Table 3
Acute and late reactions of the skin and the mucosa.

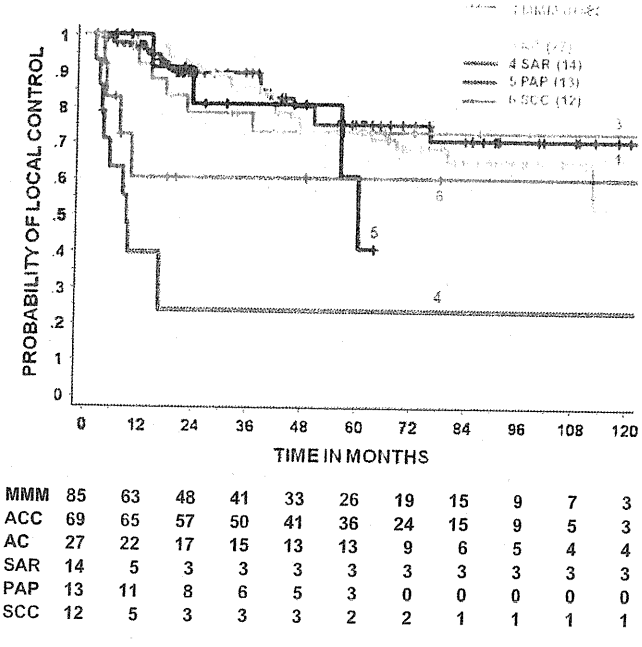
Acute skin	No.	G0	G1	G2	G3	G4
TD 57.6 GyE	215	16	103	82	14	0
TD 64.0 GyE	21	0	12	8	1	0
Total	236	16	115	90	15	0
(%)	(100)	(7)	(49)	(38)	(6)	
Acute mucosa						
TD 57.6 GyE	206	26	84	74	22	0
TD 64.0 GyE	17	1	7	7	2	0
Total	223[a]	27	91	81	24	0
(%)	(100)	(12)	(41)	(36)	(11)	
Late skin	No.	G0	G1	G2	G3	SO
TD 57.6 GyE	215	111	92	7	0	5
TD 64.0 GyE	21	11	9	0	0	1
Total	236	122	101	7	0	6
(%)	(100)	(52)	(43)	(3)		(3)
Late mucosa						
TD 57.6 GyE	206	160	39	3	0	4
TD 64.0 GyE	17	12	4	1	0	0
Total	223[a]	172	43	4	0	4
(%)	(100)	(77)	(19)	(2)		(2)

Abbreviations: GyE, gray equivalent; SO, sorted out because of short observation time.
[a] Normal mucosa of 13 cases was out of irradiated field.

Table 4
Acute tumor responses according to the histological subtype.

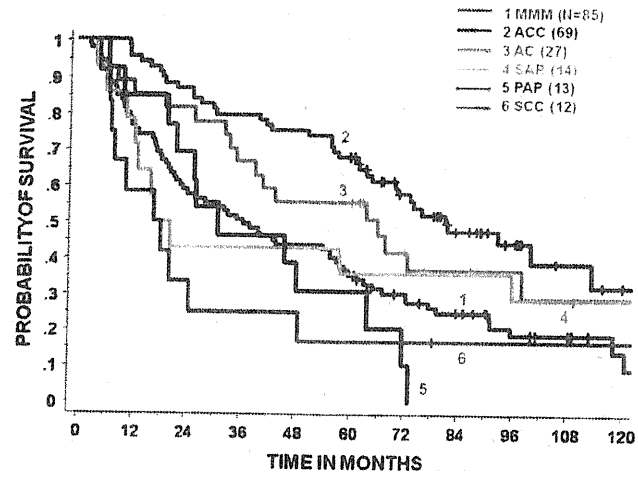
Histology	N	CR	PR	NC	PD
Mucosal malignant melanoma	85	12(14)	43(51)	30(35)	0
Adenoid cystic carcinoma	69	12(17)	32(46)	25(36)	0
Adenocarcinoma	27	1(4)	12(44)	14 (52)	0
Sarcomas	14	0	2 (14)	9(64)	3(21)
Papillary adenocarcinoma	13	0	4(31)	9(69)	0
Squamous cell carcinoma	12	3(25)	4(33)	4(33)	1(8)

Abbreviations: N, number of patients; CR, complete response; PR, partial response; NC, no change; PD, progressive diseases.
Parenthesis; percent.



MMM	85	63	48	41	33	26	19	15	9	7	3
ACC	69	65	57	50	41	36	24	15	9	5	3
AC	27	22	17	15	13	13	9	6	5	4	4
SAR	14	5	3	3	3	3	3	3	3	3	3
PAP	13	11	8	6	5	3	0	0	0	0	0
SCC	12	5	3	3	3	2	2	1	1	1	1

Fig. 1. Local control curves by histological subtype. Abbreviations: MMM, mucosal malignant melanoma; ACC, adenoid cystic carcinoma; AC, adenocarcinoma; SAR, sarcomas; PAP, papillary adenocarcinoma; SCC, squamous cell carcinoma.



MMM	85	65	50	43	37	30	23	17	10	7	3
ACC	69	66	60	55	52	45	30	19	12	6	4
AC	27	24	22	18	15	15	9	6	5	4	4
SAR	14	11	6	6	6	5	5	5	5	4	3
PAP	13	11	9	6	5	4	1	0	0	0	0
SCC	12	7	4	3	3	2	2	1	1	1	1

Fig. 2. Survival curves by histological subtype. Abbreviations: same in Fig. 1.

(SCC: 61%) vs. MMM and ACC. LC by anatomic subsites showed no statistical significance between any combinations of two anatomic subsites.

Distant metastasis

Distant metastases were seen in 35 cases (41%) of MMM, 22 cases (32%) of ACC, three cases (11%) in adenocarcinoma, and each two cases of sarcomas (14%), papillary adenocarcinoma (15%) and SCC (17%). Of these metastatic patients, local failures were seen in one case in MMM (2%), five cases in ACC (23%), each one case in adenocarcinoma (33%) and sarcomas (50%) and no failure in papillary adenocarcinoma and SCC.

Overall survival

The 5-year OS rate of 236 patients was 47% (SE: 3.2%). OS curves by histological subtype are shown in Fig. 2. There were statistical significances in survival curves (1) between ACC (5-year OS, 68%) vs. MMM (35%), papillary adenocarcinoma (31%) and SCC (17%) and (2) between adenocarcinoma (56%) vs. papillary adenocarcinoma. The 5-year OS rate of 14 sarcomas was 36%. In OS by anatomic subsite, there were statistical significances in survival curves (1) between oral cavity (58%) vs. nasal cavity (33%), pharynx (35%) and thyroid gland (27%) and (2) between orbita (68%) vs. nasal cavity, pharynx and thyroid gland.

Discussion

It is characteristic of H&N cancer to consist of a variety of histological subtypes and anatomic subsites as shown in present data. The log-rank tests for LC curves and OS curves showed the statistical significance between the histological subtypes (Figs. 1 and 2) in the C-ion RT. On the other hand, in the analysis of anatomic subsite, there were no significant differences between LC curves, but showed some significant differences between OS curves. The less favorable OS of the nasal cavity (56 patients), compared with the oral cavity and the orbita, will be caused by containing 46 MMM patients whose OS was 35%. Nine patients out of the 23 pharynx were MMMs, three were sarcomas (OS, 36%) and three were SCCs (OS, 25%).

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When the patients with ACC were treated by XRT alone, the LC rate may be around 44–66% and the OS rate is 50–79% [16,17]. The 5-year LC and OS in C-ion RT were 73% (SE; 5.8%) and 68% (SE; 5.6%) respectively. This result showed a favorite LC and compatible OS with XRT alone. There have been a lot of data of FN therapy for the locally advanced ACC. Huber et al. reported the 5-year LC rate of ACC treated by FN as 75% with grade 3/4 late morbidity as 19% [18]. Douglas et al. reported the 5-year LC rate as 57% and OS as 72% in the FN therapy for locally advanced ACC of the H&N with late grade 3/4 morbidity as 10% [19]. The LC and OS of C-ion RT may be compatible for these FN data, but we have no grade 3/4 late morbidity. This difference may be the results of dose distribution between two particles, namely charged and uncharged.

The recent reports of the 5-year LC of systemic therapy including surgery, radiation and chemotherapy for MMM are ranged between 74% and 84% [20–22]. Our result of 75% (SE; 6.2%) for 85 MMM patients may be compatible with these results. On the other hand, the OS of MMM showed unfavorable results. The review articles by Lengyel et al. showed the 5-year OS as 17–48% [23], by Bridger et al. as 10–47% [24] and by Mendenhall et al. as 20–48% [25], which were attributed mainly to a hematogenous dissemination. Our result of OS (35%) with metastatic rate of 41% showed compatible results with these reports.

Bone and soft-tissue tumor in the H&N is a rare disease. The 5-year LC rate of combined surgery and radiotherapy is 60–70% with a 5-year OS of 60% [26]. Local control of surgery alone is around 54% and that of radiotherapy alone is 43–50% [27]. Radiotherapy with a total dose less than 65 Gy showed no local control [28,29]. Results of C-ion RT showed 24% of 5 years LC and 36% of OS. This result suggested the necessity of a dose escalation study for H&N sarcomas in C-ion RT.

High LET charged particle therapy has been of interest in the treatment of H&N malignancies because of better dose localization in the tumor volume and greater biological effectiveness. Even if charged particle radiotherapy, mainly neon ions, at the University of California Lawrence Berkeley National Laboratory (LBNL) could not demonstrate an optimal neon dose for H&N malignancies, there was a trend toward better results with higher neon doses [30,31]. Also, recent preliminary data of combined photon radiotherapy and carbon ion boost show good feasible and effective results in the treatment of adenoid cystic carcinoma without severe late effects [32,33].

This paper was the first in the world to describe the results of exclusively carbon only radiotherapy for H&N malignancies. Till now, our efforts have been made to increase the patient numbers in order to produce the results that can provide cogent clinical evidence [34]. Patients numbering 236 cases and a minimum follow-up period of 60 months will be enough for noble evaluation. Although the normal tissue reactions included early grade 3 skin and mucosal reactions in approximately 10% of the subjects, late reactions were grade 2 or less. No serious toxicity related to C-ion RT was observed during the follow-up period and this result may show the physical characteristics of C-ion RT with Bragg peak.

Conclusions

In phase II clinical trial of carbon ion radiotherapy for head and neck cancer, we observed the therapeutic effectiveness for mucosal malignant melanoma and adenoid cystic carcinoma without severe morbidity of the normal tissues.

Conflict of interest statement

The author(s) indicated no potential conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.radonc.2011.12.013.

References

[1] Hirao Y, Ogawa H, Yamada S, et al. Heavy ion synchrotron for medical use—HIMAC project at NIRS—Japan. Nucl Phys 1992;A538:541c–50c.
[2] Tsujii H, Morita S, Miyamoto T, et al. Preliminary results of phase I/II carbon-ion therapy at the National Institute of Radiological Sciences. J Brachytherapy Int 1997;13:1–8.
[3] Mizoe JE, Tsujii H, Kamada T, et al. Dose escalation study of carbon ion radiotherapy for locally advanced head-and-neck cancer. Int J Radiat Oncol Biol Phys 2004;60:358–64.
[4] Wambersie A. Fast neutron therapy at the end of 1988 – a survey of the clinical data. Strahlenther Onkol 1990;166:52–60.
[5] Wambersie A, Richard F, Breteau N. Development of fast neutron therapy worldwide. Radiobiological, clinical and technical aspects. Acta Oncol 1994;33:261–74.
[6] Griffin BR, Laramore GE, Russell KJ, Griffin TW, Eenmaa J. Fast neutron radiotherapy for advanced malignant salivary gland tumors. Radiother Oncol 1988;12:105–11.
[7] Battermann JJ, Breur K, Hart GA, van Peperzeel HA. Observations on pulmonary metastases in patients after single doses and multiple fractions of fast neutrons and cobalt-60 gamma rays. Eur J Cancer 1981;17:539–48.
[8] Nguyen TD, Chassard JL, Lagarde P, et al. Results of postoperative radiation therapy in medullary carcinoma of the thyroid: a retrospective study by the French Federation of Cancer Institutes – the Radiotherapy Cooperative Group. Radiother Oncol 1992;23:1–5.
[9] Schlesinger T, Flower MA, McCready VR. Radiation dose assessments in radioiodine (131I) therapy. 1. The necessity for in vivo quantitation and dosimetry in the treatment of carcinoma of the thyroid. Radiother Oncol 1989;14:35–41.
[10] Overgaard J, Overgaard M, Hansen PV, von der Maase H. Some factors of importance in the radiation treatment of malignant melanoma. Radiother Oncol 1986;5:183–92.
[11] Kanai T, Endo M, Minohara S, et al. Biophysical characteristics of HIMAC clinical irradiation system for heavy-ion radiation therapy. Int J Radiat Oncol Biol Phys 1999;44:201–10.
[12] Tsujii H, Miyamoto T, Mizoe J, et al. Experience of carbon ion radiotherapy at NIRS. In: Kogelnik HD, editor. Progress in radiooncology. Salzburg: Austria Monduzzi Editore; 2002. p. 393–405.
[13] WHO Handbook for Reporting the Results of Cancer Treatment, Definitions of objective response. Geneva: World Health Organization Offset Publication No. 1979;48:23–25.
[14] Kaplan EL, Meier P. Non parametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457.
[15] Hasegawa A, Mizoe JE, Mizota A, et al. Outcomes of visual acuity in carbon ion radiotherapy: analysis of dose–volume histograms and prognostic factors. Int J Radiat Oncol Biol Phys 2006;64:396–401.
[16] Miglianico L, Eschwege F, Marandas P, et al. Cervico-facial adenoid cystic carcinoma: study of 102 cases. Influence of radiation therapy. Int J Radiat Oncol Biol Phys 1987;13:673–8.
[17] Mendenhall WM, Morris CG, Amdur RJ, et al. Radiotherapy alone or combined with surgery for adenoid cystic carcinoma of the head and neck. Head Neck 2004;26:154–62.
[18] Huber PE, Debus J, Latz D, et al. Radiotherapy for advanced adenoid cystic carcinoma: neutrons, photons or mixed beam? Radiother Oncol 2001;59:161–7.
[19] Douglas JG, Laramore GE, Austin-Seymour M, et al. Treatment of locally advanced adenoid cystic carcinoma of the head and neck with neutron radiotherapy. Int J Radiat Oncol Biol Phys 2000;46:551–7.
[20] Nakashima JP, Viégas CM, Fassizoli AL, et al. Postoperative adjuvant radiation therapy in the treatment of primary head and neck mucosal melanomas. ORL J Otorhinolaryngol Relat Spec 2008;70:344–51.
[21] Meleti M, Leemans CR, de Bree R, et al. Head and neck mucosal melanoma: experience with 42 patients, with emphasis on the role of postoperative radiotherapy. Head Neck 2008;30:1543–51.
[22] Wagner M, Morris CG, Werning JW, et al. Mucosal melanoma of the head and neck. Am J Clin Oncol 2008;31:43–8.
[23] Lengyel E, Gilde K, Remenar E, et al. Malignant mucosal melanoma of the head and neck – a review. Pathol Oncol Res 2003;9:7–12.
[24] Bridger AG, Smee D, Baldwin MA, et al. Experience with mucosal melanoma of the nose and paranasal sinuses. ANZ J Surg 2005;75:192–7.
[25] Mendenhall WM, Amdur RJ, Hinerman RW, et al. Head and neck mucosal melanoma. Am J Clin Oncol 2005;28:626–30.
[26] Willers H, Hug EB, Spiro IJ, et al. Adult soft tissue sarcomas of the head and neck treated by radiation and surgery or radiation alone: patterns of failure and prognostic factors. Int J Radiat Oncol Biol Phys 1995;33:585–93.
[27] Mendenhall WM, Mendenhall CM, Werning JW, et al. Adult head and neck soft tissue sarcomas. Head Neck 2005;27:916–22.
[28] Le QT, Fu KK, Kroll S, et al. Prognostic factors in adult soft-tissue sarcomas of the head and neck. Int J Radiat Oncol Biol Phys 1997;37:975–84.

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[29] Chen SA, Morris CG, Amdur RJ, et al. Adult head and neck soft tissue sarcomas. Am J Clin Oncol 2005;28:259–63.
[30] Castro JR, Reimers M. Charged particle radiotherapy of selected tumors in the head and neck. Int J Radiat Oncol Biol Phys 1988;14:711–20.
[31] Linstadt DE, Castro JR, Phillips TL. Neon ion radiotherapy: results of phase I/II clinical trial. Int J Radiat Oncol Biol Phys 1991;20:761–9.
[32] Schulz-Ertner D, Nikoghosyan A, Didinger B, et al. Therapy strategies for locally advanced adenoid cystic carcinomas using modern radiation therapy techniques. Cancer 2005;104:338–44.
[33] Schulz-Ertner D, Tsujii H. Particle radiation therapy using proton and heavier ion beams. J Clin Oncol 2007;25:953–64.
[34] Tsujii H, Kamada T, Baba M, et al. Clinical advantages of carbon-ion radiotherapy. New J Phys 2008;10:075009.

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A Phase I/II Clinical Trial of Preoperative Short-Course Carbon-Ion Radiotherapy for Patients With Squamous Cell Carcinoma of the Esophagus

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Background: Carbon-ion radiotherapy (CIR) has been under development. We report the results of a phase I/II clinical trial of preoperative CIR for esophageal squamous cell carcinoma (ESCC).

Methods: Thirty-one thoracic ESCC patients were enrolled. They were first treated with CIR. The radiation dose was escalated from the initial dose of 28.8 GyE up to 36.8. Four to 8 weeks after CIR followed by clinical evaluation of the therapy, surgery was performed. Thereafter, a pathological evaluation was made.

Results: Acute toxicity was not seen except in one case (3.2%), and there were no late toxicities. Throughout the study period, there were no cases of withdrawal due to the effects of preoperative CIR. Twelve out of 31 (38.7%) patients achieved a clinical complete response (CR) and 13 patients (41.9%) achieved a partial response. Twelve out of 31 patients (38.7%) achieved a pathological CR. The overall 1-, 3-, and 5-year survival rates in the stage I cases were 91%, 81%, and 61%, and was 100%, 85%, and 77% for the stage II, and 71%, 43%, and 29% for the stage III cases, respectively.

Conclusions: CIR showed strong local tumor control and is highly effective as a neoadjuvant therapy without severe adverse events.
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KEY WORDS: **esophageal cancer; carbon-ion radiotherapy; heavy-ion radiotherapy; neoadjuvant therapy; surgery; squamous cell carcinoma**

INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) is one of the worst malignant digestive diseases, and is the eighth most common cancer and 6th leading cause of death from cancer worldwide [1]. Although recent developments, including multi-disciplinary treatments, such as neoadjuvant therapy, three-field lymph dissection [2], and various developments in surgical techniques and management have contributed to improvement of the surgical outcome [3], it remains far from satisfactory. This is mainly because the stage of the disease is often far advanced when patients are diagnosed. Additionally, ESCC has the potential for frequent lymph node (LN) metastasis. This means that there is a high rate of postoperative recurrence and poor survival, even when curative esophagectomy is performed. In this situation, the usual strategy should be reconsidered, and a new treatment modality is urgently needed to provide further improvements in the outcome of esophageal cancer surgery.

During the past few decades, carbon-ion radiotherapy (CIR), which utilizes heavy-ion beams, has been developed as a clinical treatment [4]. CIR is attractive because carbon-ion beams have a high relative biological effectiveness (RBE) with a high linear energy transfer (LET). Furthermore, they create a very narrow high dose peak of radiation, which is called a Bragg peak. As a result of these features, targets can be selectively treated without injuring the normal tissues. Since 1995, CIR has been performed using the heavy ion medical accelerator in Chiba (HIMAC) to treat various malignant neoplasms such as prostate cancer [5], uterine cervix cancer [6], lung cancer [7], hepatoma [8], and so on at the National Institute of Radiological Sciences (NIRS). Their results proved that CIR has significant clinical advantages. However, there have not yet been any reports about ESCC. In this article, we report the world-first results of a phase I/II clinical trial of preoperative short-course CIR for patients with ESCC to evaluate the toxicity and the clinicopathologic effects of CIR.

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PATIENTS AND METHODS

Patient Eligibility

Patient eligibility criteria were determined as follows: (1) newly diagnosed thoracic ESCC without any previous treatments; (2) tumor depth, submucosa, or deeper (except T4); (3) stage, from I to IV according to the Japanese classification of esophageal cancer (9th edition); (4) curatively resectable; (5) includes measurable lesions; (6) age <80 years; (7) performance status between 0 and 2; (8) tumor size of <9 cm; (9) radiation fields include all the primary tumors and all metastatic LNs; (10) no other cancers nor active infections; (11) no serious medical or psychological problems. Because the field size of CIR is limited within 15 × 15 cm^2, only cases with metastatic LNs within 15 × 15 cm field. Therefore, cases with metastatic LNs located in distant region from the primary tumor, such as abdominal or neck metastatic LNs, were excluded to avoid influence by N-factor as much as possible. Before and after CIR treatment, blood examination and chest X-ray was performed and gastroesophageal endoscopy (GS), esophagography, computed tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI) were planned to assess the tumor depth and LN status. We defined metastatic LNs when they showed a round shape while measuring 10 mm or more in diameter in CT, or demonstrated a 3.0 or higher standardized uptake value in PET. All patients provided their informed consent and written approval was required before the enrollment. This study was approved by the NIRS Ethical Committee.

Study Design

The patients enrolled in this study were first treated with CIR using the HIMAC. The accelerator system and biological characteristics of the carbon ion beams have been described previously [9,10]. The spread-out Bragg peak (SOBP) was modulated to conform to the target volume with ridge filters, a multileaf collimator, and a compensation bolus. Before the treatment planning, metal markers (iridium seeds, 0.5 mm in diameter and 3 mm in length) were implanted endoscopically in the esophageal wall close to the proximal edge of the primary tumor and, where possible, also close to the distal edge. To reproduce the exact body position at the time of treatment, the patients were positioned in customized cradles (Moldcare; Alcare, Tokyo, Japan) and were immobilized with a low-temperature thermoplastic shell (Shellfitter; Kuraray, Osaka, Japan). Three-dimensional treatment planning with 5-mm thick CT images was performed using the HIPLAN software program, which was specially developed for carbon ion beam therapy at NIRS [11].

The planning target volume was determined on CT scanning which included the whole primary tumor with a 3 cm margin in the craniocaudal direction and metastatic LNs with a minimum of a 1cm margin. A respiratory gating system was used during the acquisition of the CT images and during the treatment [12]. CIR was administered in 8 fractions over 2 weeks. The doses of carbon ions were expressed in photon equivalent doses (GyE), which were defined as the physical doses multiplied by the RBE of the carbon ions. The RBE value of the carbon ions was assumed to be 3 at the distal part of the SOBP [10]. The radiation dose was escalated from the initial dose of 28.8 GyE in 5% increments up to 36.8 GyE when no severe adverse events (CTCAE grade3 and more) were observed. When Grade 3 or higher was observed, then the protocol committee discussed the case in order to decide whether or not to increase the dose.

Four to 8 weeks after the administration of carbon-ions, followed by clinical evaluation of the effectiveness of the therapy, an esophagectomy and LN dissection with thoracotomy and laparotomy was performed. During the peri- and postoperative course, patients were closely observed and evaluated to determine whether there were any adverse events which could be related to CIR. After surgery, the resected specimens were investigated pathologically to further evaluate the efficacy of preoperative CIR in the primary tumor. The specimens were also checked to determine whether there were any undesirable effects on the normal tissue in the radiation field. Toxicity was classified in accordance with the criteria of the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v 3.0) [13].

Endpoints

The primary objectives of this study were to evaluate the acute and late phase toxicity of CIR, and the clinicopathologic effects on the primary tumor. The effects in the normal tissue were also observed. The clinicopathologic effects on the primary tumor and in the normal tissue were evaluated clinically and pathologically. Clinical evaluation of the primary tumor was performed according to the Japanese classification of esophageal cancer (9th edition) [14]. All of the clinical evaluations were performed using blood examination, chest X-ray, GS, esophagography, CT, PET, and MRI before the surgery. For the pathological evaluation, hematoxylin-eosin staining was performed and then evaluated according to the Japanese classification of esophageal cancer (9th edition) [14]. Using these criteria, the efficacy against the primary tumor was categorized as follows: Grade 0: ineffective. No recognizable cytological or histological therapeutic effect. Grade 1: Slightly effective. Viable cancer cells account for 1/3 or more of the tumor tissue, but there is some evidence of degeneration of cancer tissue or cells. Grade 2: moderately effective. Viable cancer cells account for less than 1/3 of the tumor tissue, while other cancer cells are severely degenerated or necrotic. Grade 3: markedly effective. No viable cancer cells are evident. As secondary endpoints, the overall, cause-specific, and relapse-free survival (RFS) rates were examined.

Data Analysis

Acute toxicity was defined when toxicities including operative complications were observed within 90 days after the onset of the first treatment, and late toxicity was defined after the 91st day from the first treatment. The overall survival (OS) time was defined as the period from the first day of CIR to the date of death or the last follow-up regardless of the causes. The cause-specific survival (CSS) time was defined as the period from the first treatment to the date of death from treated esophageal cancer. The RFS time was defined as the period from the day of surgery to the day of recurrence or the last follow-up. The 1-, 3-, and 5-year OS, CSS, and RFS rates were estimated using the Kaplan–Meier method.

RESULTS

Patient Characteristics

Between July 2004 and June 2008, 31 patients from 4 facilities were enrolled in this study. The patient characteristics are shown in Table I. The mean age of patients was 65.4 years old, and the male: female ratio was 25: 6, respectively. The locations consisted of three cases of the upper thoracic, 18 of middle thoracic and 10 of the lower thoracic esophagus. The mean tumor size was 4.1 cm. Pathologically, three cases were well-differentiated squamous cell carcinoma (SCC), 22 were moderately differentiated SCC, 4 were poorly differentiated SCC, and 2 cases could not be clearly defined. Regarding the tumor depths, 12 cases were of T1, 8 of T2, and 11 of T3. A total of 22 cases were without any metastatic LNs, and 8 cases were N1 and 1 case was N2. Finally, 10 cases were classified as stage I,

TABLE I. Patient Characteristics

Age (±SD)	65.4 ± 7.1
Sex (male: female)	25:6
Tumor location	
Upper thoracic	3
Middle thoracic	18
Lower thoracic	10
Tumor size (cm, ±SD, range)	4.1 ± 1.6, 2.0–8.0
Pathological grade	
Well	3
Moderately	22
Poorly	4
Unclear	2
T category	
T1	12
T2	8
T3	11
N category	
N0	22
N1	8
N2	1
N3	0
Stage grouping	
Stage I	10
Stage II	14
Stage III	7

14 as stage II, and 7 as stage III disease, respectively. All cases were followed over 36 months after the surgery.

Acute and Late Toxicity

Several kinds of acute toxicities were observed, as shown in Table II. All of the patients except one case (3.2%) did not show any toxicity which exceeded Grade 3. Severe toxicity in the esophagus, such as esophagitis, was not observed in any of the patients, nor was any toxicity observed in the skin. In the respiratory organs, one case (3.2%) in 35.2 GyE presented Grade 3 of postoperative acute respiratory distress syndrome (ARDS) which required prolonged administration of oxygen postoperatively; however, neither a tracheostomy nor respirator was required. The correlation between CIR and ARDS was uncertain. Severe toxicity in the blood was not observed in any of the patients. The late toxicity was also closely assessed during the postoperative course; however, there were no cases which presented late toxicity of Grade 1 or higher (data not shown).

Throughout this study, there were no cases of withdrawal due to preoperative CIR. We could not define the final recommended dose (RD) due to limitations regarding the number of cases, although RD of CIR was thought to be 35.2 GyE or more based on these results. Based on the findings obtained in this study, it can be concluded that CIR followed by esophagectomy did not lead to any significant adverse effects, and did not affect either the peri- or postoperative course.

TABLE II. Acute Toxicity (National Cancer Institute Common Terminology Criteria for Adverse Events Version 3)

	Total dose (GyE)						
	28.8 (n = 5)	30.4 (n = 4)	32.0 (n = 5)	33.6 (n = 4)	35.2 (n = 12)	36.8 (n = 1)	Grade ≥3 (%)
Esophagus							0 (0)
Grade 0	0	0	0	0	0	0	
Grade 1	5	3	5	4	2	0	
Grade 2	0	1	0	0	10	1	
Grade 3	0	0	0	0	0	0	
Skin							0 (0)
Grade 0	0	0	0	0	4	0	
Grade 1	5	4	5	4	8	1	
Grade 2	0	0	0	0	0	0	
Grade 3	0	0	0	0	0	0	
Respiratory							1 (3.2)
Grade 0	5	4	5	4	11	1	
Grade 1	0	0	0	0	0	0	
Grade 2	0	0	0	0	0	0	
Grade 3	0	0	0	0	1	0	
Blood							0 (0)
Grade 0	5	4	3	4	9	0	
Grade 1	0	0	1	0	3	0	
Grade 2	0	0	1	0	0	1	
Grade 3	0	0	0	0	0	0	

Clinical and Pathologic Efficacy in the Primary Lesion

After administration of carbon-ion beams, we closely evaluated the clinical effects of therapy (Table III). In total, 12 out of the 31 (38.7%) patients achieved a clinical complete response (CR), 13 (41.9%) patients achieved a partial response (PR) and overall response rate (CR + PR) was 80.6%. According to the tumor depth, thin tumors tended to show a good response. In fact, all 12 cases of T1 and all 8 cases of T2 achieved a CR or PR (response rate, 100%), while only 5 out of the 11 cases of T3 disease achieved a CR or PR (response rate, 45.5%). A pathological response in the resected primary tumor after surgery was also seen in many patients, as shown in Table IV. In total, 12 (38.7%) patients achieved a Grade 3 response (pathological CR) and 12 (38.7%) patients achieved a Grade 2 response. The overall pathological response rate (Grade 3 + Grade 2) was therefore 77.4%. This was similar to the preoperative clinical evaluation of the response. In all dose levels except level 3, the pathological response rate was over 75%. Based on the tumor depth, the pathological response was 100% in T1 cases and 87.5% in T2 cases; however, it was 45.5% in T3 cases. Pathologically, thin tumors showed a tendency to respond well to CIR. There were no adverse findings in the normal esophageal wall of the resected specimen in the radiation field.

TABLE III. Clinical Efficacy Against the Primary Lesion

	Total dose (GyE)	Depth (No. of pts.)	CR	PR	NC, PD	Response rate (%)
Level 1	28.8	T1 (n = 3)	2	1	0	100
		T2 (n = 1)	0	1	0	
		T3 (n = 1)	0	1	0	
Level 2	30.4	T1 (n = 2)	2	0	0	75
		T2 (n = 0)				
		T3 (n = 2)	0	1	1	
Level 3	32.0	T1 (n = 1)	1	0	0	80
		T2 (n = 2)	1	1	0	
		T3 (n = 2)	0	1	1	
Level 4	33.6	T1 (n = 1)	0	1	0	100
		T2 (n = 3)	0	1	0	
		T3 (n = 0)	1	2	0	
Level 5	35.2	T1 (n = 5)	4	1	0	67
		T2 (n = 2)	1	1	0	
		T3 (n = 5)	0	1	4	
Level 6	36.8	T1 (n = 0)				100
		T2 (n = 0)				
		T3 (n = 1)	0	1	0	
Total		31	12(38.7%)	13(41.9%)	6(19.4%)	25(80.6%)

TABLE IV. Pathological Efficacy Against the Primary Lesion

	Total dose (GyE)	Depth (No. of pts.)	Grade 3	Grade 2	Grade 1,0	Response rate (%)
Level 1	28.8	T1 (n = 3)	1	2	0	80
		T2 (n = 1)	0	1	0	
		T3 (n = 1)	0	0	1	
Level 2	30.4	T1 (n = 2)	1	1	0	75
		T2 (n = 0)				
		T3 (n = 2)	0	1	1	
Level 3	32.0	T1 (n = 1)	0	1	0	60
		T2 (n = 2)	1	0	1	
		T3 (n = 2)	0	1	1	
Level 4	33.6	T1 (n = 1)	1	0	0	100
		T2 (n = 3)	3	0	0	
		T3 (n = 0)				
Level 5	35.2	T1 (n = 5)	3	2	0	82
		T2 (n = 2)	2	0	0	
		T3 (n = 5)	0	3	2	
Level 6	36.8	T1 (n = 0)				0
		T2 (n = 0)				
		T3 (n = 1)	0	0	1	
Total		31	12(38.7%)	12(38.7%)	7(22.6%)	24(77.4%)

Survival

There were 11 deaths during the observation period of this study. Of these 11 cases, one died from a simultaneous cerebral and myocardial infarction and one died from methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia, and one patient suffering from depression died from suffocation due to an unknown reason. The OS is shown in Figure 1a. In the stage I cases, the overall 1-, 3-, and 5-year survival rates were 91%, 81%, and 61%, respectively. In the stage II cases, they were 100%, 85%, and 77%, and in the stage III cases, they were 71%, 43%, and 29%, respectively. After exclusion of the cases who died due to causes that were unrelated to the primary disease, the CSS was calculated (Fig. 1b). The cause-specific 1-, 3-, and 5-year survival rates were 97%, 79%, and 71%, respectively. In the stage I cases, their 1-, 3-, and 5-year rates were 100%, 90%, and 90%, respectively. In the stage II cases, they were 100%, 85%, and 77%, and in the stage III cases, they were 83%, 50%, and 33%, respectively. The relapse-free 1-, 3-, and 5-year survival rates were 87%, 62%, and 62%, respectively. In the stage I cases, they were 100%, 80%, and 80%, respectively. In the stage II cases, they were 92%, 69%, and 69%, and in the stage III cases, they were 51%, 17%, and 17%, respectively (Fig. 1c). CSS showed better survival in Grade 3 or Grade 2 cases compared to Grade1 cases. This result indicated that patients' better survival was in proportion to pathologic effectiveness in the primary tumor (Fig. 2). The RFS in the stage III patients was comparatively worse, mainly because of postoperative recurrence, as described below.

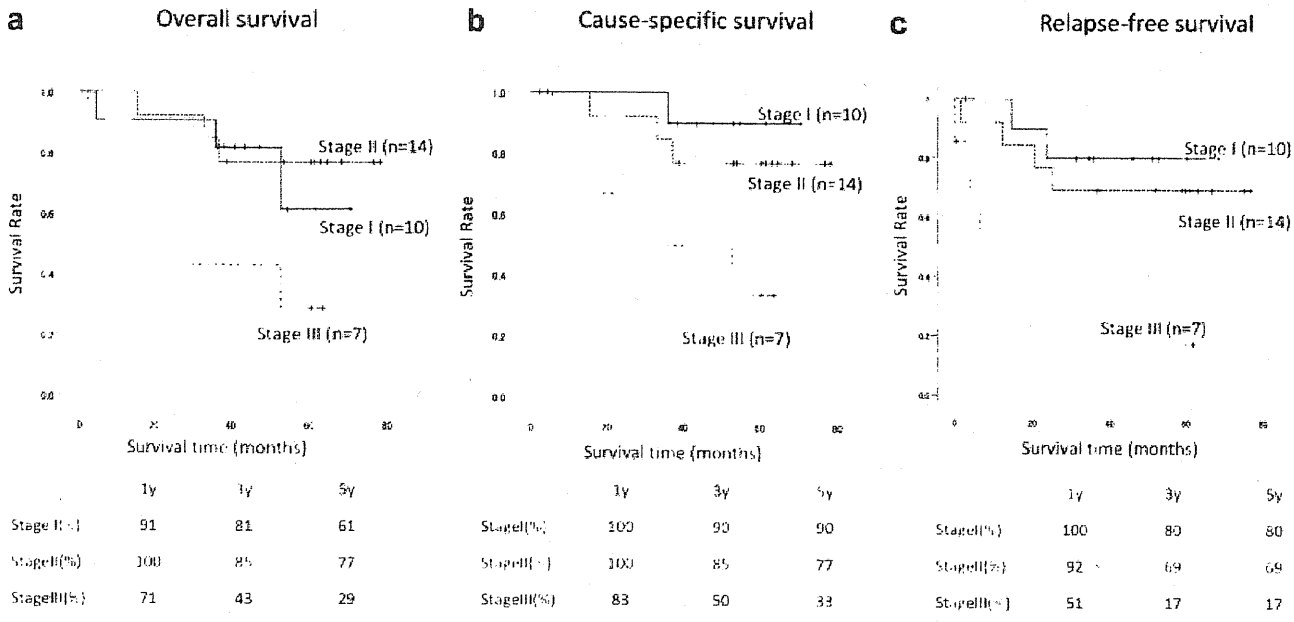
Recurrence Patterns

Patients underwent CT and endoscopy after the surgery, and at regular intervals postoperatively. In total, 11 out of the 31 cases (35%) showed recurrence during the postoperative course, as seen in Table V. The most frequent recurrence pattern was LN recurrence, which was seen in 8 out of all the 11 recurrence cases (73%). However, there were only two cases which had LN-recurrence within the field of CIR. In addition, six cases had LN-recurrence beyond the field of CIR. This means that CIR effectively controlled LN metastases. The second most frequent pattern was organ recurrence, which was seen in 6 out of the 11 cases (55%).

DISCUSSION

During the past few decades, preoperative neoadjuvant chemoradiotherapy (CRT) has been actively performed [15], although the benefits of such treatments have been controversial [16–18]. A recent meta-analysis showed the advantage of preoperative CRT, however, preoperative CRT can cause an increase in the peri- and postoperative morbidity [19,20]. Therefore, a more effective and less toxic preoperative therapeutic modality has been urgently needed.

CIR is known to have various advantages compared to conventional radiotherapy. Based on features such as the RBE, LET, and SOBP, it can be considered to be a feasible candidate that can

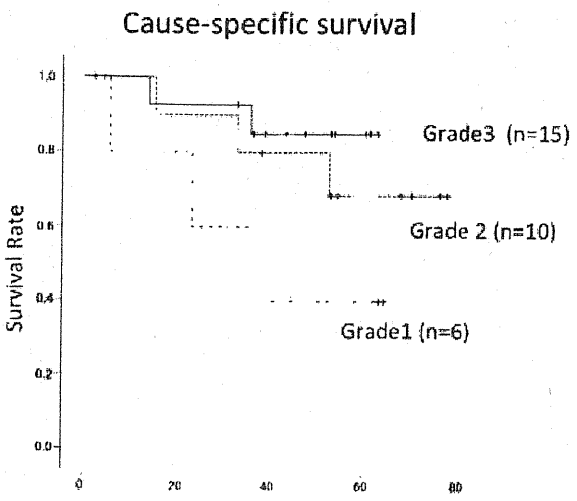


	1y	3y	5y
Stage I (%)	91	81	61
Stage II (%)	100	85	77
Stage III (%)	71	43	29

	1y	3y	5y
Stage I (%)	100	90	90
Stage II (%)	100	85	77
Stage III (%)	83	50	33

	1y	3y	5y
Stage I (%)	100	80	80
Stage II (%)	92	69	69
Stage III (%)	51	17	17

Fig. 1. (a) Overall survival. (b) Cause-specific survival. (c) Relapse-free survival.



	1y	3y	5y
Grade3(%)	100	86	86
Grade2(%)	100	80	69
Grade1(%)	80	60	40

Fig. 2. Cause-specific survival according to pathologic effectiveness in the primary tumor.

improve the outcome of esophageal surgery. Prior to this study, we performed preliminary clinical trials, for example, single use of CIR as a definitive therapy for advanced and unresectable esophageal cancer (HIMAC 9502) [21]. Regrettably, the outcome of this study did not show any advantages of CIR because most of the patients enrolled in the HIMAC 9502 study were too far advanced to be controlled by only CIR. Another reason was that the treatment course was too long for the preoperative setting. Due to the long preoperative treatment, the tumor had often progressed before surgery. Considering these points, we set the criteria for this study so that the patients could definitively undergo curative esophagectomy after short-term administration of preoperative CIR. This means we targeted clinical stage I, II, and III patients.

Our present data demonstrated the advantages of CIR in the neoadjuvant setting. First, the local tumor control was excellent, as seen in Table IV. CIR alone achieved a pathological CR (Grade 3) in 12 out of 31 (38.7%) patients. We previously showed the pathological CR rate by conventional CRT to be 24.3% even though 40 Gy of radiation and chemotherapy were concurrently administered [22]. Compared to our data and the data of the Japanese comprehensive data from the Comprehensive Registry of Esophageal Cancer in Japan 2003 [23], a smaller dose of CIR showed much better

TABLE V. Recurrence Patterns

	Cases
Negative	20
Positive	11
Local[a,b]	2/11(18%)
Organ	6/11(55%)
Lymph node	8/11(73%)

Multiple recurrences were included in each category.
[a]Surgical T4 (non-curative resection).
[b]Recurrence in anastomosis.

antitumor effects for the primary tumor. Because our previous data [22] and other reports [24,25] showed that the effectiveness in the primary tumor was closely related to better survival, this advantage provides the most significant benefit for patients as shown in Figure 2. Additionally, our study revealed that there was no histopathologic disadvantage in the normal tissue in the radiation field, and moreover, CIR prior to the surgery did not affect the peri- and postoperative morbidity, as seen in Table II.

This point is crucial because esophagectomy is often accompanied by a high rate of surgery-related morbidity and mortality [26]. When conventional radiotherapy or CRT for esophageal cancer are used, it is necessary to pay attention to the occurrence of radiation-induced toxicities, such as pleural effusion, pericardial effusion, and radiation pneumonitis [27]. Our results imply that CIR can be both a highly effective and a less toxic therapy compared to conventional radiation.

At the same time, there were still some issues that were found through our study that will need to be resolved before future progression of the treatment. First, our data indicated that there were some cases in which postoperative recurrence was observed, especially in initially advanced cases, such as clinically node positive cases and T3 cases. In recent reports, the number of metastatic LNs was found to be the most significant prognostic factor [28–31], and the outcome of esophagectomy depends on the status of LN metastasis [32] as the TNM classification applied the number of LN metastases into its N-category staging system [33]. As our results showed, the risk of postoperative LN recurrence was not completely controlled by CIR alone, although we initially eliminated cases with multiple metastatic LNs from this study. Second, the effectiveness of CIR for cases with a deeper tumor, such as T3 tumors, was found to still be unsatisfactory in the current protocol. Concerning recent trends, attention should be paid to the risk of not adding chemotherapy as a neoadjuvant therapy to control the distant metastases or LN recurrence, although precise detection of all the metastatic LNs is clinically very difficult [34]. To provide further improvements especially for advanced cases, additional chemotherapy should thus be considered. We are planning a new protocol using simultaneous chemotherapy plus CIR in the preoperative setting which could provide a stronger antitumor effect with less toxicity.

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REFERENCES

1. Ferlay J, Shin HR, Bray F, et al.: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer (J Int Cancer) 2010;127:2893–2917.
2. Isono K, Sato H, Nakayama K: Results of a nationwide study on the three-field lymph node dissection of esophageal cancer. Oncology 1991;48:411–420.
3. Akutsu Y, Matsubara H: Perioperative management for the prevention of postoperative pneumonia with esophageal surgery. Ann Thorac Cardiovasc Surg 2009;15:280–285.

4. Okada T, Kamada T, Tsuji H, et al.: Carbon ion radiotherapy: Clinical experiences at National Institute of Radiological Science (NIRS). J Radiat Res 2010;51:355–364.
5. Ishikawa H, Tsuji H, Kamada T, et al.: Carbon ion radiation therapy for prostate cancer: Results of a prospective phase II study. Radiother Oncol: J Eur Soc Ther Radiol Oncol 2006;81: 57–64.
6. Kato S, Ohno T, Tsujii H, et al.: Dose escalation study of carbon ion radiotherapy for locally advanced carcinoma of the uterine cervix. Int J Radiat Oncol Biol Phys 2006;65:388–397.
7. Miyamoto T, Baba M, Yamamoto N, et al.: Curative treatment of Stage I non-small-cell lung cancer with carbon ion beams using a hypofractionated regimen. Int J Radiat Oncol Biol Phys 2007;67:750–758.
8. Kato H, Tsujii H, Miyamoto T, et al.: Results of the first prospective study of carbon ion radiotherapy for hepatocellular carcinoma with liver cirrhosis. Int J Radiat Oncol Biol Phys 2004;59:1468–1476.
9. Kanai T, Furusawa Y, Fukutsu K, et al.: Irradiation of mixed beam and design of spread-out Bragg peak for heavy-ion radiotherapy. Radiat Res 1997;147:78–85.
10. Kanai T, Endo M, Minohara S, et al.: Biophysical characteristics of HIMAC clinical irradiation system for heavy-ion radiation therapy. Int J Radiat Oncol Biol Phys 1999;44:201–210.
11. Endo M, Koyama-Ito H, Minohara S, et al.: HIPLAN—A heavy ion treatment planning system at HIMAC. J Jpn Soc Ther Radiol Oncol 1996;8:231–238.
12. Minohara S, Kanai T, Endo M, et al.: Respiratory gated irradiation system for heavy-ion radiotherapy. Int J Radiat Oncol Biol Phys 2000;47:1097–1103.
13. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf. In, 2006.
14. Japanese Society for Esophageal Diseases (ed): "Guide Lines for the Clinical and Pathologic Studies on Carcinoma of the Esophagus." Tokyo: Kanehara, 1999.
15. van Heijl M, van Lanschot JJ, Koppert LB, et al.: Neoadjuvant chemoradiation followed by surgery versus surgery alone for patients with adenocarcinoma or squamous cell carcinoma of the esophagus (CROSS). BMC Surg 2008;8:21.
16. Jin HL, Zhu H, Ling TS, et al.: Neoadjuvant chemoradiotherapy for resectable esophageal carcinoma: A meta-analysis. World J Gastroenterol 2009;15:5983–5991.
17. Burmeister BH, Smithers BM, Gebski V, et al.: Surgery alone versus chemoradiotherapy followed by surgery for resectable cancer of the oesophagus: A randomised controlled phase III trial. Lancet Oncol 2005;6:659–668.
18. Wijnhoven BP, van Lanschot JJ, Tilanus HW, et al.: Neoadjuvant chemoradiotherapy for esophageal cancer: A review of meta-analyses. World J Surg 2009;33:2606–2614.
19. Courrech Staal EF, Aleman BM, Boot H, et al.: Systematic review of the benefits and risks of neoadjuvant chemoradiation for oesophageal cancer. Br J Surg 2010;97:1482–1496.
20. Fiorica F, Di Bona D, Schepis F, et al.: Preoperative chemoradiotherapy for oesophageal cancer: A systematic review and meta-analysis. Gut 2004;53:925–930.
21. Research Center of Charged Particle Therapy. National Institute of Radiological Sciences (ed): "Phase I/II clinical trial of preoperative heavy ion therapy for esophageal cancer (9502)." Chiba, 2000.
22. Akutsu Y, Matsubara H, Shuto K, et al.: Clinical and pathologic evaluation of the effectiveness of neoadjuvant chemoradiation therapy in advanced esophageal cancer patients. World J Surg 2009;33:1002–1009.
23. Ozawa S, Tachimori Y, Baba M, et al.: Comprehensive Registry of Esophageal Cancer in Japan, 2003. Esophagus 2011;8: 9–29.
24. Le Prise E, Etienne PL, Meunier B, et al.: A randomized study of chemotherapy, radiation therapy, and surgery versus surgery for localized squamous cell carcinoma of the esophagus. Cancer 1994;73:1779–1784.
25. Apinop C, Puttisak P, Preecha N: A prospective study of combined therapy in esophageal cancer. Hepato-Gastroenterol 1994;41:391–393.
26. Jamieson GG, Mathew G, Ludemann R, et al.: Postoperative mortality following oesophagectomy and problems in reporting its rate. Br J Surg 2004;91:943–947.
27. Kato K, Muro K, Minashi K, et al.: Phase II study of chemoradiotherapy with 5-fluorouracil and cisplatin for stage ii–iii esophageal squamous cell carcinoma: JCOG trial (JCOG 9906). Int J Radiat Oncol Biol Phys 2011;81:684–690.
28. Twine CP, Lewis WG, Morgan MA, et al.: The assessment of prognosis of surgically resected oesophageal cancer is dependent on the number of lymph nodes examined pathologically. Histopathology 2009;55:46–52.
29. Wilson M, Rosato EL, Chojnacki KA, et al.: Prognostic significance of lymph node metastases and ratio in esophageal cancer. J Surg Res 2008;146:11–15.
30. Greenstein AJ, Litle VR, Swanson SJ, et al.: Prognostic significance of the number of lymph node metastases in esophageal cancer. J Am Coll Surg 2008;206:239–246.
31. Akutsu Y, Matsubara H: The significance of lymph node status as a prognostic factor for esophageal cancer. Surg Today 2011;41:1190–1195.
32. Miyata H, Yamasaki M, Takiguchi S, et al.: Pre- and post-therapy nodal status equally affects survival of patients with oesophageal squamous cell carcinoma receiving preoperative chemoradiation. Oncol Rep 2010;23:1331–1337.
33. Rice TW, Rusch VW, Ishwaran H, et al.: Cancer of the esophagus and esophagogastric junction: Data-driven staging for the seventh edition of the American Joint Committee on Cancer/ International Union Against Cancer Cancer Staging Manuals. Cancer 2010;116:3763–3773.
34. Nishimaki T, Tanaka O, Ando N, et al.: Evaluation of the accuracy of preoperative staging in thoracic esophageal cancer. Ann Thorac Surg 1999;68:2059–2064.

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RESEARCH Open Access

In vitro characterization of cells derived from chordoma cell line U-CH1 following treatment with X-rays, heavy ions and chemotherapeutic drugs

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Abstract

Background: Chordoma, a rare cancer, is usually treated with surgery and/or radiation. However, very limited characterizations of chordoma cells are available due to a minimal availability (only two lines validated by now) and the extremely long doubling time. In order to overcome this situation, we successfully derived a cell line with a shorter doubling time from the first validated chordoma line U-CH1 and obtained invaluable cell biological data.

Method: After isolating a subpopulation of U-CH1 cells with a short doubling time (U-CH1-N), cell growth, cell cycle distribution, DNA content, chromosome number, p53 status, and cell survival were examined after exposure to X-rays, heavy ions, camptothecin, mitomycin C, cisplatin and bleocin. These data were compared with those of HeLa (cervical cancer) and U87-MG (glioblastoma) cells.

Results: The cell doubling times for HeLa, U87-MG and U-CH1-N were approximately 18 h, 24 h and 3 days respectively. Heavy ion irradiation resulted in more efficient cell killing than x-rays in all three cell lines. Relative biological effectiveness (RBE) at 10% survival for U-CH1-N was about 2.45 for 70 keV/μm carbon and 3.86 for 200 keV/μm iron ions. Of the four chemicals, bleocin showed the most marked cytotoxic effect on U-CH1-N.

Conclusion: Our data provide the first comprehensive cellular characterization using cells of chordoma origin and furnish the biological basis for successful clinical results of chordoma treatment by heavy ions.

Background

Chordoma is a rare malignant bone tumor accounting for only 1 to 4% of all primary malignant bone tumors [1]. Chordoma originates from notochordal remnants and has slower local growth and metastasizes less frequently than other bone and soft tissue malignant tumors [2]. Chordoma is not easy to control because of its anatomic location and propensity for spreading extensively. Complete radical resection produces better local control compared with subtotal resection and chemotherapy [1,2]. Some case studies reported that photon, proton, and charged particle carbon radiotherapy may delay possible recurrence after incomplete resection and may also be able to control the tumor [3-13]. A phase II study of 9-nitro-camptothecin in patients with advanced chordoma showed that it possessed modest activity in delaying progression with unresectable or metastatic chordoma [14]. Several reports suggested that PI3K/AKT/TSC1/TSC2/mTOR pathway and EGFR are potential therapeutic targets for chordoma [15,16]. One report showed that the combination with topoisomerase II inhibitor razoxane enhances the effectiveness of chordoma radiotherapy [17].

It is sometimes difficult to perform complete radical resection of chordoma tumors, depending on anatomic location or grade of tumor spreading. Because of the lower effectiveness of chemotherapy, radiotherapy is a useful treatment tool, and thus information on cellular

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radiosensitivities to photon and/or charged particles is urgently needed.

Despite the accumulation of data from the clinical side, there is a scarcity of information from the biology side because of the difficulty in obtaining basic cell biological data from the two currently available chordoma lines; the first cell line has been available for the last few years and the second one became available from the Chordoma foundation a few month ago. Another big obstacle is extremely long doubling time of chordoma cells. The first validated chordoma cell line, U-CH1, isolated by a German group, presented a long cell doubling time (~ 7 days) and chromosome instability and rearrangement [18]. U-CH1-N, a subpopulation derived from U-CH1 chordoma cells at National Institute of Radiological Sciences (NIRS), has acceptably shorter cell doubling time that enabled us to carry out *in vitro* cell biological research such as clonogenic cell survival assay. This study is the first to report the measurement of *in vitro* cellular radiosensitivity, heavy ion biological effectiveness, and responses to chemotherapy agents for a sacral chordoma cell line.

Methods

Cell lines and culture conditions

The chordoma cell line U-CH1 was kindly supplied by the Chordoma Foundation in Greensboro, NC, USA. U87-MG and HeLa cell lines were obtained from ATCC, USA. Cells were cultured in MEM-alpha (Gibco, Japan) supplemented with 10% fetal bovine serum (FBS, Sigma, Japan) and 1% antibiotics and antimicotics (Gibco, Japan), and they were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air.

U-CH1-N cells and cell doubling time

Original U-CH1 cells had 7 days of doubling time in Iscove/RPMI (4:1) medium with 10% FBS in collagen-coated flasks [18]. In order to perform clonogenic colony formation assay, at least 7 cell divisions are required to obtain colony containing more than 50 cells. If we use the original U-CH1, it will take at least 2 months to get countable colonies. Therefore, we adapted U-CH1 in alpha-MEM medium supplemented with 10% FBS under normal culture conditions in tissue culture plastic flasks, similar to the other two cell lines. After three weeks we isolated fast growing subpopulation of U-CH1, and designated as "U-CH1-N" (N for NIRS). To measure the cell doubling time, cells were seeded at 5000 cells per T12.5 flask, and their number was counted at regular intervals.

Comparison of parental and subpopulation of U-CH1, chromosome and p53 analysis

U-CH1-N cells were verified for their characteristics on karyotyping compared with their original U-CH1 cells. U-CH1-N cells were cultured with 0.1 μg/ml Colcemid for 6 hours to harvest metaphase chromosomes. Samples were treated in hypotonic solution, 75 mM KCl, for 20 min at 37°C and fixed in 3:1 (methanol: acetic acid) fixation solution three times. Spread metaphase chromosomes were stained with Giemsa solution, and the chromosome number was observed under a microscope.

Genomic DNA from parental U-CH1 and faster growing subpopulation U-CH1-N was isolated with Qiagen Blood & Cell Culture DNA mini kit (Qiagen, Japan). The genomic regions of the p53 gene were amplified by PCR using KOD plus polymerase (TOYOBO, Japan) with the following primers: hTP53AF (5'-ccattcttttcctgctccacaggaagccga-3') and hTP53BR (5'-ggctaagctatgatgttccttagattaggt-3') for exons 2 - 9, hTP53CF (5'-ctgtataggtacttgaagtgcagtttctac -3') and hTP53CR (5'-ttgtaaactaacccttaactgcaagaacat -3') for exons 10 and 11. The conditions of PCR were: 94°C, 2 min, 35 cycles of 94°C (15 sec) and 68°C (4 min). The amplified DNA fragments (approximate 3.6 kb and 1.5 kb) were subjected to sequencing reaction using the p53 exon-specific primers supplied by Nippon Gene (Toyama, Japan) and Big-Dye Terminator Cycle Sequencing FS Ready Reaction Kit V3.1 (Applied Biosystems, Foster City, CA). The nucleotide sequence was evaluated by genetic analyzer PRISM 310 (Applied Biosystems) and verified on both strands. The nucleotide sequence data of TP53 determined in the present study were deposited to DDBJ/EMBL/Genbank as a following accession ID; AB511810.

Flow cytometry

Randomly dividing sample cultures were fixed in 70% ethanol and kept at -20°C until analysis. PI-stained 10,000 cells were analyzed by BD FACSCalibur (Beckton Dickinson, Japan) to obtain the DNA content histogram. Cell cycle characteristics were analyzed by Modfit program on Mac OS 9. The DNA content was compared with Chinese Hamster Ovary cells that have diploid DNA content and were calculated as 50 as an arbitrary unit.

Irradiation and chemical treatment for Colony formation assay

Cells were irradiated with TITAN x-ray irradiator with 200 kVp, 20 mA, 0.5 cm of Al and Cu filter (Shimadzu, Japan). Heavy ion treatment was performed by HIMAC (Heavy Ion Medical Accelerator in Chiba). The accelerated ions used in this study were carbon ions (290 MeV/n), neon (400 MeV/n), silicon (490 MeV/n), argon (500 MeV/n), and iron ions (500 MeV/n). The details concerning the beam characteristics of carbon-ion beams, biological irradiation procedures, and dosimetry have been described elsewhere [19,20]. We used several

kinds of beams having different LET values, using Lucite absorbers with various thicknesses to change the energy of the beams. At the sample position, we estimated the LET values of carbon (13, 30, 50, 70 keV/μm), neon (31, 70, 120 keV/μm), silicon (55, 150, 250 keV/μm), argon (100 keV/μm), and iron (200 keV/μm). Taking fragmentations into consideration, dose was calculated from fluence [21-23]. Asynchronously dividing cells cultured in T12.5 flasks were irradiated at room temperature. For chemical treatment, cycling cells in T12.5 culture flasks were exposed to series of concentration of bleocin, a single component of bleomycin family group A (Calbiochem, Japan), which induces DNA strand breaks, camptothecin (CPT, Sigma, Japan) which is a Topoisomerase I inhibitor, mitomycin C (MMC, Funakoshi, Japan) which induces DNA crosslink, or cisplatin (Nippon Kayaku, Japan) which induces DNA crosslink for 1 hour at 37°C.

After exposure to ionizing radiation or chemical treatment, cells were trypsinized and re-plated in P-100 cell culture dishes. HeLa and U87-MG cells were cultured for 10 to 14 days, and U-CH1-N cells were kept in an incubator for 3 to 4 weeks. Plating efficiency of U-CH1-N, U87-MG, and HeLa cells were 4.8%, 32%, and 70%, respectively. After colonies were formed, cells were fixed with 100% ethanol and stained with crystal violet solution. Colonies were observed under microscope and colonies containing more than 50 cells were counted as survivors. Cell survival assay was carried out 2 to 4 times independently. Radiation exposed cell survival curves were fitted with linear quadratic model by PRISM5 software on MacOSX10.6. Error bars indicate standard error of the means.

Results

Cellular doubling time, chromosome number, and p53 status of U-CH1-N

The original U-CH1 cell line had a 7-day doubling time under culture medium and conditions originally used. We used the identical cell culture conditions for all three different tumor cell lines to avoid complexities arising from different growth conditions among them. The doubling time for U-CH1-N derived from U-CH1 at NIRS was about three days as against 7 days for the parental U-CH1 cell line. This reduced doubling time is still significantly longer than 21.5 hours for U87-MG and 18 hours for HeLa cells (Figure 1A). This shortened doubling time for U-CH1-N enabled us to carry out essential in vitro experiments including the colony formation assay to determine cell survival fraction against ionizing radiation and anti-tumor chemicals.

Our chromosome analysis of U-CH1-N cells showed that the distribution of chromosome numbers are practically identical to the numbers measured for the original U-CH1 cells (Figure 1B) [18]. Original U-CH1 had 75 chromosomes per cell on average, and our U-CH1-N cells averaged 75.34 chromosomes per cell.

The DNA sequencing data of the p53 gene of U-CH1-N was compared with the human wild-type TP53 gene MM_000546. It was revealed that one allele of p53 had a mutation carrying a C > G substitution at nucleotide residue 412 within exon 4, converting the corresponding amino acid from proline to arginine (Figure 1C). Parental U-CH1 carried exactly the same heterozygous mutation in p53 gene.

Cell Cycle Distribution and DNA Content

Both cell cycle distribution and DNA profile were measured by a flow cytometer. The results are summarized in Table 1. DNA profile showed that U-CH1-N and HeLa were near tetraploid (about 100 and 90, respectively) compared with almost normal diploid DNA content (about 60) of U87-MG. Cell cycle distribution in chordoma cells showed a significantly high ratio in G1-phase, very different from the DNA profile patterns of the other two cell lines. These showed a greater number of cells in G1-phase (75%) and a smaller number in S-phase (13.3%). The slow growth speed of U-CH1-N may have a relationship with the long resting time before DNA synthesis in G1-phase.

Cellular Radiosensitivity and Relative Biological Effectiveness

Asynchronous cell cultures were irradiated with various kinds of ionizing radiations (X-rays, carbon-ions 13 keV/μm, carbon-ions 70 keV/μm, iron-ions 200 keV/μm). Because of long cellular doubling time, the colony size of U-CH1-N was generally smaller than HeLa and U87 cells, even when a longer incubation time was allowed to form colonies. Nevertheless, by the time of fixation, we were able to observe U-CH1-N colonies containing more than 100 cells with or without irradiation. p53 mutated HeLa cells were the most resistant to all kinds of ionizing radiation among these cell lines; U87-MG and U-CH1 revealed similar radiosensitivity (Figure 2). From these D_{10} (radiation dose to kill 90% of irradiated cells) values, we calculated the relative biological effectiveness (RBE) of heavy charged particles compared to x-rays (Figure 3). RBE was obtained from D_{10} of x-rays divided by D_{10} of heavy ions with certain LET. RBE values of U-CH1-N cell line were not significantly different from ones of either HeLa or U87-MG by t-test.

Extended Relative Biological Effectiveness Study for U-CH1-N

In order to understand the detailed RBE values in this chordoma cell line, 11 different qualities of photon and ion beams were employed to obtain cell survival curves

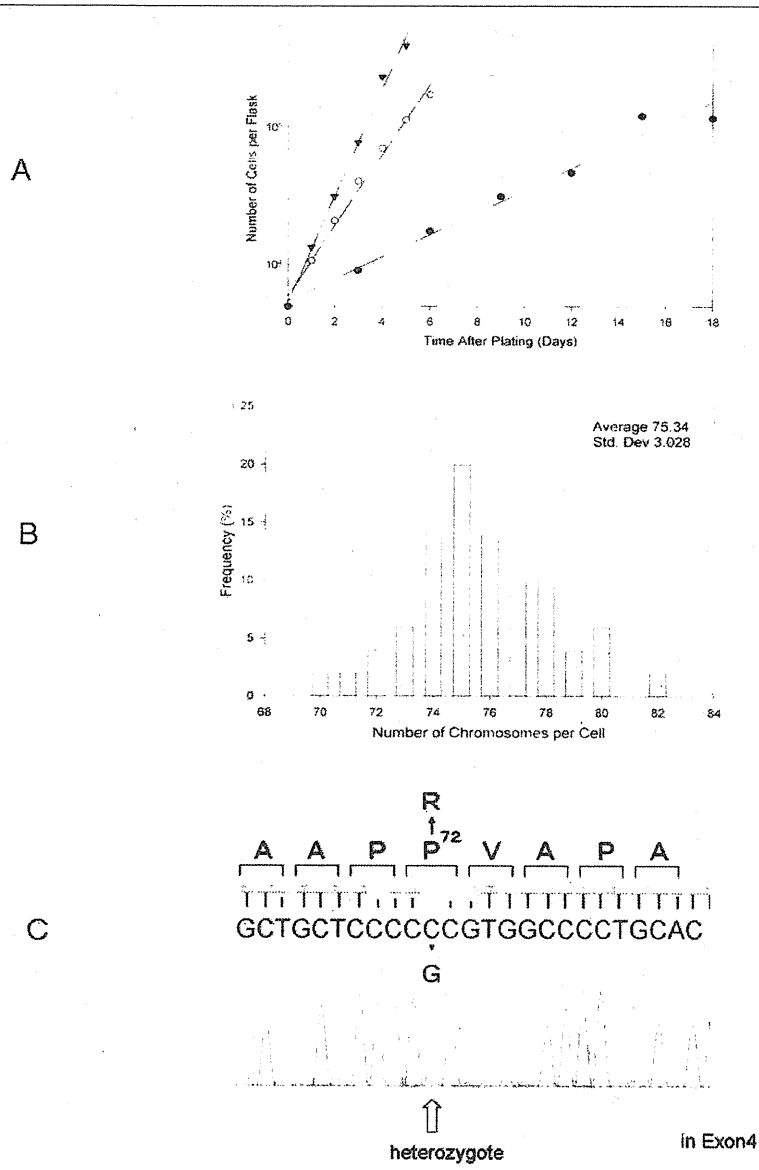

Figure 1 Characters of cell lines. A) Growth curves for three cell lines. Black reverse triangle indicates HeLa cells, white circle indicates U87-MG glioma cells, and black circle presents chordoma origin U-CH1-N cells. B) Chromosome number of chordoma origin U-CH1-N cells. 50 metaphase chromosomes were scored to obtain average chromosome number. C) The substitution C at 412 (NM_000546) to 'G.' was detected in the 4th exon of the p53 gene in U-CH1-N cells. Note that 'C' from wild-type allele is also detected. Deduced amino acid sequence is indicated at the top, where the mutation deduces Proline at 72 to Arginine.

Table 1 Cell cycle distribution and DNA contents of the three cell lines

Cell line	G1-phase	S-phase	G2/M-phase	DNA content*
HeLa	52.10%	30.30%	17.60%	~90
U87-MG	64.70%	23.00%	12.30%	~60
U-CH1-N	75.00%	13.30%	11.70%	~100

Data were obtained by flow cytometry analysis. *Arbitrary unit, a standard DNA content in normal diploid CHO cells (2n, G1-phase) is equal to 50.

(Figure 4). Calculated RBE values from D_{10} were plotted against LET (Figure 5). The RBE values increased up to LET near 200 keV/μm and decreased afterwards. The maximum RBE was approximately 3.86 at LET 200 keV/μm of iron beam.

Sensitivity to Genotoxic Chemical Agents

Figure 6 shows the survival curves of the four chemical agents. Although camptothecin, mitomycin C and

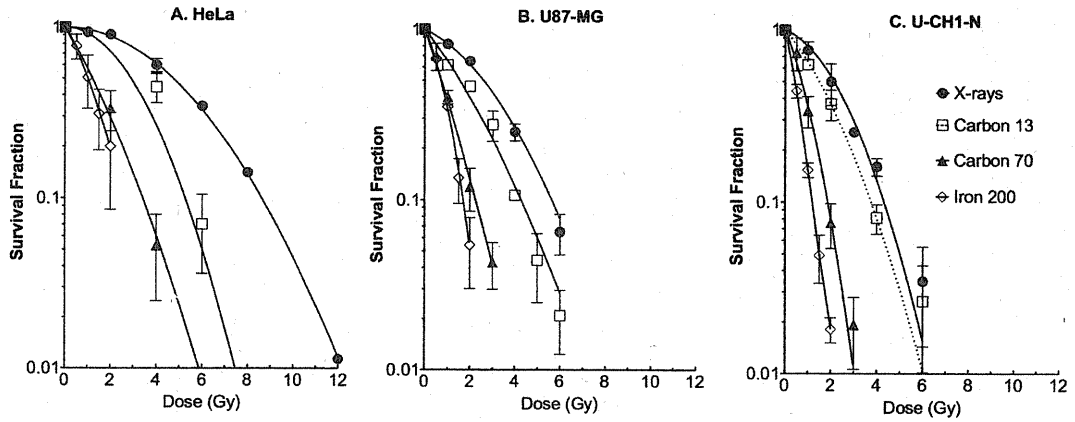

Figure 2 Survival fraction after ionizing radiation exposure. Cells were irradiated to X-rays or heavy ions having different LET. Black circle indicates X-rays, white square indicates Carbon LET 13 keV/μm, black triangle indicates Carbon LET 70 keV/μm, and white diamond indicates Iron LET 200 keV/μm. Error bars indicate standard errors of the mean of three independent experiments.

cisplatin did not reveal strong cytotoxic effects for the particular cell lines under the treatment condition (1 hour, 37°C) we used, bleocin showed a distinct cell inactivation effect for U-CH1-N cells. This trend was also observed in U87-MG cells to less extent, but HeLa cells showed a very resistant phenotype to bleocin.

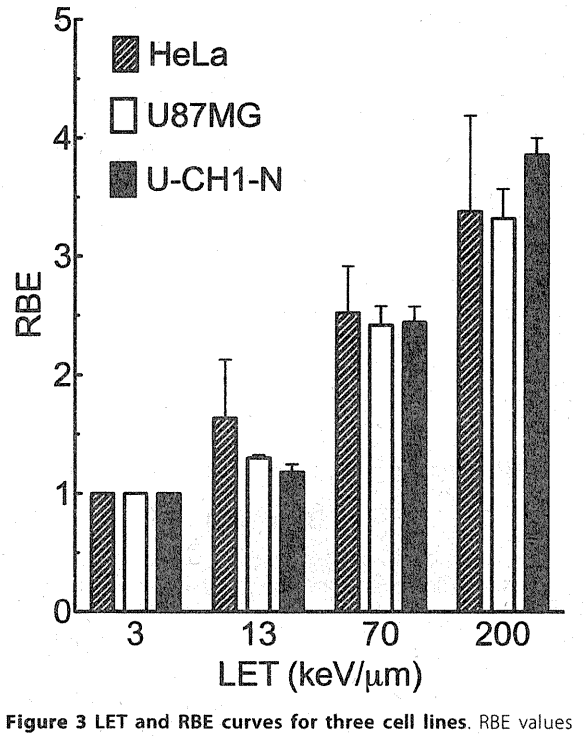

Figure 3 LET and RBE curves for three cell lines. RBE values were calculated from dose to get 10% survival fractions. LET 3 indicate 3 keV/μm for X-rays, 13 and 70 indicate 13 keV/μm or 70 keV/μm for carbon ions, and 200 indicates 200 keV/μm for iron-ions. Error bars indicate standard errors.

Discussion

Chordoma is a rare tumor and information on its cellular radiobiology as well as chemotoxicity is still lacking. This study revealed that the chordoma cell line U-CH1-N *in vitro* cell culture condition was within the normal radiosensitivity range. We also examined the sensitivity to four different therapeutic agents. The higher sensitivity to ionizing radiation and bleocin, may suggest that chordoma cells are a good target for agents producing DNA double strand breaks. The results with other chemicals (cisplatin, mitomycin C, and camptothecin) indicate that chordoma cells are likely to have a normal repair mechanism other than the repair system needed for DNA double strand breaks. In general, p53 mutation confers a potential to change cellular radiosensitivity, increasing resistance due to reduced apoptosis induction by the inactivated p53 pathway [24-27]. HeLa cells have p53 mutation [28], while U87-MG cells have wild-type p53 [29] and show non-resistant phenotype. Judging from the cell survival data, we suspected that U-CH1-N cell line may have wild-type p53. We sequenced the p53 gene from parental U-CH1 and subpopulation U-CH1-N, and found that both cell lines retain a wild-type allele of p53 gene, although our sequence result exhibited a heterozygous mutation C > G, causing an amino acid substitution of proline 72 to arginine (Figure 1). Since the substitution has not been reported to confer any dominant negative effects of the gene [30], we estimated that this mutation hardly affect cellular radiosensitivity from cell cycle checkpoint or apoptosis induction [31].

U-CH1-N had slow growing and poor plating efficiency compared with other two tumor cells. In spite of

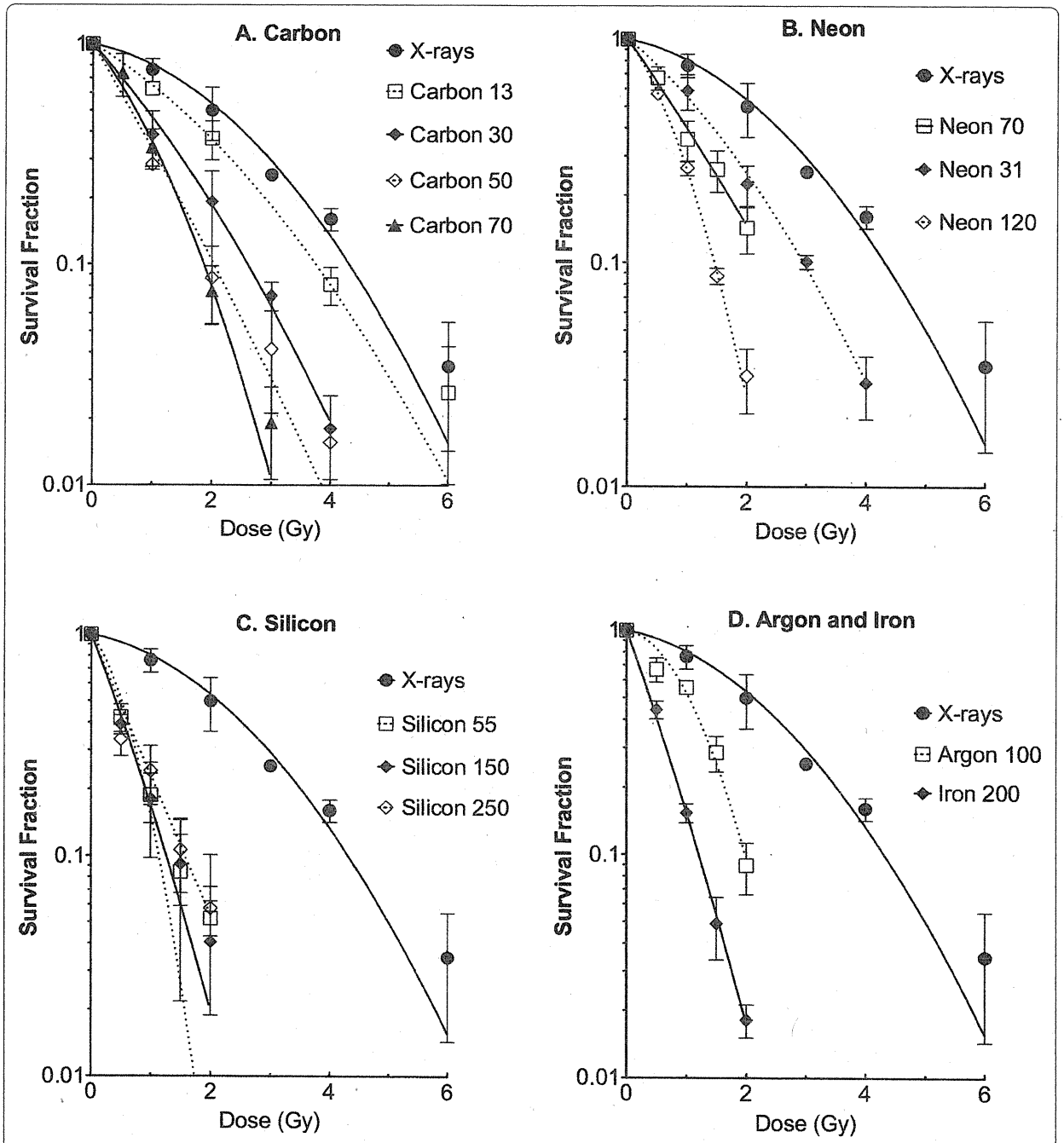

Figure 4 Survival fraction of U-CH1-N cells after high LET heavy-ions. A) X-rays and carbon-ions (LET 13, 30, 50, and 70 keV/μm), B) X-rays and neon-ions (LET 13, 70, and 120 keV/μm), C) X-rays and silicon-ions (LET 55, 150, and 250 keV/μm), and D) X-rays, argon-ions (LET 100 keV/μm), and iron-ions (LET 200 keV/μm). Error bars indicate standard error of the means from three or four independent experiments.

these problems, we were still able to evaluate the radiosensitivity of U-CH1-N. The majority of colonies without irradiation contained more than 200 cells (more than 8 doublings), and the most of the colonies from irradiated cells contained more than 100 cells (more than 7 doublings) for U-CH1-N. Small colonies with 10-20 cells (less than 4 doublings) observed after irradiation were eliminated from survivors. If chordoma cells in general would have normal radiosensitivity as observed in U-CH1-N, the regular photon radiation therapy may

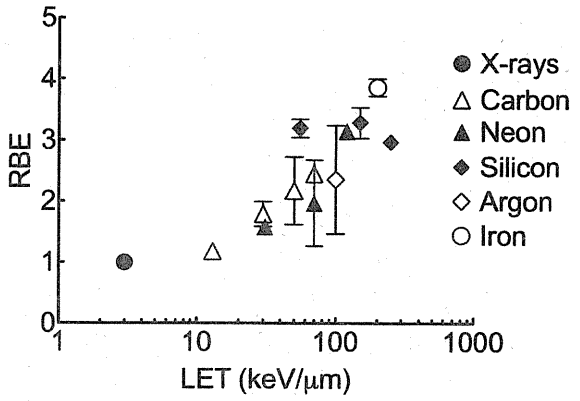

Figure 5 Assembled LET and RBE relationship for U-CH1-N cells. RBE values were calculated from 10% survival points. Error bars indicate standard error. Black circle indicates X-rays, white triangle; carbon-ions, black triangle; neon-ions, black diamond; silicon ions, white diamond; argon-ions, and white circle; iron-ions.

control chordoma easily, although the location and the size could be a problem. However, the recurrence seems to be a big problem for chordoma patients after conventional radiotherapy [2,5].

It is possible that the poor tumor control associated with chordoma may be due to hypoxic effects and/or cancer stem cells which are resistant to ionizing radiation and chemical agents in *in vivo* tumor environment [32,33]. Chordoma tumors tend to be very large when they are diagnosed because of unnoticeable symptoms during the early stage. It is reasonable to consider that chordoma tumors contain a large fraction of hypoxic area. Recently, a PET (positron emission tomography) study revealed a substantial volume of chordoma is hypoxic [34]. Hypoxic regions within tumors are known to be radioresistant [35-37]. The clinical use of heavy charged particles with a spread out Bragg peak (SOBP) containing LET higher than 50 keV/μm could

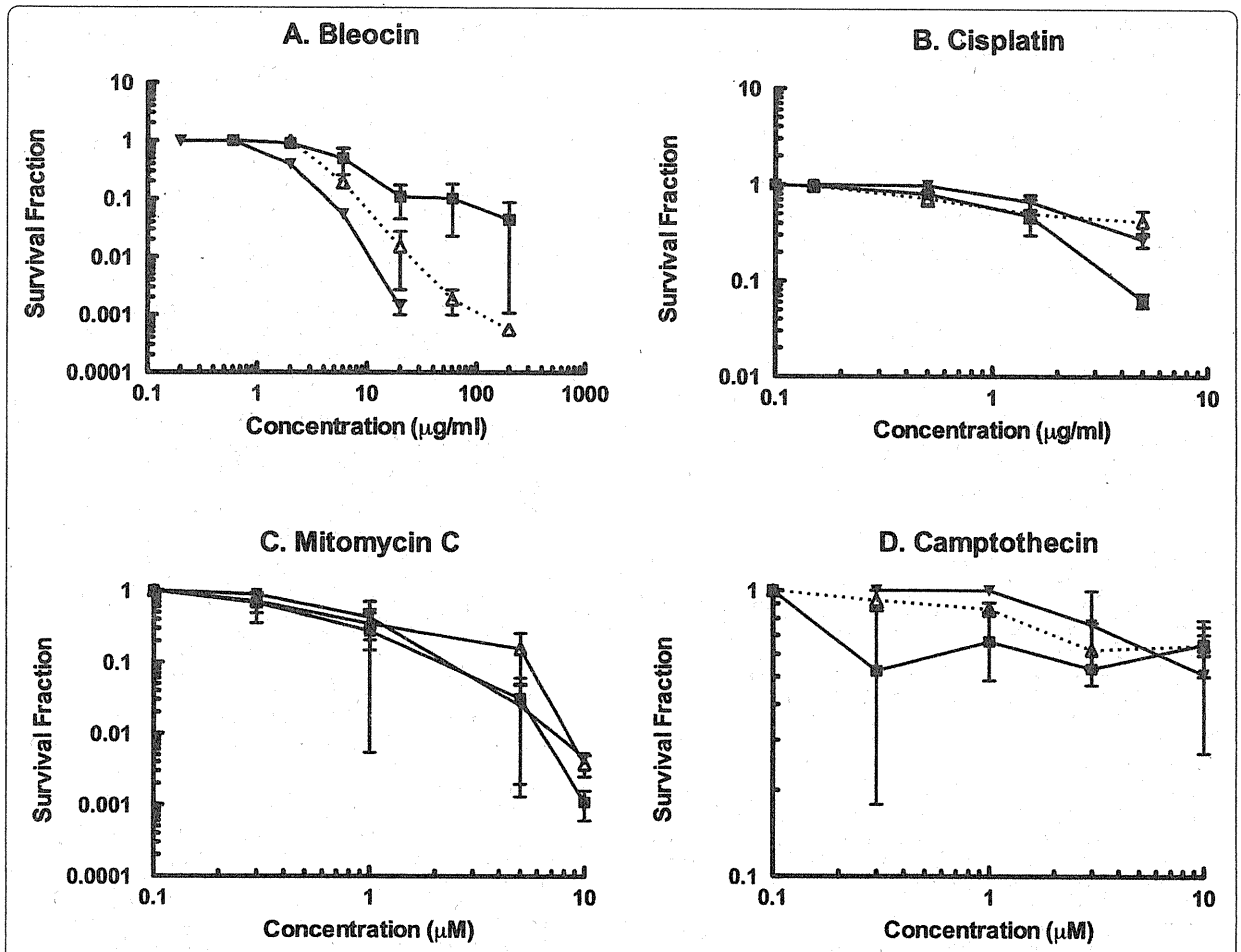

Figure 6 Survival fraction after exposure to various genotoxic agents. A) bleocin, B) cisplatin, C) mitomycin C, and D) camptothecin. Black square represents HeLa cells, white triangle indicates U87-MG cells, and black reverse triangle indicates U-CH1-N chordoma cell line. Cells were exposed to drugs at 37° for 1 hour. 2-4 independent experiments were carried out.

overcome the hypoxic tumor fraction [21]. In general, at low LET irradiation such as X-rays or gamma-rays, the Oxygen Enhancement Ratio (OER) is between 2.5 to 3. As the LET increases, the OER falls slowly until the LET exceeds about 60 keV/μm, after which the OER decreases rapidly and reaches unity by the time the LET reaches to about 200 keV/μm [38]. High LET exposure could overcome low oxygen concentrations which give radio-resistance in tumor populations, and thus this kind of radiation can control tumors with a better efficiency, but increasing LET means also high RBE to normal tissue [39]. Therefore, with high RBE for tumor control and the reduced OER, chordoma becomes a very attractive target for heavy charged particle therapy. The successful treatment of chordoma by carbon ions at our institute may be attributed to such characteristics even SOBP carbon ions are not as high RBE or low OER as monoenergetic high LET carbon beam [23,40].

Conclusion

This study has comprehensively characterized the first validated chordoma cell line, U-CH1. Our next step will be to test more cell lines to verify our results; in vivo xenograft model with U-CH1-N should also be considered in the near future. Nonetheless, this is the first report presenting the extensive *in vitro* cellular studies including radiation and chemical cell survival/toxicity curves with the cell line originating from chordoma.

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Authors' contributions

TAK and AT performed most of the experiments and analyzed the data. MU helped in experimental design. AF performed p53 sequencing experiment in Figure 1 and helped prepare the manuscript. TK and HT provided help in experimental design and preparation of the manuscript. TAK and RO oversaw all the experiments and prepared the manuscript.

Declaration of competing interests

The authors declare that they have no competing interests.

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References

1. Azzarelli A, Quagliuolo V, Cerasoli S, Zucali R, Bignami P, Mazzaferro V, Dossena G, Gennari L: **Chordoma: natural history and treatment results in 33 cases.** *J Surg Oncol* 1988, **37(3)**:185-191.
2. Sundaresan N, Galicich JH, Chu FC, Huvos AG: **Spinal chordomas.** *J Neurosurg* 1979, **50(3)**:312-319.
3. Catton C, O'Sullivan B, Bell R, Laperriere N, Cummings B, Fornasier V, Wunder J: **Chordoma: long-term follow-up after radical photon irradiation.** *RadiotherOncol* 1996, **41(1)**:67-72.
4. Cheng EY, Ozerdemoglu RA, Transfeldt EE, Thompson RC Jr: **Lumbosacral chordoma. Prognostic factors and treatment.** *Spine (Phila Pa 1976)* 1999, **24(16)**:1639-1645.
5. Combs SE, Ellerbrock M, Haberer T, Habermehl D, Hoess A, Jakel O, Jensen A, Klemm S, Munter M, Naumann J, Nikoghosyan A, Oertel S, Parodi K, Rieken S, Debus J: **Heidelberg Ion Therapy Center (HIT): Initial clinical experience in the first 80 patients.** *Acta Oncol* 2010, **49(7)**:1132-1140.
6. Fagundes MA, Hug EB, Liebsch NJ, Daly W, Efird J, Munzenrider JE: **Radiation therapy for chordomas of the base of skull and cervical spine: patterns of failure and outcome after relapse.** *Int J Radiat Oncol Biol Phys* 1995, **33(3)**:579-584.
7. Henderson FC, McCool K, Seigle J, Jean W, Harter W, Gagnon GJ: **Treatment of chordomas with CyberKnife: georgetown university experience and treatment recommendations.** *Neurosurgery* 2009, **64(2 Suppl)**:A44-A53.
8. Imai R, Kamada T, Tsuji H, Sugawara S, Serizawa I, Tsujii H, Tatezaki S: **Effect of carbon ion radiotherapy for sacral chordoma: results of Phase I-II and Phase II clinical trials.** *Int J Radiat Oncol Biol Phys* 2010, **77(5)**:1470-1476.
9. Imai R, Kamada T, Tsuji H, Yanagi T, Baba M, Miyamoto T, Kato S, Kandatsu S, Mizoe JE, Tsujii H, Tatezaki S: **Carbon ion radiotherapy for unresectable sacral chordomas.** *Clin Cancer Res* 2004, **10(17)**:5741-5746.
10. Schoenthaler R, Castro JR, Petti PL, Baken-Brown K, Phillips TL: **Charged particle irradiation of sacral chordomas.** *Int J Radiat Oncol Biol Phys* 1993, **26(2)**:291-298.
11. Schulz-Ertner D, Nikoghosyan A, Didinger B, Karger CP, Jakel O, Wannenmacher M, Debus J: **Treatment planning intercomparison for spinal chordomas using intensity-modulated photon radiation therapy (IMRT) and carbon ions.** *Phys Med Biol* 2003, **48(16)**:2617-2631.
12. Schulz-Ertner D, Nikoghosyan A, Thilmann C, Haberer T, Jakel O, Karger C, Scholz M, Kraft G, Wannenmacher M, Debus J: **Carbon ion radiotherapy for chordomas and low-grade chondrosarcomas of the skull base. Results in 67 patients.** *Strahlenther Onkol* 2003, **179(9)**:598-605.
13. Sciubba DM, Chi JH, Rhines LD, Gokaslan ZL: **Chordoma of the spinal column.** *Neurosurg Clin N Am* 2008, **19(1)**:5-15.
14. Chugh R, Dunn R, Zalupski MM, Biermann JS, Sondak VK, Mace JR, Leu KM, Chandler WF, Baker LH: **Phase II study of 9-nitro-camptothecin in patients with advanced chordoma or soft tissue sarcoma.** *JClinOncol* 2005, **23(15)**:3597-3604.
15. Hof H, Welzel T, Debus J: **Effectiveness of cetuximab/gefitinib in the therapy of a sacral chordoma.** *Onkologie* 2006, **29(12)**:572-574.
16. Presneau N, Shalaby A, Idowu B, Gikas P, Cannon SR, Gout I, Diss T, Tirabosco R, Flanagan AM: **Potential therapeutic targets for chordoma: PI3K/AKT/TSC1/TSC2/mTOR pathway.** *Br J Cancer* 2009, **100(9)**:1406-1414.
17. Rhomberg W, Bohler FK, Novak H, Dertinger S, Breitfellner G: **A small prospective study of chordomas treated with radiotherapy and razoxane.** *Strahlen ther Onkol* 2003, **179(4)**:249-253.
18. Scheil S, Bruderlein S, Liehr T, Starke H, Herms J, Schulte M, Moller P: **Genome-wide analysis of sixteen chordomas by comparative genomic hybridization and cytogenetics of the first human chordoma cell line, U-CH1.** *Genes Chromosomes Cancer* 2001, **32(3)**:203-211.
19. Kanai T, Endo M, Minohara S, Miyahara N, Koyama-ito H, Tomura H, Matsufuji N, Futami Y, Fukumura A, Hiraoka T, Furusawa Y, Ando K, Suzuki M, Soga F, Kawachi K: **Biophysical characteristics of HIMAC clinical irradiation system for heavy-ion radiation therapy.** *Int J Radiat Oncol Biol Phys* 1999, **44(1)**:201-210.
20. Kanai T, Furusawa Y, Fukutsu K, Itsukaichi H, Eguchi-Kasai K, Ohara H: **Irradiation of mixed beam and design of spread-out Bragg peak for heavy-ion radiotherapy.** *Radiat Res* 1997, **147(1)**:78-85.
21. Kanai T, Matsufuji N, Miyamoto T, Mizoe J, Kamada T, Tsuji H, Kato H, Baba M, Tsujii H: **Examination of GyE system for HIMAC carbon therapy.** *Int J Radiat Oncol Biol Phys* 2006, **64(2)**:650-656.

22. Matsufuji N, Fukumura A, Komori M, Kanai T, Kohno T: **Influence of fragment reaction of relativistic heavy charged particles on heavy-ion radiotherapy.** *Phys Med Biol* 2003, **48(11)**:1605-1623.
23. Matsufuji N, Kanai T, Kanematsu N, Miyamoto T, Baba M, Kamada T, Kato H, Yamada S, Mizoe JE, Tsujii H: **Specification of Carbon Ion Dose at the National Institute of Radiological Sciences (NIRS).** *J Radiat Res(Tokyo)* 2007, **48(Suppl A)**:A81-A86.
24. Canman CE, Chen CY, Lee MH, Kastan MB: **DNA damage responses: p53 induction, cell cycle perturbations, and apoptosis.** *Cold Spring Harb Symp Quant Biol* 1994, **59**:277-286.
25. Canman CE, Kastan MB: **Role of p53 in apoptosis.** *Adv Pharmacol* 1997, **41**:429-460.
26. Canman CE, Wolff AC, Chen CY, Fornace AJ Jr, Kastan MB: **The p53-dependent G1 cell cycle checkpoint pathway and ataxia-telangiectasia.** *Cancer Res* 1994, **54(19)**:5054-5058.
27. Gueven N, Becherel OJ, Birrell G, Chen P, DelSal G, Carney JP, Grattan-Smith P, Lavin MF: **Defective p53 response and apoptosis associated with an ataxia-telangiectasia-like phenotype.** *Cancer Res* 2006, **66(6)**:2907-2912.
28. Crawford LV, Pim DC, Gurney EG, Goodfellow P, Taylor-Papadimitriou J: **Detection of a common feature in several human tumor cell lines–a 53,000-dalton protein.** *Proc Natl Acad Sci USA* 1981, **78(1)**:41-45.
29. Kondo S, Barna BP, Kondo Y, Tanaka Y, Casey G, Liu J, Morimura T, Kaakaji R, Peterson JW, Werbel B, Barnett GH: **WAF1/CIP1 increases the susceptibility of p53 non-functional malignant glioma cells to cisplatin-induced apoptosis.** *Oncogene* 1996, **13(6)**:1279-1285.
30. Willis A, Jung EJ, Wakefield T, Chen X: **Mutant p53 exerts a dominant negative effect by preventing wild-type p53 from binding to the promoter of its target genes.** *Oncogene* 2004, **23(13)**:2330-2338.
31. Naka T, Boltze C, Kuester D, Schulz TO, Schneider-Stock R, Kellner A, Samii A, Herold C, Ostertag H, Roessner A: **Alterations of G1-S checkpoint in chordoma: the prognostic impact of p53 overexpression.** *Cancer* 2005, **104(6)**:1255-1263.
32. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN: **Glioma stem cells promote radioresistance by preferential activation of the DNA damage response.** *Nature* 2006, **444(7120)**:756-760.
33. Kruuv J, Sinclair WK: **X-ray sensitivity of synchronized Chinese hamster cells irradiated during hypoxia.** *Radiat Res* 1968, **36(1)**:45-54.
34. Park SA, Kim HS: **F-18 FDG PET/CT evaluation of sacrococcygeal chordoma.** *Clin Nucl Med* 2008, **33(12)**:906-908.
35. Brurberg KG, Skogmo HK, Graff BA, Olsen DR, Rofstad EK: **Fluctuations in pO2 in poorly and well-oxygenated spontaneous canine tumors before and during fractionated radiation therapy.** *Radiother Oncol* 2005, **77(2)**:220-226.
36. Cooper RA, Carrington BM, Loncaster JA, Todd SM, Davidson SE, Logue JP, Luthra AD, Jones AP, Stratford I, Hunter RD, West CM: **Tumour oxygenation levels correlate with dynamic contrast-enhanced magnetic resonance imaging parameters in carcinoma of the cervix.** *Radiother Oncol* 2000, **57(1)**:53-59.
37. Nordsmark M, Bentzen SM, Rudat V, Brizel D, Lartigau E, Stadler P, Becker A, Adam M, Molls M, Dunst J, Terris DJ, Overgaard J: **Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study.** *Radiother Oncol* 2005, **77(1)**:18-24.
38. Davis MA, Little JB: **RBE and OER of the heavy ionizing particles released during 10beta-neutron capture therapy.** *Rev Latino am Microbiol Parasitol (Mex)* 1968, **10(2)**:207.
39. Bassler N, Jakel O, Sondergaard CS, Petersen JB: **Dose- and LET-painting with particle therapy.** *Acta Oncol* 2010, **49(7)**:1170-1176.
40. Tsujii H, Mizoe J, Kamada T, Baba M, Tsuji H, Kato H, Kato S, Yamada S, Yasuda S, Ohno T, Yanagi T, Imai R, Kagei K, Hara R, Hasegawa A, Nakajima M, Sugane N, Tamaki N, Takagi R, Kandatsu S, Yoshikawa K, Kishimoto R, Miyamoto T: **Clinical Results of Carbon Ion Radiotherapy at NIRS.** *J Radiat Res(Tokyo)* 2007, **48(Suppl A)**:A1-A13.

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CLINICAL INVESTIGATION

CARBON ION RADIATION THERAPY IMPROVES THE PROGNOSIS OF UNRESECTABLE ADULT BONE AND SOFT-TISSUE SARCOMA OF THE HEAD AND NECK

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Purpose: To evaluate the safety and efficacy of carbon ion radiotherapy (C-ion RT) with 70.4 GyE for unresectable bone and soft-tissue sarcoma of the adult head and neck.

Methods and Materials: Twenty-seven patients (mean age, 46.2 years) were enrolled in this prospective study on C-ion RT with 70.4 GyE/16 fractions (fr) between April 2001 and February 2008. The primary end points were acute and late reactions of normal tissues, local control rate, and overall survival rate. The secondary end point was efficacy of the treatment in comparison to historical results with 57.6 or 64.0 GyE/16 fr.

Results: The 3-year local control rate and overall survival rate for all patients were 91.8% (95% confidence interval [CI] = 81.0–100%) and 74.1% (95% CI = 57.5–90.6%), respectively. Acute reaction of Grade 3 or more was observed in only 1 patient. With regard to late reactions, visual loss was observed in 1 patient and a Grade 3 reaction of the maxillary bone was observed in 4 patients. A comparison with historical results revealed that the local control rate with 70.4 GyE was significantly higher than that with 57.6 or 64.0 GyE (3-year, 91.8% vs. 23.6%, $p < 0.0001$). Furthermore, the overall survival with 70.4 GyE tended to be higher than that with 57.6 or 64.0 GyE (3-year, 74.1% vs. 42.9%, $p = 0.09$).

Conclusion: C-ion RT with 70.4 GyE/16 fr for bone and soft-tissue sarcoma of the adult head and neck appears to be effective with acceptable toxicities in comparison to conventional RT and C-ion RT with lower doses. © **2010 Elsevier Inc.**

Sarcoma, Head-and-neck cancer, Carbon ion radiation therapy, Osteosarcoma, Particle radiation therapy.

INTRODUCTION

Bone and soft-tissue sarcomas of the head and neck are mesenchymal malignant neoplasms accounting for less than 10% of all bone and soft-tissue sarcomas and only about 1% of all head-and-neck neoplasms (1–5). There are many histologic subtypes of sarcomas, which present with a variety of clinical characteristics. Depending on the subtype and characteristics of the individual tumor, treatment may require a combination of surgery, radiation therapy, and chemotherapy. Given their complexity, these head-and-neck sarcomas are best treated in sarcoma units where an expert multidisciplinary approach to management is available.

Conventional radiation therapy improves local tumor control and in selected cases brings about a complete cure with acceptable adverse effects (6–10). However, the prognosis for local control and survival for patients with unresectable sarcomas is still poor (11, 12). Furthermore, patients with sarcomas originating in the head-and-neck region have the lowest overall survival rates (13).

Carbon ion radiation therapy (C-ion RT) was initiated at the National Institute of Radiological Sciences in 1994 (14). Carbon ions exhibit high linear energy transfer and

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show good dose-localizing properties in comparison to other ion species (15, 16). The physical characteristics of carbon ions offer the theoretical benefit of more localized delivery of a radiation dose and greater relative biological effectiveness (RBE) than that of photons. The improved dose distribution can potentially be exploited either by allowing higher radiation doses to the tumor with less radiation-induced normal tissue toxicity or by reducing adverse effects at equivalent effective doses. Either of these two approaches is appealing, particularly when a critical host structure is in close proximity to the tumor. Decreasing doses to normal tissues is always important, especially in the head and neck, because the organs at risk are usually close to the target volume in this region.

Previously, a prospective study on C-ion RT for head-and-neck malignant tumors employing 57.6 or 64 Gray equivalent (GyE) in 16 fractions (fr) was conducted between April 1997 and March 2001 (17). Fourteen patients with sarcoma were enrolled in the previous study, and it was shown that the 3-year local control rate with 57.6 or 64.0 GyE (<30%) was significantly lower than that of other histologic tumors. Therefore, for further improving the local control of sarcoma, it was decided to increase the total dose, and 70.4 GyE/16 fr was selected as the prescribed dose in the subsequent study.

In this article, the results of a prospective clinical study on C-ion RT using 70.4 GyE/16 fr for unresectable bone and soft-tissue sarcoma in the adult head and neck are described. The results are also compared with those obtained in the previous study for 14 patients treated by C-ion RT with 57.6 or 64.0 GyE/16 fr.

METHODS AND MATERIALS

A total of 27 patients were enrolled in the present prospective clinical study between April 2001 and February 2008.

Eligibility criteria and ethics

The treatment protocol for the present study was reviewed and approved by the National Institute of Radiological Sciences Ethical Committee on Human Clinical Research and all patients signed an informed consent form. The eligibility criteria were: 1) histologically confirmed sarcoma, 2) tumor deemed to be medically inoperable by the referring surgeons or declined surgery, 3) age between 18 and 79 years, 4) Karnofsky performance status score of ≥60, 5) N0M0 status, 6) tumor being grossly measurable, 7) no chemotherapy having been performed within the past 2 weeks, and 8) no serious medical or psychological conditions precluding safe administration of treatment.

Carbon ion irradiation technique

Doses of carbon ions were expressed in photon equivalent doses (GyE), which were defined as the physical doses multiplied by the RBE of the carbon ions. The biological flatness of the spread-out Bragg peak was normalized by the survival fraction of human salivary gland tumor cells at the distal region of the spread-out Bragg peak, where the RBE of carbon ions was assumed to be 3.0 (16).

The patients were positioned in customized cradles (Moldcare; Alcare, Tokyo, Japan) and immobilized with a low-temperature thermoplastic shell (Shellfitter; Kuraray, Osaka, Japan). A set of 2.5-mm-thick computed tomography (CT) images was taken for treatment planning with the immobilization devices. CT imaging alone is inadequate for detection of extension of the tumor. Therefore, magnetic resonance imaging (MRI) was routinely used for identification of the tumor, after fusing it with the planning CT. Determination of gross target volume (GTV) was based on contrast-enhanced MRI. The clinical target volume (CTV) had minimum margins of 5.0 mm added around the GTV. The CTV and organs at risk (*e.g.*, eyeball wall, optic nerve, optic chiasma, brain stem) were outlined on the planning CT images to permit dose-volume histogram analysis. Three-dimensional treatment planning was performed using HIPLAN software (18). The planning target volume had margins of 3.0–5.0 mm added around the CTV. A typical dose distribution is shown in Figure 1.

Irradiation was carried out once per day, 4 days per week (Tuesday–Friday) in C-ion RT. The prescribed total dose to the center of the CTV was 70.4 GyE in 16 fr over 4 weeks at a fraction size of 4.4 GyE.

Follow-up

The patients were followed up by CT or MRI every 1-2 months for the first 6 months after C-ion RT and every 3-6 months thereafter. The overall survival and local control rates were calculated from the first day of C-ion RT.

End points

The primary end points of the present study were to estimate the acute and late reactions of normal tissues, the local control rate, and the overall survival rate of adult patients with bone and soft-tissue sarcoma. Acute reactions of normal tissues were classified according to the National Cancer Institute-Common Toxicity Criteria scoring system (version 2.0), by which the maximum reactions within 3 months after initiation of C-ion RT were scored. Late reactions were classified according to the Radiation Therapy Oncology Group/

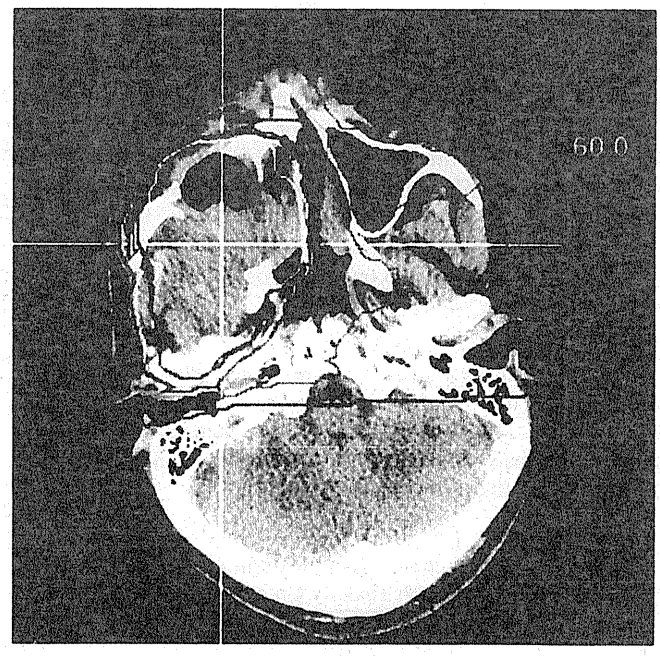

Fig. 1. Dose distribution on the computed tomography image for treatment planning. Isodose lines indicating 95%, 90%, 70%, 60%, 50%, 30%, and 10% dose areas (from inside to outside).