens were slightly counterstained with hematoxylin, and finally dehydrated and mounted. As a positive control, a known positive control (breast cancer) specimen was handled in parallel. As a negative control, a specimen was treated without a primary antibody in the incubation step.

Evaluation

All immunohistochemical stained slides were pathologically assessed by one of the authors (K.O.), without knowledge of the clinical data. The cell nuclei and cytoplasm of more than 500 tumor cells were evaluated in each specimen. Only the viable glioblastoma areas were evaluated, while the necrotic and non-viable areas were excluded. Nuclear and cytoplasm survivin scores were evaluated with cell positivity and staining intensity. Scores were defined as follows, 0 (no staining), 1 (less than 50% of cell positivity and any intensity), 2 (more than 50% of cell positivity and weak to moderate intensity) and 3 (more than 50% of cell positivity and strong intensity). The correlation between nuclear and cytoplasmic survivin scores and overall survival were evaluated.

Statistical analysis

The overall survival period was calculated from the date of operation to the date of death or last follow-up. The Kaplan–Meier method was used for the overall survival curves, and differences were statistically analyzed with the Log-rank test. The multivariate analysis for overall survival was applied with the Cox proportional hazard model with a 95.0% CI. The differences were considered statistically

significant at $P < 0.05$. All analyses were performed with SPSS 11.0 for Windows.

Results

The overall survival rates of all patients at 3 and 5 years were 11.1% and 5.9%, respectively (Fig. 1). The median survival period was 12 months. The overall survival based on clinical characteristics and survivin scores were shown in Table 1. The 3-year survival rates of patients aged <60 and ≥60 were 11.5% and 10.3%, respectively ($P = 0.26$). The 3-year survival rates of male and female were 5.4% and 19.2%, respectively ($P = 0.71$). As for the extent of surgery, the 3-year survival rates of patients performed gross total resection was 22.2%, significantly higher than 6.7% performed partial resection and biopsy ($P = 0.02$). The 3-year survival rates of patients irradiated with BED ≤72.0 and with BED >72.0 were 9.7% and 13.6%, respectively ($P = 0.40$). The 3-year survival rates of patients on which chemotherapy was performed was 11.6%, significantly higher than 10.0% patients on which chemotherapy was not performed ($P = 0.04$).

Nuclear and cytoplasm survivin staining were noted in 47 and 58 patients, respectively (Fig. 2). The nuclear survivin score 0, 1, 2 and 3, were 19 patients (28.8%), 26 patients (39.4%), 9 patients (13.6%) and 12 patients (18.2%), respectively. The 3-year overall survival rate of the nuclear survivin score 3 was 0%, significantly lower than 11.6% of the nuclear survivin score ≤2 ($P = 0.0003$, Fig. 3). The cytoplasm survivin score 0, 1, 2 and 3, were 8 patients (12.1%), 47 patients (71.2%), 6 patients (9.1%) and 5 patients (7.6%), respectively. These cytoplasm survivin scores did not correlate with the prognosis. With regard to histology, the 3-year overall survival rates in glioblastoma multiforme and giant cell glioblastoma were 11.4% and 0 %, respectively ($P = 0.90$). The other pathological features, including the extent of necrosis, vascularity, cell density and existence of giant cells were not significant prognostic factors ($P = 0.94$, $P = 0.71$, $P = 0.13$ and $P = 0.64$, respectively).

Table 1 Results of univariate analysis of prognostic factors

Variable	No. of patients	3-year survival rate (%)	P
Age			
≥60 years old	29	10.3	0.26
<60 years old	37	11.5	
Gender			
Male	40	5.4	0.71
Female	26	19.2	
Extent of surgery			
PR and biopsy	48	6.7	0.02
GTR	18	22.2	
BED			
≤72.0	44	9.7	0.40
>72.0	22	13.6	
Chemotherapy			
Yes	43	11.6	0.04
No	20	10.0	
(Unknown)	3		
Nuclear survivin score			
3	12	0	0.0003
≤2	54	11.6	

GTR gross total resection, *PR* partial resection, *BED* biological effective dose

The results of multivariate survival analysis for the clinical characteristics and the nuclear survivin score were given in Table 2. The factor with the strongest impact on overall survival was nuclear survivin score ($P = 0.003$, hazard ratio = 0.35).

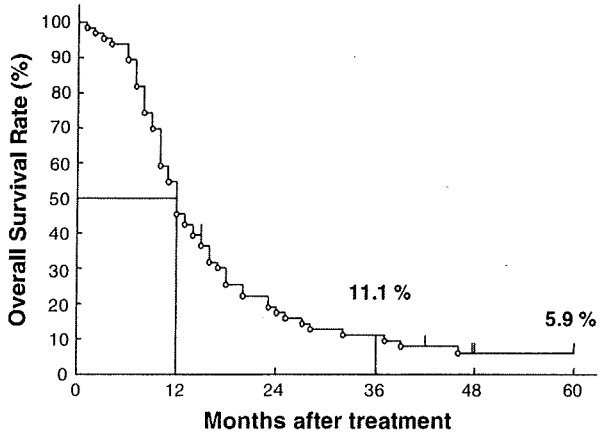

Fig. 1 The overall survival rate of 66 patients with glioblastoma

Discussion

Sasaki et al. [15] reported that survivin expression increased in parallel with malignant grade in astrocytic tumors. Kajiwara et al. [16] also reported that survivin expression was significantly associated with malignant grade, and they concluded that survivin might play an important role in the oncogenesis and progression of astrocytic tumors. They mentioned that the median survival period for patients with survivin positive tumors was shorter than that for patients with survivin negative tumors in astrocytic tumors [16]. Saito et al. [17] reported that simultaneous survivin expression in both nucleus and cytoplasm was worth prognosis in high-grade

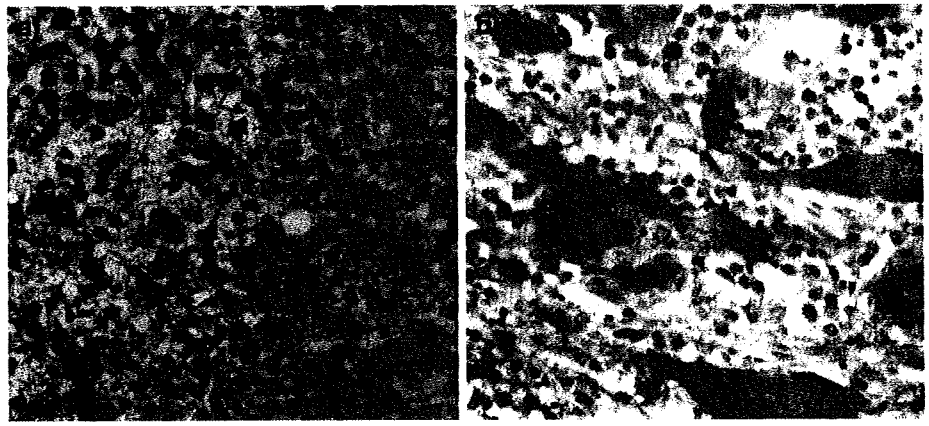

Fig. 2 Immunohistochemical staining of nuclear (**a**) and cytoplasmic (**b**) survivin. (Original magnification: 400×)

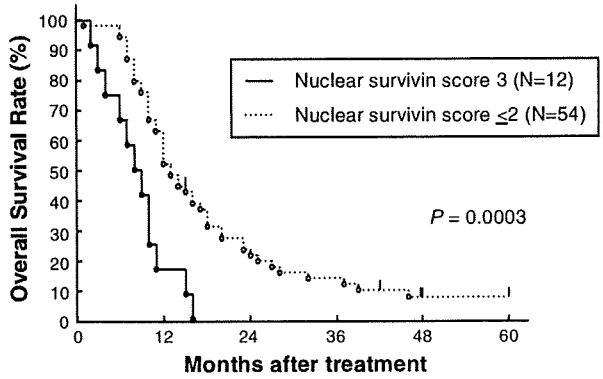

Fig. 3 The overall survival rate of 66 patients with glioblastoma and nuclear survivin score. The overall survival rate of patients with the nuclear survivin score = 3 was significantly lower than patients with the score ≤2 ($P = 0.0003$)

Table 2 Results of multivariate analysis of prognostic factors

Variable	HR (95% CI)	*P*
Extent of surgery (biopsy and PR vs. GTR)	0.60 (0.33–1.08)	0.09
Chemotherapy (Yes vs. No)	0.82 (0.46–1.45)	0.48
Nuclear survivin score (3 vs. ≤2)	0.35 (0.18–0.70)	0.003

HR hazard ratio, *CI* confidence interval, *PR* partial resection, *GTR* gross total resection

astrocytomas. Uematsu et al. [18] compared the survivin index and MIB-1 index, and investigated a positive linear correlation of them was weak. And they mentioned that survivin index was more sensitive marker to predict survival than MIB-1 index in low grade gliomas.

Previously 4 studies have been attempted among the immunohistochemical analyses of glioblastoma. Das et al. [19] firstly reported that the survivin was highly expressed in glioblastoma, but did not discuss the relationship to prognosis. Xie et al. [20] reported a difference in the survivin expression between primary glioblastoma and secondary glioblastoma, but also did not mention prognosis. Preusser et al. [21] reported that survivin did not seem to be useful as a prognostic factor in 104 patients with glioblastoma. Recently, Mellai et al. [22] showed that a positive linear correlation was found between survivin expression and proliferation, whereas inverse correlation did not found between survivin expression and apoptosis. However, they did not indicate the correlation between survivin expression and survival. The present study is the first report to show that the nuclear survivin score correlate with a worse prognosis in the patients with glioblastoma.

Fortugno et al. [23] reported that survivin existed in 2 distinct nuclear and cytoplasmic subcellular pools in human cervical carcinoma HeLa cells. Nuclear survivin has been proposed to serve in the maintenance of the integrity of the mitotic spindle in HeLa cells [24]. In many immunohistochemical studies, nuclear survivin expression has been shown to be an unfavorable factor for prognosis, including prostate cancer [4], rectal cancer [25], oesophageal squamous cell carcinoma [26], colorectal carcinoma [27], soft-tissue sarcomas [28], breast cancer [29], laryngeal squamous cell carcinoma [30], hepatocellular carcinoma [31], ovarian carcinoma [32] and non-small cell lung carcinoma [7, 8]. Shinohara et al. [7] suggested that strong nuclear staining of survivin may represent increased mitotic events, hence resulting in poor survival. Recently, it was reported that survivin has 3 splicing variants, survivin-ΔEx3, survivin-2B, and survivin wild type [33, 34]. Survivin-ΔEx3 exists in the nucleus, and nuclear survivin has been considered active in the maintenance of the integrity of the mitotic spindle. Yamada et al. [35] reported that survivin-ΔEx3 was significantly more increased in malignant brain tumors than benign brain tumors in vitro setting. Both survivin-2B and survivin wild type exist in the cytoplasm, and cytoplasmic survivin has been considered to be anti-apoptotic factor [36]. However, Islam et al. [37] reported that survivin wild type can inhibit apoptosis and survivin-2B does not. Immunohistochemically, a few studies have reported cytoplasmic survivin expression to be

an unfavorable factor in patients with colorectal cancer [5], pancreatic cancer [6], and oral squamous cell carcinoma [38]. Previously, we reported that cytoplasmic survivin expression was an unfavorable factor in patients with cervical squamous cell carcinomas [39]. However in this study cytoplasmic expression is not a prognostic factor for survival in patients with glioblastoma. Shinohara et al. [7] suggested that cytoplasmic staining might represent the combined level of 2 functionally opposing variants of survivin wild type and survivin-2B, and therefore, as a result, cytoplasmic survivin expression was not a significant prognostic marker. Cytoplasmic survivin score was tended to be higher in relatively large cells (alike a giant cell; data not shown). In this report, we showed correlations between prognosis and the pathological features, including the existence of giant cells. We additionally analyzed the correlation between cytoplasmic survivin score and the existence of giant cells. Then, higher survivin score was significantly correlated with the existence of giant cells (Pearson test; $P = 0.048$). Though the existence of giant cells does not correlate to tumor phenotype exactly, cytoplasmic survivin score might have a correlation with tumor phenotypes.

Survivin has been expected to serve as a treatment target in various malignancies. Yang et al. [40] reported that apoptosis was induced when the activity of survivin was blocked by the expression of dominant-negative mutant survivin in various tumors, including pancreatic cancer cell lines, breast cancer cell lines, and a colon carcinoma cell line, but not normal cell lines. Ansell et al. [41] reported that cell growth was significantly inhibited by using an antisense oligonucleotide approach in aggressive non-Hodgkin's lymphomas. Van Houdt et al. [42] reported that transcriptional targeting therapy of the survivin promoter significantly inhibited the growth of glioma xenografts in vivo.

Survivin expression was associated with radiation resistance in various malignancies. Chakravarti et al. reported that survivin enhanced double-strand DNA break repair and tumor cell metabolism, and thereby survivin suppressed radiation-induced cell death in primary human glioblastoma [43]. Saito et al. [44] showed that survivin suppression by small interfering RNA (siRNA) enhanced radiosensitivity in glioma cells. And they analyzed the cell death induced by radiation was mitotic cell death correlated with chromosome instability. Rodel et al. [25] reported that the attenuation of survivin mRNA and protein by siRNA enhanced radiation sensitivity in colorectal cancer cell lines. They suggested that anti-survivin strategies improved the radiation response, and may be ultimately correlated with the better outcome [25, 43, 44].

In conclusion, we suggest that nuclear survivin score is a useful biomarker to predict survival outcome in patients with glioblastoma.

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Inoperable Pancoast tumors treated with hyperthermia-inclusive multimodality therapies

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ABSTRACT

Purpose: This study aimed to assess the feasibility, efficacy and complication of hyperthermia-inclusive multimodality therapies for patient with inoperable Pancoast tumor.
Material and methods: Five patients with inoperable Pancoast tumor were treated with hyperthermia-inclusive multimodality therapies. They received thermoradiotherapy with/without chemotherapy. Radiation therapy was delivered using 10 MV X-rays with total dose of 68–70 Gy. In the latter half of the radiation therapy hyperthermia was performed for 2–4 sessions once a week with 8 MHz radiofrequency device.
Results: For primary response, 4 tumors showed partial response to the treatment with the exception of 1 tumor who showed stable disease. Only one patient was with a short follow-up period (9 months), all other patients survived 3 years or more without recurrence. Of them, 2 patients were recognized with local recurrence at 38.7 and 42.7 months after treatment and died at 66.9 and 78.5 months after treatment. The other 2 patients are disease-free survivor for 4 and 5 years after treatment. No severe non-hematological toxicity was observed in each patient.
Conclusion: These data suggested that hyperthermia-inclusive multimodality therapies might be a promising approach for inoperable Pancoast tumor.

1. Introduction

Non-small-cell lung carcinomas (NSCLC) of the superior sulcus, which frequently termed Pancoast tumors, are an uncommon lung cancer accounting for only a slight percentage of all lung cancer cases [1]. These tumors cause specific symptoms and signs, and are associated with patterns of failure that differ from those seen for NSCLC tumors in other nonapical locations, and originally considered to be fatal. During the past 40 years the development of various effective combined modality treatments including new surgical approaches have greatly increased the local control and the overall survival for patients having these tumors [2]. At first, in 1956 Chardack and MacCallum reported the case of a patient who survived long term after surgical resection followed by a postoperative radiation [3]. Subsequently, several authors reported that preoperative radiation facilitated the surgical resection and proposed that such a combined treatment was potentially curative [4,5]. Since then preoperative radiation followed by surgical resection has been considered as a standard treatment approach for Pancoast tumors. However, numerous reported data showed that his treatment was associated with a relatively high incomplete resection rate (30–50%). These high incomplete resection rates doomed the patient to inadequate local control and overall survival of approximately 30% in 5 years. Recently, the addition of chemotherapy to preoperative radiation has improved the resectability and survival rates [6–8]. In a large prospective multicenter phase II trial which tested the feasibility of chemoradiotherapy induction for Pancoast tumors showed that the overall survival in 5-year was 44% for all patients and increased to 54% after complete resection [9]. Although this strategy is very attractive and encouraging, operative mortality is reported and showed that all cases are not always eligible for surgery. Inoperable Pancoast tumor which was treated with radiation therapy alone or in combination with chemotherapy has associated with poor prognosis [6,10,11]. Since the local tumor control is an important goal of the primary treatment, more aggressive treatment applied is advocated to improve the prognosis.

Heat is cytotoxic for cells in an environment with hypoxic and low pH, conditions that are specifically found within tumor tissue, due to insufficient blood perfusion [12]. Therefore, we hypothesized

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