Radiotherapy for Localized Hormone-refractory Prostate Cancer in Japan

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Abstract. *Background: The role of radiotherapy for patients with localized hormone-refractory cancer has not been well documented. Materials and Methods: The Patterns of Care Study in Japan examined the records of 311 patients with prostate cancer treated with radiotherapy during the period 1996-1998. Of them, 61 patients (19.6%) with regionally localized hormone-refractory cancer were selected. Local progression or biochemical failure was observed after a median duration of 15.9 months of androgen deprivation. At the time of radiotherapy, 49 patients (80.4%) had T3-4 tumors and 15 (26.8%) had regional lymph node metastases. External beam radiotherapy was performed with a median total dose of 60 Gy. Results: Although distant metastases or regional lymph node metastases were seen in 22 patients (36.1%), local progression was observed in one patient (1.6%). The five-year overall and progression-free survival rates were 51.6% and 43.5%, respectively. Conclusion: Radiotherapy had an excellent local control rate for hormone-refractory cancer.*

Androgen ablation alone for prostate cancer is only palliative; it may be used as the initial treatment for patients with prostate cancer because of a short life expectancy, advanced stage, or patient preference. While most patients will have remission by androgen ablation, such remissions do not last and eventually hormone-refractory disease will develop. This clinical situation is frequently encountered in Japan, because most Japanese patients with prostate cancer have high-risk disease and hormonal therapy is frequently preferred as the primary treatment (1, 2).

Local progression is one of the most common types of disease progression. It has been reported that node-positive prostate cancer treated with androgen ablation alone had actuarial local progression rates at 5 and 8 years of 32% and 51%, respectively (3). Radiotherapy may be used to treat local progression with curative intent or to release urinary obstructive symptoms as a palliative treatment (4-8). However, little information exists on the efficacy of radiotherapy in the management of hormone refractory prostate cancer. In particular, the role of radiotherapy for patients with regionally localized hormone-refractory cancer has not been well documented.

The Patterns of Care Study (PCS), a widely known quality assurance program in the United States (9), was conducted in Japan (10), in an attempt to obtain data on the national standards of radiotherapy for several diseases, including prostate cancer. A total of 311 prostate cancer patients were surveyed (2), which included 61 patients (19.6%) who received radiotherapy after clinical or biological progression on hormonal therapy.

The purpose of the current study was to examine the patterns of treatment and clinical course of hormone-refractory prostate cancer patients treated with radiotherapy in Japan and to investigate whether radiotherapy is a reasonable treatment option.

Materials and Methods

The methods used in data collection for the PCS have been previously described (2, 11). From a stratified Facilities Master list, a random sample of radiotherapy facilities was selected. Each of the 50 randomly chosen facilities was visited and a total of up to

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Key Words: Radiotherapy, hormone-refractory prostate cancer, Patterns of Care Study.

Table I. *Patients and disease characteristics at the time of radiation therapy.*

No. of patients	61
Age	
Median	73 years
Range	55-86 years
Differentiation	
Well	6 (9.8%)
Moderate	33 (54.1%)
Poor	19 (31.1%)
Unknown	3 (4.9%)
T-Stage/1997 UICC	
T1	0 (0%)
T2	9 (14.8%)
T3	27 (44.3%)
T4	22 (36.1%)
Unknown	3 (4.9%)
N-Stage/1997 UICC	
N0	45 (73.8%)
N1	15 (24.6%)
Unknown	1 (1.6%)
Pretreatment PSA level	
Median	36.8 ng/ml
Range	1.5-211 ng/ml

PSA, prostate-specific antigen; UICC, International Union Against Cancer

Table II. *Treatment characteristics.*

Hormonal therapy	
Content	
Orchiectomy	21 (34.4%)
Estrogen agent	12 (19.7%)
LH-RH agonist	44 (72.1%)
Antiandrogen	37 (60.7%)
Chemotherapy	
Yes	20 (32.8%)
No	39 (63.9%)
Unknown	2 (3.3%)
Radiotherapy	
Equipment	
Cobalt 60	1 (1.6%)
Linear accelerator	57 (93.4%)
Microtron	2 (3.3%)
Unknown	1 (1.6%)
Beam energy (MV)	
Cobalt 60	1 (1.6%)
<10	13 (21.3%)
10-18	39 (63.9%)
>18	6 (9.8%)
Unknown	2 (3.3%)
Technique	
AP/PA only	21 (34.4%)
4 fields	18 (29.5%)
Moving beam	18 (29.5%)
Others/Unknown	4 (6.6%)

LH-RH, luteinizing hormone-releasing hormone; AP/PA, anterior-posterior.

20 medical records from each institute were randomly selected and reviewed. The following eligibility criteria were used in the process survey. The patients were required to have adenocarcinoma of the prostate without evidence of distant metastases; they must have been treated with radiotherapy between January 1996 and December 1998; the patients must not have been diagnosed with any other malignancy nor have been previously treated with radiotherapy. The total number of prostate cancer patients surveyed was 311, which included patients who had a radical prostatectomy or hormonal therapy prior to radiotherapy.

In this paper, we report the patterns of treatment and outcome of 61 patients with hormone-refractory cancer. All these patients received androgen ablation alone initially, followed by radiotherapy for local or biological progression in the absence of distant metastasis.

Patients were categorized as having progression after radiotherapy if they developed local, pelvic nodal, or distant failure. For living patients, the median follow-up was 17 months (range 1-70 months).

Statistical analyses were performed using the Statistical Analysis System at the PCS statistical center (12). The overall survival rate and the progression-free survival rate were calculated from the first day of radiotherapy using the Kaplan-Meier method. Log-rank statistics were used to identify significant prognostic factors for survival. Cox's proportional hazard model was used in multivariate analysis. The Radiotherapy Oncology Group (RTOG) late toxicity scales were used to assess the late morbidity.

Results

Patient and disease characteristics. Patient characteristics are shown in Table I. Only 9.8% of patients had well-differentiated adenocarcinoma. Biopsy Gleason scores were: <7 in 4 patients (6.6%), =7 in 4 patients (6.6%), >7 in 3 patients (5.0%) and not available in 50 patients (82.0%). More than 80% of the patients had T3 or T4 tumors at the time of radiotherapy. Fifteen patients (24.6%) were diagnosed as having regional lymph node involvement by computed tomography. No pathologic confirmation of nodal metastasis was obtained by pelvic lymph node dissection or needle biopsy. The median PSA level before radiotherapy was 36.8 ng/ml.

Treatment. The method of androgen ablation is shown in Table II. Luteinizing hormone-releasing agonists and antiandrogen agents were frequently used, but estrogen agents were given in 19.7% of patients. Orchidectomy was performed in 34.4% of patients. The median duration of androgen ablation before radiotherapy was 16.8 months (range 1-105.1 months). Twenty patients (32.8%) were also treated with chemotherapy including estramustine.

Regarding radiotherapy, a linear accelerator of ≥ 10 MV was used in 73.7% of patients. The radiotherapy technique varied, with less than 30% of men treated with AP/PA-only

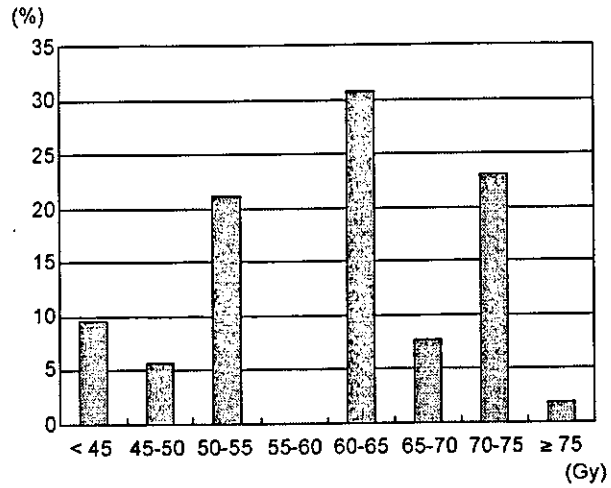

Figure 1. *Distribution of radiation doses delivered to the prostate.*

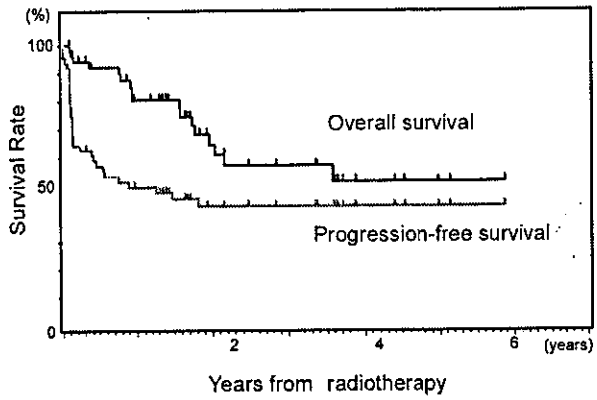

Figure 2. *Overall and progression-free survival curves for patients with hormone-refractory prostate cancer.*

portals. The distributions of the doses delivered are shown in Figure 1. The median dose delivered to the prostate was 60.0 Gy. Nineteen patients (31.1%) received low doses (less than 55.0 Gy) as palliative treatment. The median dose per fraction was 2.0 Gy (range 1.5-3.0 Gy).

Twenty-two patients (36.1%) received treatment to the pelvic lymph nodes to the level of L5 or S1, 20 (32.8%) received treatment to the pelvic lymph nodes to the level lower than S1, and 19 (31.1%) received irradiation only to the prostate.

Outcome. The overall and progression-free survival rates were 51.6% and 43.5% at five years, respectively (Figure 2). Fifteen patients died of prostate cancer and one died of intercurrent diseases. Clinically, there were one local and distant, 2 regional, 3 regional and distant, and 17 distant failures after completion of radiotherapy. Local progression was observed in only one patient (1.6%), who was treated with a dose of 40 Gy to the prostate. Regional lymph node metastases or distant metastases were seen in 22 patients (36.1%). Four patients had a consecutive rising PSA profile without clinical progression after radiotherapy.

The prognostic factors found to be associated with improved overall survival by univariate analysis were T2-3 tumors (p=0.001), N-0 disease (p=0.001) and dose ≥ 60 Gy (p=0.004). However, multivariate analysis showed that T stage (p=0.001), N stage (p=0.016), total dose (p=0.026), and pelvic irradiation (p=0.028) were significant prognostic factors (Table III).

Late morbidity of RTOG Grade 1-2 was seen in 8 patients (13.1%). There were no cases of Grade 3-4 toxicity. Late rectal toxicity was seen in 5 patients and late urinary toxicity was seen in 3 patients.

Discussion

In patients with prostate cancer treated with androgen ablation, disease progression mainly occurs in the prostate, regional and distant lymph nodes and bone. Some patients have progression only in the primary lesion as the first site of failure (3) and such patients may be referred for radiotherapy. However, reports concerning radiotherapy for hormone refractory prostate cancer are limited (4-8). In Japan, a considerable number of patients have been treated with androgen ablation alone (1, 13), although androgen ablation is only palliative. The PCS study in Japan, which surveyed a total of 311 prostate cancer patients without metastasis, revealed that 61 patients (19.6%) received radiotherapy because of hormone-refractory disease. Therefore, it is beneficial to examine the role of radiotherapy for hormone refractory prostate cancer.

The dose-response in patients who receive irradiation for hormone refractory prostate cancer has not been established clearly. Kraus found that doses of 4000 to 5000 rads provided satisfactory palliation with less morbidity in 33 patients with locally invasive prostate cancer including hormone-refractory disease (4). Kawakami *et al.* reported that palliative doses of 27-38 Gy to treat symptomatic hormone-refractory progression in 10 patients were sufficient to control local disease and these effects lasted for more than 11 months (7). In the present study, although 19 patients (31.1%) received < 55 Gy, local control was excellent. A radiation dose of 30-50 Gy appears to be effective for just suppressing progression of the tumor or releasing urinary obstructive symptoms. On the other hand, higher doses with curative intent may be used for patients who have a fairly prolonged survival. Furuya *et al.* treated 11 patients with local progression by external irradiation at a dose of 50-66.6 Gy (5). Seven out of 11 patients died because of distant metastases and none suffered

Table III. *Univariate and multivariate analysis of prognostic factors.*

Prognostic factor	n*	3-y overall survival(%)	Univariate *P*-value	Multivariate *P*-value	risk ratio
Age (<70 *vs.* ≥ 70 y)	14/44	51.6/64.5	0.965	0.523	0.678
Pre-RT PSA (<20 *vs.* ≥20 ng/mL)	42/16	54.0/80.4	0.173	0.704	0.764
Differentiation (well/moderate *vs.* poor)	40/18	60.7/61.9	0.911	0.130	0.360
T stage (T2-3 *vs.* T4)	36/22	77.8/34.4	0.001	0.001	8.166
N stage (N0 *vs.* N1)	45/13	72.6/16.9	0.001	0.016	3.920
Total dose (<60 *vs.* ≥60 Gy)	25/33	37.8/75.4	0.004	0.026	0.240
Pelvis irradiation (yes *vs.* no)	22/36	76.7/52.4	0.052	0.028	5.064
Chemotherapy (yes *vs.* no)	20/38	34.5/70.3	0.060	0.670	1.259

*Because some data were missing, the total numbers of patients may be less than the actual numbers.
Abbreviations: RT = radiotherapy; PSA = prostate-specific antigen.

from local progression. Lankford *et al.* examined 29 patients with hormone-refractory prostate cancer treated with radiotherapy and showed that the 3-year local control rate after irradiation of > 60 Gy was 90%, compared to only 29% for those receiving ≤ 60 Gy (6). They recommended an aggressive approach to palliation which is justified by the maintenance of freedom from local symptoms. In the present study, radiation dose was one of the prognostic factors that affected survival. For those who have a long life expectancy, a radiation dose ≥ 60 Gy should be considered.

A patient with hormone refractory prostate cancer has a poor prognosis, even if the prostate cancer is localized regionally. The most common pattern of failure in patients treated with radiotherapy is distant metastasis. In the present study, 22 patients (36.1%) showed regional or distant metastases and the 5-year overall survival rate was 51.6%. Lankford *et al.* demonstated that there were 6 local and 14 regional or distant failures following loco-regional radiotherapy in 29 patients with localized hormone refractory prostate cancer and the 4-year survival rate was 39% (6). Analysis of 29 patients with prostate-confined hormone-refractory cancer by Sanguineti *et al.* demonstrated that 4 patients (13.8%) developed local progression after radiotherapy with a median dose of 70 Gy, while distant or regional metastases were seen in 16 patients (55.2%) (8). The overall survival at three years was 46.1%. Despite its ability to suppress the disease, radiotherapy has a limited capacity to prolong survival. Other adjuvant treatments combined with radiotherapy may have a central role in the treatment strategy in the future.

Pelvic nodal irradiation may be considered in patients at high risk of pelvic nodal metastases (14). Because patients with hormone-refractory cancer have a high risk of lymph node involvement, it seems likely that pelvic irradiation would be beneficial. In this study, pelvic irradiation was associated with an improved survival in multivariate analysis but not in univariate analysis. Taking into account the high rate of distant failure, the necessity for pelvic irradiation is still controversial.

To our knowledge, this is the largest series of regionally localized hormone-refractory prostate cancer patients treated with radiotherapy. From previously published papers and our data, the conclusions to be derived may be as follows: (1) Radiotherapy has a good local control rate for hormone-refractory prostate cancer; (2) the prognosis of these patients is poor, in particular, due to distant metastasis. Fundamentally, androgen ablation is only palliative. Therefore, even if a patient with hormone refractory prostate cancer has a localized disease, radiotherapy should be palliative. However, there may be some exceptions. Stage D1 disease treated with androgen ablation initially may recur locally (3). During neoajuvant hormonal therapy, PSA levels may increase before scheduled irradiation starts. In these cases, radiotherapy with curative intent must be a reasonable treatment approach.

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References

1 Kumamoto Y, Tsukamoto T, Umehara T, Shimazaki J, Fuse H, Oshima H, Takeuchi H, Yoshida O, Okada K, Saito Y, Kanetake H, Harada M and Tamiya T: Clinical studies on endocrine therapy for prostatic carcinoma (2): prognosis of patients with prostatic carcinoma given endocrine therapy, and analyses on causes of death and side effect of endocrine therapy. Acta Urol Jpn *36*: 285-293, 1990 (in Japanese).

2 Nakamura K, Teshima T, Takahashi Y, Imai A, Koizumi M, Mitsuhashi N, Inoue T and Japanese PCS Working Subgroup of Prostate Cancer: Radical radiation therapy for prostate cancer in Japan: A Patterns of Care Study Report. Jpn J Clin Oncol *33*: 122-126, 2003.

3 Zagars GK, Sands ME, Pollack A and von Eschenbach AC: Early androgen ablation for stage D1 (N1-3, M0) prostate cancer: prognostic variables and outcome. J Urol *151*: 1330-1333, 1994.
4 Kraus PA, Lytton B, Weiss RM and Prosnitz LR: Radiation therapy for local palliative treatment of prostatic cancer. J Urol *108*: 612-614, 1972.
5 Furuya Y, Akakura K, Akimoto S, Ichikawa T and Ito H: Radiotherapy for local progression in patients with hormone-refractory prostate cancer. Int J Urol *6*: 187-191, 1999.
6 Lankford SP, Pollack A and Zagars GK: Radiotherapy for regionally localized hormone refractory prostate cancer. Int J Radiat Oncol Biol Phys *33*: 907-912, 1995.
7 Kawakami S, Kawai T, Yonese J, Yamauchi T, Ishibashi K and Ueda T: Palliative radiotherapy for local progression for hormone refractory stage D2 prostate cancer. Jpn J Urol *84*: 1681-1684, 1993 (in Japanese).
8 Sanguineti G, Marcenaro M, Franzone P, Orsatti M, Foppiano F and Vitale V: Radiotherapy for prostate-confined hormone refractory prostate cancer (Abstr.). Int J Radiat Oncol Biol Phys *51(Suppl. 1)*: 280, 2001.
9 Hanks GE, Coia LR and Cury J: Patterns of Care Studies –past, present, and future-. Semin Radiat Oncol 7: 97-100, 1997.
10 Teshima T: Patterns of care study in radiation oncology –history, methodology, and important results in the United States. J Jpn Soc Ther Radiol Oncol *11*: 247-254, 1999(in Japanese).
11 Teshima T, Abe M, Ikeda H, Hanks GE, Owen JB, Hiraoka M, Hirokawa Y, Oguchi M, Nishio M, Yamashita T, Niibe H, Masuda K, Watanabe S and Inoue T: Patterns of Care Study of radiation therapy for esophageal cancer in Japan: Influence of the stratification of institution on the process. Jpn J Clin Oncol *28*: 308-313, 1998.
12 SAS Procedure Reference, version 6. 1st ed. Tokyo, SAS Institute in Japan. 1995.
13 Tanaka M, Murakami S, Suzuki N, Oikawa T, Kinsui H, Hamano S and Shimazaki J: Trends of prostate cancer: comparison between 1986-90 and 1991-95 at Asahi General Hospital. Hinyokika Kiyo *44*: 775-780, 1998(in Japanese).
14 Roach M, Lu JD, Lawton C, Hsu IC, Machtay M, Seider MJ, Rotman M, Jones C, Asbell SO, Valicenti R, Han S, Thomas Jr CR and Shipley WS: A phase III trial comparing whole-pelvic to prostate only radiotherapy and neoadjuvant to adjuvant total androgen suppression: preliminary analysis of RTOG 9413(Abstr.) Int J Radiat Oncol Biol Phys *51(Suppl. 1)*: 3, 2001.

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Radical External Beam Radiotherapy for Prostate Cancer in Japan: Preliminary Results of the 1999–2001 Patterns of Care Process Survey

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Background: A Patterns of Care Study (PCS) has been conducted to evaluate the standards of practice for prostate cancer patients treated with radiotherapy in Japan. This study examines the influence of institutional stratification on the process of care for patients receiving radical external beam radiotherapy for prostate cancer in the 1999–2001 PCS in Japan. These PCS results were compared with those of the 1999 PCS in the USA.

Methods: A national survey of 36 institutions was conducted using two-stage cluster sampling and detailed information was accumulated on 305 clinically localized prostate cancer patients who received radiotherapy between 1999 and 2001. Of these, 181 patients treated with radical external beam radiotherapy were selected and the preliminary results were analyzed. Institutions were classified as A1 (academic institutions treating ≥430 patients a year) or B1 (non-academic institutions treating ≥130 patients a year).

Results: In both A1 and B1 institutions, more than 80% of the patients had intermediate or unfavorable risk diseases. There were no significant differences in the patients' disease characteristics between A1 and B1 institutions, while the institutional stratification significantly affected the patterns of radiotherapy; such as the beam energy (≥10 MV, A1 89.9%, B1 72.2%; $P = 0.0022$), the use of a CT simulator (A1 91.0%, B1 80.0%; $P = 0.0340$) and the administration of conformal therapy (A1 85.0%, B1 20.5%; $P < 0.0001$). The median number of full-time equivalent (FTE) radiation oncologists was 2.7 in A1 institutions and only 0.7 in B1 institutions. Median radiation doses of 66.00 Gy (A1 institutions) and 69.00 Gy (B1 institutions) were delivered and hormonal therapy was commonly used before, during and after radiotherapy, with a mean duration of 1.3 years (88.0% in A1 institutions; 90.0% in B1 institutions). In comparing the results of PCS in Japan (1999–2001) with those in the USA (1999), patients in Japan were found to have more advanced primary diseases with higher PSA levels than those in the USA. The median prescribed dose to the primary tumor was not significantly different between the two countries (69.00 Gy in Japan and 70.45 Gy in the USA). Conversely, almost half of the patients in the USA were treated with higher prescription dose levels (≥72 Gy), whereas only 9.4% of the Japanese patients received these dose levels. Hormonal therapy was used more frequently in Japan (88.1% of the patients) than in the USA (50% of the patients). Most of the Japanese patients with a favorable prognosis (72.0%) were treated with hormonal therapy, compared with 30% in the USA. On the other hand, most of the patients in the unfavorable risk group were treated with radiotherapy in conjunction with hormonal therapy both in Japan (91.1%) and the USA (81%).

Conclusions: During the period 1999–2001, the majority of the prostate cancer patients treated in Japan with radical external beam radiotherapy had advanced diseases and institutional stratification significantly affected the patterns of radiotherapy. In both academic and non-academic institutions, radiotherapy in conjunction with long-term hormonal therapy was commonly used. In comparison with the 1999 PCS in the USA, Japanese patients had more advanced diseases, but the higher prescribed doses (≥72 Gy) were less common in Japan. Administration rates of hormonal therapy for favorable risk patients were different between Japan and the USA. On the other hand, for unfavorable risk patients, radiotherapy in conjunction with hormonal therapy appeared to be an accepted approach both in Japan and in the USA.

Key words: patterns of care study – prostatic carcinoma – type of institution – radiation therapy – hormone therapy

INTRODUCTION

The Patterns of Care Study (PCS) national survey is a retrospective study designed to establish national practice processes for selected malignancies over a specific time period (1–3). In

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addition to documenting practice process, the PCS is important in developing and spreading national guidelines for cancer treatment. This helps to promote a more uniform care process in the country. The PCS is also designed to complement clinical trials that enhance the standard of care for cancer patients (1,4).

To improve the quality of radiation oncology nationwide in Japan, the PCS has been imported from the USA and the first Japanese version of a PCS for esophageal and uterine cervical cancer has been functioning since July 1996 (5–7). In September 1998, the Japanese PCS started a nationwide survey of patients with breast, lung, esophageal and uterine cervical cancer who were treated between 1995 and 1997 (8–10). One year later, the Japanese PCS began the first nationwide process survey of prostate cancer patients who underwent radiotherapy between 1996 and 1998. This involved 162 prostate cancer patients who were treated by radical external beam radiotherapy between 1996 and 1998. The results revealed that there were significant proportions of high-risk diseases in the patient group and that hormonal therapy was prescribed frequently in Japan (11).

Since entering the PSA era, it is possible to detect earlier stages of prostate cancer and there is a better chance of successfully treating early-stage patients with prostate cancer than ever before. Moreover, the use of radiotherapy to treat prostate cancer recently has become much more common, because a significant amount of new radiation treatment planning technology and methodology has become available. Therefore, the optimal management of radiotherapy for prostate cancer patients has been a major concern in Japan. However, we have not been able to evaluate properly the updated national practice processes of radiotherapy for prostate cancer in Japan owing to the limited information available. In July 2002, PCS audits for prostate cancer patients treated between 1999 and 2001 were started in Japan. The preliminary results of this PCS were evaluated in May 2003 and detailed information regarding 181 patients who underwent radical external beam radiotherapy has already been collected, even though the 1999–2001 PCS survey is still ongoing. Therefore, we analyzed these preliminary results of radical external beam radiotherapy for clinically localized prostate cancer, focusing especially on the influence of institutional stratification on the process of care and also comparing the PCS results with the 1999 PCS reported in the USA (12).

SUBJECTS AND METHODS

The preliminary results of the 1999–2001 PCS concerning radiotherapy for Japanese prostate cancer patients were evaluated. The PCS involved an extramural audit survey of 36 institutions using stratified two-stage cluster sampling and collected specific information on 305 patients with prostate cancer who were treated with radiotherapy between 1999 and 2001. The Japanese PCS developed an original data format in collaboration with the American College of Radiology (ACR, Philadelphia, PA). The following eligibility criteria were used in this survey: the patients were required to have adenocarcinoma of the prostate without evidence of distant metastasis; they must have been treated with radiotherapy between 1999 and 2001; and they must not have been diagnosed with any other malignancy or have been previously treated with radiotherapy. The PCS surveyors consisted of 20 radiation oncologists from academic institutions. For each institution surveyed, one radiation oncologist visited and surveyed data by reviewing patients' charts. In order to validate the quality of the collected data, the PCS utilized an Internet mailing list including all the surveyors. In-site real time checks and adjustments of the data input were available to each surveyor to the PCS committee (13).

On the basis of the Japanese facility master list of 1999 (14), the 1999–2001 PCS stratified the institutions as follows: A1, academic institutions treating ≥430 patients a year; A2, <430 patients; B1, non-academic institutions treating ≥130 patients a year; and B2, <130 patients. The 1999–2001 PCS was scheduled to collect the data for 80 institutions, including A1, A2, B1 and B2 institutions, with two-stage cluster sampling. However, at the time of analysis, the PCS had collected the data on 36 institutions, including only those with A1 and B1 stratifications. Therefore, in the current study, we analyzed the data concerning the A1 and the B1 institutions. Among the 305 patients surveyed in the current PCS, 181 patients who were treated with radical external beam radiotherapy were selected for analysis and preliminary results for these patients were reported. Patients who had received prior prostatectomy and patients with hormone-refractory prostate cancer were excluded from this analysis.

With regard to the risk groups for prostate cancer, the 1999 PCS in the USA categorized patients into the following risk groups: favorable – absence of adverse features (PSA <10, Gleason score <6 and T stage <3); the presence of one or more of these features classified patients into the intermediate and unfavorable groups, respectively (12). In the current study, because 35% (64 of 176 patients) of the data regarding the Gleason combined score were missing, we used the tumor differentiation instead of the Gleason combined score as a factor to evaluate the risk group. Therefore, Japanese patients were categorized into the following risk groups: favorable – absence of adverse features (PSA <10, not poorly differentiated and T stage <3); the presence of one or more of these features classified patients into the intermediate and unfavorable groups, respectively.

Statistical analyses were performed using the Statistical Analysis System at the PCS statistical center (15). Statistical significance was tested using the chi-squared test and Student's *t*-test. A probability level of 0.05 was chosen for statistical significance.

RESULTS

Patient and Disease Characteristics

Patient and disease characteristics were separated according to the stratified institutions, as shown in Table 1. There were no significant differences in the disease characteristics, such as pretreatment PSA level, tumor differentiation, Gleason combined score and T stage. In both A1 and B1 institutions, >80% of the patients had intermediate or unfavorable risk diseases. The main reasons given for selection of radiotherapy were patient preference, advanced or high-risk disease, medical contraindication and old age.

Treatment Characteristics

Treatment characteristics of the stratified institutions are shown in Table 2. Institutional stratification was closely related to the infrastructure of radiation oncology such as equipment and personnel and significantly affected the patterns of radiotherapy, such as beam energy (≥10 MV, A1 89.9%, B1 72.2%; $P = 0.0022$), the use of a CT simulator (A1 91.0%, B1 80.0%; $P = 0.0340$) and the administration of conformal therapy (A1 85.0%, B1 20.5%; $P < 0.0001$). Median radiation doses of 66.00 Gy (A1 institutions) and 69.00 Gy (B1 institutions) were delivered and the proportion of patients who received total doses of <60 Gy was 2.8% (A1 4.0%, B1 1.3%). Pelvic irradiation was performed in 29.7% of the patients in the A1 institutions and 53.8% in the B1 institutions ($P = 0.0011$). The median number of full-time equivalent (FTE) radiation oncologists was 2.7 in A1 institutions and only 0.7 in B1 institutions.

Hormonal therapy was commonly used before, during and after radiotherapy with a mean duration of 1.3 ± 1.0 years (88.0% in A1 institutions; 90.0% in B1 institutions). Luteinizing hormone-releasing hormone (LH-RH) agonist and antiandrogen were frequently used as hormonal agents. In contrast, chemotherapy in general was not administered in both institutions (9.0% in A1 institutions; 4.2% in B1 institutions).

Comparison Between the Results in Japan and Those in the USA

Comparisons of PCS results between Japan (1999–2001) and the USA (1999) (12) are shown in Table 3. Patients in Japan were found to have more advanced primary diseases with higher PSA levels than those in the USA. In Japan, the percentage of patients with favorable, intermediate and unfavorable tumors were 15.2, 37.0 and 47.9%, respectively, compared with 40, 39 and 21%, respectively, in the USA. Conformal radiotherapy was administered to 56.7% of the patients in Japan and 85% in the USA. The median prescribed dose was not significantly different between the two countries (Japan 69.00 Gy, USA 70.45 Gy). In contrast, almost half of the patients in the USA (48%) were treated with higher prescription dose levels (≥72 Gy), while only 9.4% of the Japanese patients received these dose levels. With regard to hormonal therapy, 88.1% of the patients in Japan and 50% in the USA were treated with hormonal therapy. In Japan, the percentages of patients with favorable, intermediate and unfavorable tumors treated in conjunction with hormonal therapy were 72.0, 91.8 and 91.1%, respectively, compared with 30, 54 and 81, respectively, in the USA (Fig. 1). For the favorable risk group, most of the patients (72.0%) in Japan were treated with hormonal therapy, whereas only 30% of these patients received hormonal therapy in the USA. On the other hand, for the unfavorable risk group, >80% of the patients were treated with radiotherapy in conjunction with hormonal therapy both in Japan (91.1%) and in the USA (81%).

DISCUSSION

The 1999–2001 PCS revealed that in Japan, more than 80% of the patients treated with radical external beam radiotherapy had intermediate or unfavorable risk diseases and institutional stratification did not significantly affect the disease characteristics, such as pretreatment PSA levels, Gleason combined score and T stage. Conversely, the current study has demonstrated significant differences in the practice process of radiotherapy for prostate cancer, according to the stratification of the institutions. Significant differences were found in the beam energy, the use of a CT simulator and the administration of conformal therapy. These differences in process indicate that the quality of radiotherapy in academic institutions was significantly higher than that in non-academic institutions. Results of a process survey carried out to evaluate treatment of cancers at different disease sites, such as cancer of the esophagus and uterine cervix, have also been reported and significant differences in the patterns of process were found according to the stratification of the institutions (6,7). The processes in the non-academic institutions in Japan were closely related to structural immaturity, especially in terms of equipment and personnel. In the non-academic institutions, CT simulators and conformal therapy were used for only 80.0 and 20.5% of the patients, respectively, compared with 91.0 and 85.0%, respectively, in the academic institutions. Moreover, in these non-academic institutions, less than one full-time equivalent radiation oncologist has been managing many of these patients (6,7). This PCS survey revealed that the median number of FTE radiation oncologists was 2.7 in A institutions, but only 0.7 in B institutions. Therefore, in order to provide good-quality radiotherapy, facilities need appropriate treatment planning capabilities. Modern radiotherapy requires a CT simulator or conformal therapy in order to improve the target dose distribution, while concomitantly reducing the normal tissue dose (16). Moreover, the number of patients treated with radiotherapy has increased in every institutional stratification, with an overall increase of 1.4-fold over the past 10 years (17). Therefore, the number of FTE radiation oncologists on duty must be increased, especially in non-academic institutions in Japan.

This study indicates that radiotherapy in conjunction with long-term hormonal therapy was almost routinely (88.1% of the patients surveyed) administered to Japanese patients who

Table 1. Patients' and disease characteristics

	Stratification of institutions		Significance (*P*)
	A (*n* = 101)	B (*n* = 80)	
Age (years)			
Median (min.–max.)	72.8 (58.6–90.6)	72.4 (49.7–98.6)	
Mean ± SD	72.5 ± 5.7	73.9 ± 10.4	0.2508
KPS (%)			
Median (min.–max.)	90 (80–100)	90 (70–100)	
Mean	90.2 ± 5.0	89 ± 8.4	0.2383
Missing	5	0	
Pretreatment PSA level (ng/ml, %)			
Median (min.–max.)	18.6 (3.2–324.0)	23.0 (1.9–517.5)	
Mean ± SD	39.1 ± 57.2	53.5 ± 80.3	0.1701
<4	2/98 (2.0%)	1/75 (1.3%)	
4–<10	32/98 (32.7%)	16/75 (21.3%)	
10–<20	16/98 (16.3%)	17/75 (22.7%)	0.3551
≥20	48/98 (49.0%)	41/75 (54.7%)	
Missing	3	5	
Differentiation			
Well	23/99 (23.2%)	20/75 (26.7%)	
Moderate	36/99 (36.4%)	27/75 (36.0%)	0.341
Poor	33/99 (33.3%)	27/75 (36.0%)	
Unknown	7/99 (7.1%)	1/75 (1.3%)	
Missing	2	5	
Gleason combined score (%)			
2–6	32/70 (45.7%)	16/47 (34.0%)	
7	13/70 (18.6%)	13/47 (27.7%)	0.3624
8–10	25/70 (35.7%)	18/47 (38.3%)	
Missing	31	33	
Clinical T stage			
TX	5/98 (5.1%)	2/75 (2.7%)	
T0	0/98 (0.0%)	0/75 (0.0%)	
T1	8/98 (8.2%)	4/75 (5.3%)	
T2	35/98 (35.7%)	39/75 (52.0%)	0.2587
T3	44/98 (44.9%)	26/75 (34.7%)	
T4	2/98 (2.0%)	3/75 (4.0%)	
Unknown	4/98 (4.1%)	1/75 (1.3%)	
Missing	3	5	
Clinical N stage			
NX	1/98 (1.0%)	0/73 (0.0%)	
N0	91/98 (92.9%)	69/73 (94.5%)	
N1	4/98 (4.1%)	3/73 (4.1%)	0.8339
Unknown	2/98 (2.0%)	1/73 (1.4%)	
Missing	3	7	
Stage/JUA 2001 (%)			
A	2/100 (2.0%)	2/78 (2.6%)	
B	46/100 (46.0%)	42/78 (53.9%)	
C	49/100 (49.0%)	31/78 (39.7%)	0.5997
D	3/100 (3.0%)	2/78 (2.6%)	
Other (C–D1)	0/100 (0.0%)	1/78 (1.3%)	
Missing	1	2	
Risk group* (%)			
Favorable	17/96 (17.7%)	8/69 (11.6%)	
Intermediate	34/96 (35.4%)	27/69 (39.1%)	0.5520
Unfavorable	45/96 (46.9%)	34/69 (49.3%)	
Missing	5	11	
Reason for selection of radiotherapy			
Patient preference	33/96 (34.3%)	23/78 (29.5%)	
Advanced or high-risk disease	25/96 (26.0%)	24/78 (30.8%)	
Medical contraindication	10/96 (16.7%)	13/78 (16.7%)	0.0079
Old age	16/96 (16.7%)	12/78 (15.4%)	
Others	1/96 (1.0%)	6/78 (7.7%)	
N/A or unknown	11/96 (11.5%)	0/78 (0.0%)	
Missing	5	2	

KPS = Karnofsky performance status; PSA = prostate-specific antigen; JUA = Japan Urological Association.
*Favorable = meet all conditions below; Intermediate = meet 2 conditions; Unfavorable = meet only 1 or no conditions: (1) PSA <10; (2) not poorly differentiated; (3) T stage <3.

Table 2. Treatment characteristics

	Stratification of institutions		Significance (*P*)
	A (*n* = 101)	B (*n* = 80)	
Radiotherapy			
Energy ≥10 MV (%)			
Yes	89/99 (89.9%)	57/79 (72.2%)	0.0022
Missing	2	1	
Were portal films or electric portal images used (%)			
Yes	86/98 (87.8%)	51/80 (63.8%)	0.0002
Missing	3	0	
All field treated each day (%)			
Yes	80/101 (79.2%)	45/80 (56.3%)	0.0009
CT simulator (%)			
Yes	91/100 (91.0%)	64/80 (80.0%)	0.0340
Missing	1	0	
Conformal therapy (%)			
Yes	85/100 (85.0%)	16/78 (20.5%)	<0.0001
Missing	1	2	
Pelvic irradiation (%)			
Yes	30/101 (29.7%)	43/80 (53.8%)	0.0011
Radiation dose (cGy)			
Median (min.–max.)	6600 (1400–7600)	6900 (3000–8000)	
Mean ± SD	6686.5 ± 775.0	6738.9 ± 568.6	0.6141
Missing	1	0	
Higher prescription dose levels (≥72 Gy) (%)			
Yes	14/100 (14.0%)	3/80 (3.8%)	0.0195
Missing	1	0	
Hormonal therapy			
Yes	88/100 (88.0%)	72/80 (90.0%)	0.6714
Missing	1	0	
Content (%)			
Orchiectomy	13/87 (14.9%)	11/67 (16.4%)	0.8024
Missing	1	5	
Estrogen agent	3/84 (3.6%)	15/61 (24.6%)	0.0002
Missing	4	11	
LH-RH agonist	68/87 (78.2%)	66/72 (91.7%)	0.0199
Missing	1	0	
Antiandrogen	63/88 (71.6%)	54/71 (76.1%)	0.5255
Missing	0	1	
Period (%)			
Before RT	80/88 (90.9%)	59/71 (83.1%)	0.1398
Missing	0	1	
During RT	73/88 (83.0%)	68/72 (94.4%)	0.0254
Missing	0	0	
After RT	67/88 (76.1%)	64/72 (88.9%)	0.0372
Missing	0	0	
Duration* (days)			
Median (min.–max.)	0.9 (0.1–4.8)	1.7 (0.2–2.8)	
Mean ± SD	1.2 ± 1.2	1.5 ± 0.8	0.2583
Chemotherapy			
Yes	9/100 (9.0%)	3/72 (4.2%)	0.2196
Missing	1	8	

CT = computed tomography; RT = radiotherapy; LH-RH = lutein hormone-releasing hormone.

Table 3. Patient and disease characteristics: comparison of PCS results between Japan and the USA

	Japan 1999–2001	USA 1999*
No. of institutions	36	47
No. of patients	181	320
Age (years)		
Median (min.–max.)	72.5 (49.7–98.6)	72 (49–86)
Mean ± SD	73.1 ± 8.2	
Missing	0	
Pretreatment PSA level (ng/ml)		
Med (min.–max.)	20.2 (1.9–517.5)	–
Mean ± SD	45.3 ± 68.3	
<4	3/173 (1.7%)	11%
4–<10	48/173 (27.8%)	57%
10–<20	33/173 (19.1%)	20%
≥20	89/173 (51.5%)	12%
Missing	8	
Gleason combined score		
2–6	48/117 (41.0%)	53%
7	26/117 (22.2%)	29%
8–10	43/117 (36.8%)	18%
Missing	64	
T stage		
TX	7/173 (4.1%)	6%
T0	0/173 (0.0%)	6%
T1	12/173 (6.9%)	43%
T2	74/173 (42.8%)	34%
T3	70/173 (40.5%)	6%
T4	5/173 (2.9%)	0.30%
Unknown	5/173 (2.9%)	6%
Missing	8	
Risk group (%)		
Favorable	25/165 (15.2%)†	40%‡
Intermediate	61/165 (37.0%)†	39%‡
Unfavorable	79/165 (47.9%)†	21%‡
Missing	16	
Conformal therapy		
Yes	101/178 (56.7%)	85%
Missing	3	
Radiation dose (cGy)		
Median (min.–max.)	6900 (1400–8000)	7045 (2700–7920)
Mean ± SD	6709.8 ± 689.6	–
Missing	1	
Higher prescription dose levels (≥72 Gy)		
Yes	17/180 (9.4%)	48%
Missing	1	
Administration of pelvic irradiation		
Yes	73/181 (40.3%)	28%
Hormonal therapy		
Yes	141/160 (88.1%)	50%

*Ref. 12.
†Favorable = meet all three conditions below; Intermediate = meet 1 condition; Unfavorable = meet only 1 or no conditions: (1) PSA <10, (2) not poorly differentiated; (3) T stage <3. ‡Favorable = meet all 3 conditions below; Intermediate = meet 1 condition; Unfavorable = meet only 1 or no conditions: (1) PSA <10, (2) Gleason score <6, (3) T stage <3.

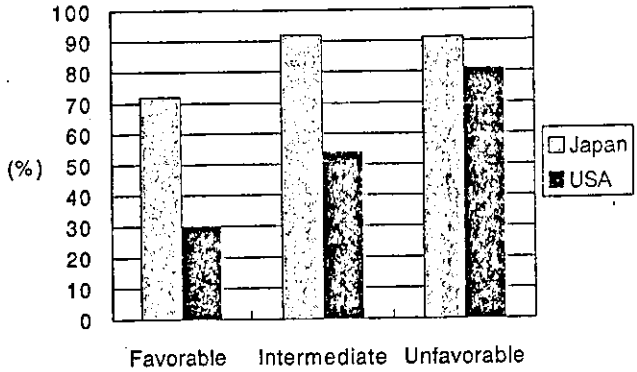

Figure 1. Hormonal therapy distribution according to the risk groups for prostate cancer patients in Japan and in the USA.

were treated between 1999 and 2001. In the 1996–1998 PCS reported by Nakamura et al. (11), the administration rate of hormonal therapy was also high (85.8% of the patients surveyed). These results indicate that the administration rate of hormonal therapy continues to be high during these periods. However, it has been acknowledged that conventional radiotherapy alone has little curative potential in high-risk prostate cancer (18). Taking into account the high percentage of high-risk patients in the current study, the therapeutic strategy of long-term hormonal therapy with radiotherapy may be appropriate for most Japanese patients. However, prolonged hormonal therapy may lead to side effects such as impotence, hot flushes, fatigue and osteoporosis. Investigation into the optimal timing and duration of hormonal therapy should be carried out in the future.

When compared with the PCS results in the USA, patients in Japan had more advanced diseases than did those in the USA. Japanese patients had higher pretreatment PSA levels and an advanced T stage and the incidence of unfavorable risk patients in Japan was 47.9%, compared with 21% in the USA. However, it is not known whether these differences were caused by the differences in access to care or to biological difference in the tumors themselves, between the patients in Japan and in the USA. Further investigation of the different disease characteristics between individuals into the two countries would be worthwhile.

The median radiation dose employed in Japan (69.00 Gy) was not significantly different from that used in the USA (70.45 Gy). However, almost half of the patients in the USA were treated with higher prescription dose levels (≥72 Gy), whereas only 9% of the Japanese patients received these higher doses. Moreover, the proportion of Japanese patients who received total doses of <60 Gy was only 2.8%. These results indicate that most Japanese patients received the irradiation doses with a small range just around 69 Gy. One reason for this may be the lower incidence of conformal therapy in Japan, especially in B1 institutions. Conformal radiotherapy was administered to 85% of the patients in the USA, whereas only 20.5% of the patients in the B1 institutions received this treatment in Japan. Another reason may be the high incidences of hormonal therapy in Japan. At present, many Japanese radia-

tion oncologists may consider the higher dose levels (≥72 Gy) unnecessary for prostate cancer patients when combined with long-term hormonal therapy.

As for the risk groups and the incidences of hormonal therapy, the administration of hormonal therapy for favorable risk patients was found to be different in Japan and the USA. Only 30% of these patients were treated with hormonal therapy in the USA (Fig. 1). Several studies in the USA have indicated that radical radiotherapy alone could control the disease in patients with a favorable risk status. Zietman et al. indicated that total doses of <71 Gy were sufficient to control the disease when the pretreatment PSA level was <10 ng/ml (19). Hanks et al. found that prostate cancer patients with pretreatment PSA <10 ng/ml did not benefit from dose escalation above 70 Gy (20). Therefore, radical external beam radiotherapy without hormonal therapy has been a primary treatment for patients with favorable risk diseases in the USA. On the other hand, 72% of the patients in the favorable risk group were treated with long-term hormonal therapy in Japan (Fig. 1). The high rate of health insurance coverage (21,22) and fewer side effects of estrogen therapy (23,24) for Japanese people may explain the frequent administration of hormonal therapy in Japan. However, hormonal therapy was found to be unnecessary for favorable risk patients in the USA (19,20). Therefore, radical external beam radiotherapy without hormonal therapy should be a treatment of choice for favorable risk patients also in Japan.

On the other hand, the significantly increased use of hormonal therapy for high-risk patients in the USA reflects the penetration and growing acceptance of clinical trial results that have demonstrated the efficacy of these treatment approaches (25). The randomized trial RTOG 8610 (26,27) showed an increase in disease-free survival at 2 years of 76 vs 62% survival for locally advanced prostate cancer patients treated with neoadjuvant total androgen blockade plus radiation vs radiation therapy alone. In Japan, hormonal therapy was administered to >90% of the patients with unfavorable risk diseases. Therefore, for the unfavorable risk group, radiotherapy in conjunction with hormonal therapy appears to be an accepted approach both in Japan and in the USA.

The analysis of these 1999–2001 PCS results delineates the patterns of radiotherapy for prostate cancer patients treated with radical external beam radiotherapy between 1999 and 2001 in Japan. Moreover, we compared the Japanese results with those in the USA and these data will be informative to establish where we stand now and where we should go in the future. However, this report analyzes preliminary results of a survey of only 36 A1 and B1 institutions. In the meantime, the same PCS survey is still collecting more information on prostate cancer patients including A2 and B2 institutions. When we have analyzed the data from the additional institutions, we will report the updated results. The national Practice of Care Standard will be represented more accurately when data from a larger number of prostate cancer patients are included.

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References

1. Hanks GE, Coia LR, Curry J. Patterns of care studies: past, present and future. *Semin Radiat Oncol* 1997;7:97–100.
2. Owen JB, Sedransk J, Pajak TF. National averages for process and outcome in radiation oncology: methodology of the patterns of care study. *Semin Radiat Oncol* 1997;7:101–7.
3. Tanisada K, Teshima T, Ohno Y, Inoue T, Abe M, Ikeda H, et al. Patterns of care study quantitative evaluation of the quality of radiotherapy in Japan. *Cancer* 2002;95:164–71.
4. Inoue T. Quality assurance of radiotherapy and its clinical assessment. *Jpn J Clin Oncol* 2002;32:497–505.
5. Tanisada K, Teshima T, Ikeda H, Abe M, Owen JB, Hanks GE, et al. A preliminary outcome analysis of the patterns of care study in Japan for esophageal cancer patients with special reference to age: non surgery group. *Int J Radiat Oncol Biol Phys* 2000;46:1223–33.
6. Teshima T, Abe M, Ikeda H, Hanks GE, Owen JB, Yamada S, et al. Patterns of care study of radiation therapy for cervix cancer in Japan: the influence of the stratification of institution on the process. *Jpn J Clin Oncol* 1998;28:388–95.
7. Teshima T, Abe M, Ikeda H, Hanks GE, Owen JB, Hiraoka M, et al. Patterns of care study of radiation therapy for esophageal cancer in Japan: influence of the stratification on the process. *Jpn J Clin Oncol* 1998;28: 308–13.
8. Uno T, Sumi M, Ikeda H, Teshima T, Yamashita M, Inoue T. Radiation therapy for small-cell lung cancer: results of the 1995–1997 patterns of care process survey in Japan. *Lung Cancer* 2002;35:279–85 .
9. Uno T, Sumi M, Sawa Y, Teshima T, Hara R, Ikeda H, et al. Process of care and preliminary outcome in limited-stage small-cell lung cancer: results of the 1995–1997 patterns of care study in Japan. *Int J Radiat Oncol Biol Phys* 2003;55:626–32.
10. Gomi K, Oguchi M, Hirokawa Y, Kenjo M, Ogata T, Takashima Y, et al. Process and preliminary outcome of a patterns-of-care study of esophageal cancer in Japan: patterns treated with surgery and radiotherapy. *Int J Radiat Oncol Biol Phys* 2003;56:813–22.
11. Nakamura K, Teshima T, Takahashi Y, Imai A, Koizumi M, Mitsuhashi N, et al. Radical radiotherapy for prostate cancer in Japan: a patterns of care study report. *Jpn J Clin Oncol* 2003;33:122–6.
12. Zelefsky MJ, Moughan J, Owen J, Zietman A, Hanks GE. Changing trends in the national practice for external beam radiotherapy for clinically localized prostate cancer: the 1999 patterns of care survey for prostate cancer. *Int J Radiat Oncol Biol Phys* 2002;54(Suppl):8.
13. Kinoshita K, Teshima T, Ohno Y, Inoue T, Yamashita T, Hiraoka M, et al. Logical checking function increases the accuracy of data entry in the patterns of care study. *Strahlenther Onkol* 2003;179:107–12.
14. JASTRO Database Committee. Present status of radiotherapy in Japan – the regular structure survey in 1999. *J Jpn Soc Ther Radiol Oncol* 2001; 13:227–35 (in Japanese).
15. SAS Procedure Reference, Version 6, 1st edition. Tokyo: SAS Institute 1995.
16. Sumi M, Ikeda H, Tokuuye K, Kagami Y, Murayama S, Tobisu K. et al. External radiotherapy with three-dimensional conformal boost after neoadjuvant anfrogen suppression for patients with locally advanced prostatic carcinoma. *Int J Radiat Oncol Biol Phys* 2000;48:519–28.

17. Iami A, Teshima T, Ohno Y, Inoue T, Yamashita T, Mitsuhashi N, et al. The future demand for and structural problems of Japanese radiotherapy. *Jpn J Clin Oncol* 2001;31:135–41.
18. Pollack A, Zagars GK. Androgen ablation in addition to radiation therapy for prostate cancer: is there true benefit? *Semin Radiat Oncol* 1998;8:95–106.
19. Zietman AL. Radiation therapy or prostatectomy: an old conflict revisited in the PSA era; a radiation oncologist's view-point. *Semin Radiat Oncol* 1998;8:81–6.
20. Hanks GE, Schultheiss TE, Hanlon AL, Hunt M, Lee WR, Epstein BE, et al. Optimization of conformal radiation treatment of prostate cancer: report of a dose escalation study. *Int J Radiat Oncol Biol Phys* 1997;37: 543–50.
21. Takara K. The origins and development of public health in Japan. In: Detels R, McEwen J, Omenn GS, editors. Oxford Textbook of Public Health, 3rd edition, vol 1. The Scope of Public Health. New York: Oxford University Press 1997;55–72.
22. OECD Health Data 2001. A Comparative Analysis of 30 OECD Countries. http://www.oecd.org/els/health/.
23. Kumamoto Y, Tsukamoto T, Umehara T. Shimazaki J, Fuse H, Oshima H, et al. Clinical studies on endocrine therapy of prostatic carcinoma (2): prognosis of patients with prostatic carcinoma given endocrine therapy and analyses of causes of death and side effects of endocrine therapy. *Hinyokika Kiyo* 1990;36:285–93 (in Japanese).
24. Sakai H, Minami Y, Kanetaka H, Saito Y, Ito K, Suzuki T, et al. Endocrine therapy for prostate cancer: 1980–1989. *Nishinihon J Urol* 1995;57:592–5 (in Japanese).
25. Bolla M, Gonzalez D, Warde P, Dubois JB, Mirimanoff RO, Storme G, et al. Improved survival in patients with locally advanced prostate cancer treated with radiotherapy and goserelin. *N Engl J Med* 1997;337:295–300.
26. Pilepich MV, Krall JM, al-Sarraf M, John MJ, Doggett RL, Sause WT, et al. Androgen deprivation with radiation therapy compared with radiation therapy alone for locally advanced prostatic carcinoma: a randomized comparative trial of the Radiation Therapy Oncology Group. *Urology* 1995;45:616–23.
27. Pilepich MV, Caplan R, Byhardt RW, Lawton CA, Gallagher MJ, Mesic JB, et al. Phase III trial of androgen suppression using goserelin in unfavorable-prognosis carcinoma of the prostate treated with definitive radiotherapy: report of Radiation Therapy Oncology Group Protocol 85-31. *J Clin Oncol* 1997;15:1013–121.

Particle Irradiation Suppresses Metastatic Potential of Cancer Cells

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Abstract

Particle radiotherapy such as proton and carbon ion has been producing promising clinical results worldwide. The purpose of this study was to compare metastatic capabilities of malignant tumor cells after irradiation with photon, proton, and carbon ion beams to clarify their ion beam–specific biological effects. We examined the biological properties of highly aggressive HT1080 human fibrosarcoma cells to assess their metastatic processes in terms of cell adhesion capability to extracellular matrix, expression of integrins, cell migration, cell invasive capability, and matrix metalloproteinase-2 activity *in vitro*. We then assessed the metastatic capabilities of LM8 mouse osteosarcoma irradiated with carbon ion or photon beam in the syngeneic mice. Both proton and carbon ion irradiation decreased cell migration and invasion in a dose-dependent manner and strongly inhibited matrix metalloproteinase-2 activity. On the other hand, lower X-ray irradiation promoted cell migration and invasion concomitant with up-regulation of αVβ3 integrin. For cancer cells treated with carbon ion irradiation, the number of pulmonary metastasis was decreased significantly *in vivo*. These findings suggest that particle irradiation suppresses metastatic potential even at lower dose, whereas photon irradiation promotes cell migration and invasive capabilities at lower dose level, and provide preclinical evidence that ion beam radiotherapy may be superior to conventional photon beam therapy in possible preventive effects on metastases of irradiated malignant tumor cells. (Cancer Res 2005; 65(1): 113-20)

Introduction

Metastasis, the biggest threat to survival for patients with solid tumors, is the spread of tumor cells from the original growth to the other sites in the body. Metastatic processes of malignant tumor cells generally consist of (i) detachment of cells from the primary tumor, (ii) migration to extracellular matrices, (iii) degradation of basement membrane, (iv) invasion into blood vessels, (v) circulation in blood flow, (vi) escape to extravascular matrices, and (vii) implantation to target organs. These processes are based upon a number of biological characteristics associated with various molecular changes involving proteinases, adhesion molecules, and cell motility factors.

The integrin family of adhesion molecules is extracellular matrix receptors consisting of α and β chains that form various heterodimers with distinct cellular and adhesive characteristics. Integrin-mediated adhesion to extracellular matrix triggers intracellular signaling pathways to modulate cell proliferation, shape, migration, invasion, and survival (1, 2). The β1 integrin subfamily consists of a receptor subunit associated with several α subunits resulting in a broad spectrum of receptors for a variety of potential ligands (3, 4). The vitronectin receptor, αVβ3 integrin, also seems to be associated with increased invasiveness (5, 6).

Matrix metalloproteinases (MMP) constitute a family of Zn^{2+}-dependent enzymes essential for extracellular matrix turnover under normal and pathologic conditions. Especially MMP-2 can degrade type IV collagen, one of the major components of the basement membrane, resulting in the promotion of tumor invasion and metastasis (7). One of the mechanisms of this process is that MMP-2 directly binds to αVβ3 integrin and thus localizes in a proteolytically active form on the surface of invasive cells (8).

In the clinic, ionizing radiation has been established as a highly effective modality used in the local control of tumor growth. However, several authors have reported that photon beam irradiation enhanced metastatic processes of malignant tumor cells at sublethal dose (9–13). New types of radiation sources, particle beams such as proton and carbon ion, may be expected to be a new modality of cancer treatment. Particle therapy has the advantage, in theory, over conventional photon beam that the tumor can be targeted with extreme precision, without damage to normal surrounding tissue, either superficial or deep, thereby allowing for an extraordinary escalation of dosage to the tumor. Carbon ion with high linear energy transfer has been shown more effective than photon and proton for cell-killing effect (14–16). Only a few studies have been conducted of the effects of particle beams on functioning of cells with metastatic potential. Our group was the first to report that carbon beam irradiation inhibited *in vitro* angiogenesis even at sublethal dose (17).

We show metastatic potential after irradiation with photon, proton, and carbon ion beams to elucidate particle-specific biological effects. Here, we report that particle irradiation suppresses metastatic potential, whereas photon irradiation promoted cell migration and invasive capabilities at lower dose level.

Materials and Methods

Cell Culture and Reagents. Highly aggressive HT1080 human fibrosarcoma (American Type Culture Collection, Rockville, MD) and LM8 mouse osteosarcoma (18), a highly metastatic Dunn cell subline which was kindly given by Dr. Yoshikawa (Osaka University, Osaka, Japan), were maintained in DMEM medium (Nihonseiyaku, Tokyo, Japan) with 10% fetal bovine serum and penicillin/streptomycin (Invitrogen, Carlsbad, CA) at 37°C in a humidified atmosphere of 10% CO_2 and 90% air. The MMP inhibitor GM6001 was purchased from Chemicon (Temecula, CA).

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Irradiation. Cell irradiation with 190 MeV/nucleon proton beams was done at the Hyogo Ion Beam Medical Center in Japan. Cells were irradiated at the center of Bragg peaks modulated to 6-cm widths. The irradiation system and biophysical characteristics of proton beams have been detailed elsewhere (19).

For carbon ion irradiation, cells were treated with 290 MeV/nucleon carbon ion beams at 6-cm spread-out Bragg peak center from the Heavy Ion Medical Accelerator in Chiba at the National Institute of Radiological Sciences in Japan. The irradiation system for carbon ion at Heavy Ion Medical Accelerator in Chiba and the physical characteristics of the beam have been described elsewhere (20, 21).

For photon irradiation, 4 MV X-ray from the linear accelerator at Osaka University Graduate School of Medicine was used with a delivered dose rate of ~1.8 Gy/min.

Colony Formation Assay. Survival curves were obtained by means of standard colony formation assay. Irradiated cells were plated onto triplicate 60-mm-diameter plastic dishes aiming for 80 to 100 colonies per dish. After 10 to 12 days of incubation, colonies were fixed with 10% formalin and stained with crystal violet. Colonies with >50 cells were scored as a surviving colony.

Cell Adhesion Assay. Plastic plates (96 wells) were coated with 10 μg/mL of collagen, laminin, fibronectin, and vitronectin (IWAKI, Chiba, Japan) in PBS (Invitrogen) for 2 hours at 37°C and then treated with 3% bovine serum albumin for 1 hour at 37°C, or were coated with only bovine serum albumin for negative control. The cells (2×10^5 cells/mL) in serum-free DMEM containing 0.1% bovine serum albumin were then added and incubated for 2 hours at 37°C. After removal of the medium, a 0.04% crystal violet solution was added and incubation was conducted for 10 minutes at room temperature. The wells were washed thrice with PBS and 20 μL of Triton X-100 were added for permeabilization. Finally, distilled water was then added for a total quantity of 100 μL, and the number of adherent cells was assessed with a microplate reader (measurement wavelength = 550 nm and reference wavelength = 630 nm).

Flow Cytometry. For αVβ3 and β1 integrin analysis, cells in DMEM supplemented with 1% fetal bovine serum and 0.03% sodium azide were incubated with a monoclonal antibody against mouse monoclonal antibody αVβ3 and β1 (Chemicon), for 30 minutes at 4°C. After washing with DMEM as described above, the cells were incubated with FITC-conjugated mouse IgG (DAKO, Copenhagen, Denmark) for 30 minutes at 4°C. After washing, cells were resuspended with the same medium and analyzed using a FACSCalibur (Beckton Dickson, Heidelberg, Germany) with CellQuest software (Beckton Dickson). Finally, cell surface fluorescence for individual integrin receptors was obtained.

Chemotaxis Assay. Chemotaxis was assessed with a 48-microwell chemotaxis chamber (Neuro Probe, Gaithersburg, MD) that was set a polycarbonate filter of 8-μm pores coated with 10 μg/mL fibronectin. The cells were trypsinized, resuspended in 0.1% bovine serum albumin and adjusted to a final concentration of 1×10^6 cells/mL. The cells (5×10^4) were added to the upper well, which was placed into a lower well containing medium with 10% fetal bovine serum as a chemoattractant. After 3 hours of incubation at 37°C, cells remaining on the upper membrane surface were removed with a cotton swab. The cells that had migrated to the bottom of the filter were fixed with formalin and stained with hematoxylin. Cell migration was quantitated by counting the number of stained nuclei in four random fields at 20× magnification with a microscope.

Matrigel Invasion Assay. Invasion of cancer cells was assessed by measuring the invasion of cells through transwell inserts with 8-μm pores coated with Matrigel (Becton Dickinson). Irradiated cells were trypsinized, washed twice with DMEM supplement with 0.1% bovine serum albumin, and 200 μL of cell suspension (5×10^5 cells/mL) per condition were added to the upper well. DMEM supplement with 10% fetal bovine serum (700 μL) as a chemoattractant was added to the lower well. The number of cells that had invaded to the lower surface of the Matrigel-coated membrane was counted in four random fields under a microscope.

Gelatin Zymography. MMP-2 activity was analyzed as detailed elsewhere (22). After irradiation, cells were washed twice with PBS and incubated with serum-free DMEM for 24 hours. After the medium had been centrifuged to remove corpuscular material, supernatant was collected, frozen in liquid nitrogen, and stored at −80°C. Samples were mixed with SDS sample buffer without heating or reduction and applied to 8% polyacrylamide gels containing 0.1% gelatin. After electrophoresis, gels were renatured by soaking for 45 minutes at room temperature in 2.5% Triton X-100 with gentle agitation and then incubated for 12 hours at 37°C in buffer containing 5 mmol/L $CaCl_2$ and 1 μmol/L $ZnCl_2$. Gelatinolytic activity made the bright bands visible at M_r 72,000 for the pro form and M_r 62,000 for the active form of MMP-2.

Animal and Tumor Model. LM8 cells were irradiated with 290 MeV/nucleon carbon ion beams or 4 MV X-ray (proton irradiation was not done because of restricted irradiation time). Cells were harvested by treatment with trypsin-EDTA (Invitrogen), washed twice with serum-free DMEM, and suspended in serum-free DMEM. Irradiated LM8 mouse osteosarcoma cells, 10^5 cells in 0.05 mL, were injected s.c. into the hind limbs or inoculated into tail vein of 8- to 10-week-old female specific pathogen-free C3H/HeJ mice (Charles River, Yokohama, Japan). In s.c. tumor, tumor volume (mm^3) was measured with calipers and calculated according to the formula: 1/2 × length × width^2. Mice injected s.c. or i.v. were euthanized 15 or 30 days after injection. Lung tumor formation was observed under a dissecting stereomicroscope, and the number of lung tumors was counted. These experiments were repeated twice.

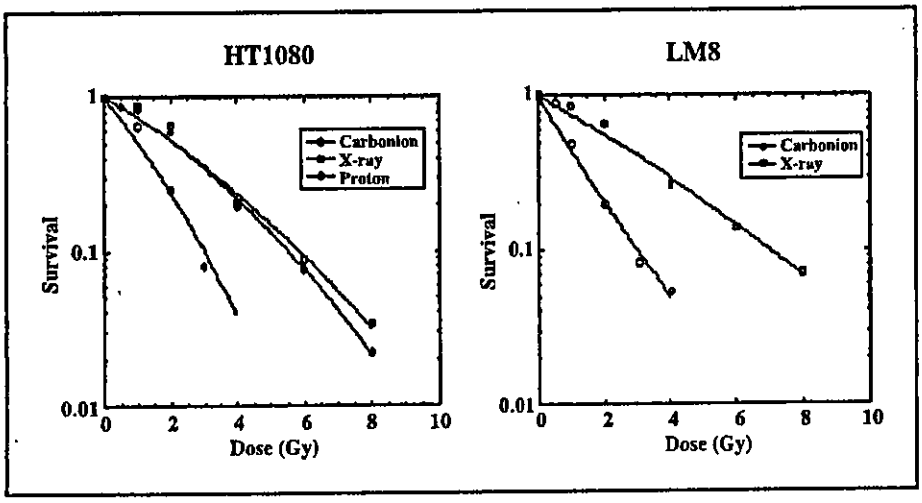

Figure 1. Clonogenic survival curves after photon, proton, or carbon beam irradiation for cancer cells. Surviving fractions against physical doses were plotted and fitted to surviving curves using the following linear-quadratic model: $SF = \exp(-\alpha D - \beta D^2)$, where SF is the surviving fraction and D is the physical dose. Survival curve for proton irradiation was examined only in HT1080 cells.

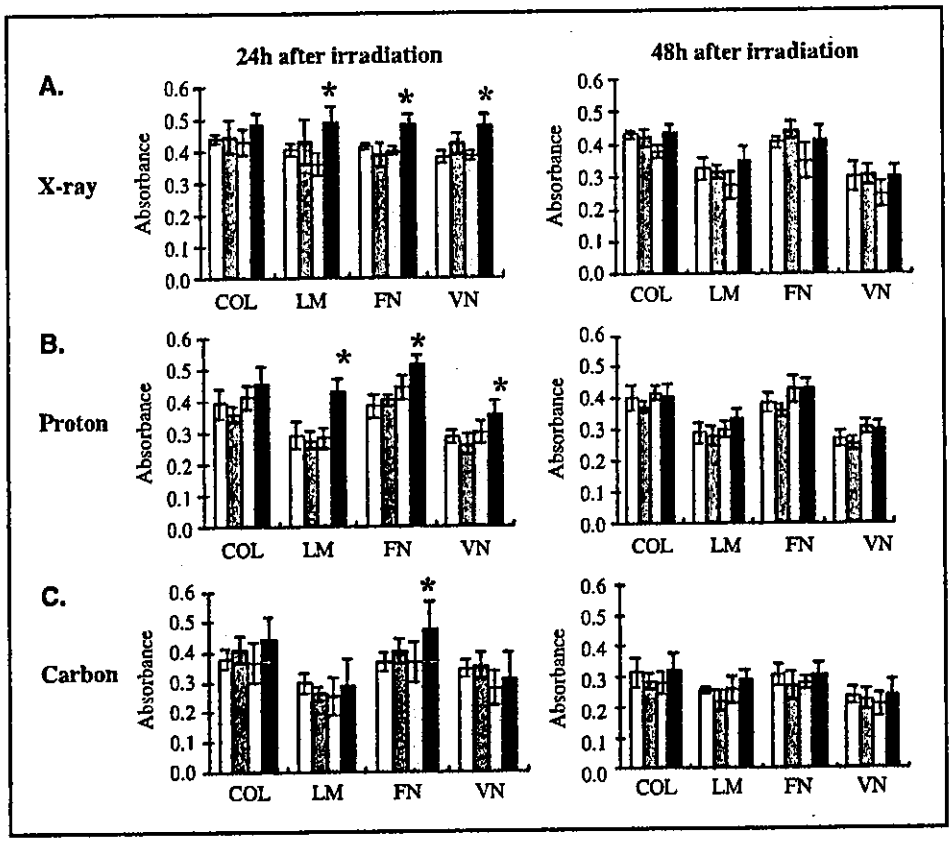

Figure 2. Effects of irradiation on adhesion of cancer cells to collagen (*COL*), laminin (*LM*), fibronectin (*FN*), or vitronectin (*VN*). *A*, cells were untreated or irradiated with photon beams. *White bar*, untreated control cells; *dark gray bar*, cells irradiated at 0.5 Gy; *light gray bar*, cells irradiated at 2 Gy; and *black bar*, cells irradiated at 8 Gy. *B*, cells were untreated or irradiated with proton beam. *White bar*, untreated control cells; *dark gray bar*, cells irradiated with 0.5 Gy; *light gray bar*, cells irradiated with 2 Gy; and *black bar*, cells irradiated with 8 Gy. *C*, cells were untreated or irradiated with carbon ion beam. *White bar*, untreated control cells; *dark gray bar*, cells irradiated at 0.2 Gy; *light gray bar*, cells irradiated at 1 Gy; and *black bar*, cells irradiated at 4 Gy. *Columns*, mean; *bars*, ± SD. *, $P < 0.05$ (Student's *t* test, compared with untreated cell).

Statistics. The results were expressed as mean values with SDs of at least three independent experiments, except indicated elsewhere. The statistical significance was tested by means of Student's *t* test or ANOVA where appropriate. $P < 0.05$ was considered statistically significant.

Results

Survival Curves of Cancer Cells. To require biologically equivalent doses for each radiation quality, we first examined clonogenic survival using the colony formation assay. For HT1080 cells, the relative biological effectiveness values, calculated by the D10 relative to X-ray, were to be 1.1 for proton irradiation and 1.9 for carbon irradiation (Fig. 1). The corresponding relative biological effectiveness measured by the D10 relative to X-rays for the LM8 cells was 2.3 for carbon irradiation (Fig. 1). Therefore, in subsequent assays, we applied the physical doses of carbon ion or proton to half or the same physical doses for X-ray.

The Expression Levels of Integrin and Adhesion to Extracellular Matrix. Cell adhesion assays were done to assess tumor cell adhesion capabilities to extracellular matrix proteins (collagen, fibronectin, laminin, and vitronectin). The adhesion capability to fibronectin, laminin, and vitronectin of cells irradiated with 8 Gy for X-ray or proton had significantly increased 24 hours after irradiation (Fig. 2*A* and *B*). Irradiation with 4 Gy for carbon at 24 hours after irradiation showed a significantly higher attachment to fibronectin compared to the nontreatment controls (Fig. 2*C*). On the other hand, no significant changes were observed 48 hours after X-ray, proton, and carbon ion irradiation (Fig. 2*A-C*). Mean fluorescence intensity (% control) of integrins that plays crucial roles in cell adhesion to extracellular matrices, cell migration, and invasion was analyzed by flow cytometry analyses. The expression levels of β1 integrin did not show significant differences for X-ray, proton, and carbon ion irradiation at 24 hours after irradiation (Table 1). After delivery of more than 4 Gy of each type of irradiation, the amount of β1 integrin was increased, although not significantly. The expression levels of αVβ3 integrin were not changed by either proton or carbon ion irradiation (Table 1). However, for cells irradiated with 0.5 Gy of X-ray, the amount of αVβ3 integrin was significantly increased compared with untreated controls (Table 1).

Effects of Irradiation on Cell Migration and Invasion. Cell migration and invasion are fundamental components of tumor

Table 1. Effects of irradiation on the expression levels of β1 and αVβ3 integrin in cancer cells by flow cytometric analysis

Integrin		0.5 (0.2) Gy	2 (1) Gy	8 (4) Gy
β1	X-ray	94 ± 7	107 ± 8	131 ± 21
	Proton	93 ± 2	94 ± 4	118 ± 11
	Carbon	106 ± 15	103 ± 16	115 ± 12
αVβ3	X-ray	132 ± 10*	119 ± 20	102 ± 9
	Proton	95 ± 14	87 ± 8	98 ± 11
	Carbon	97 ± 9	101 ± 18	106 ± 6

NOTE: Number in parentheses represents the physical dose of carbon ion.
*$P < 0.05$ (Student's *t* test, compared with untreated cell).

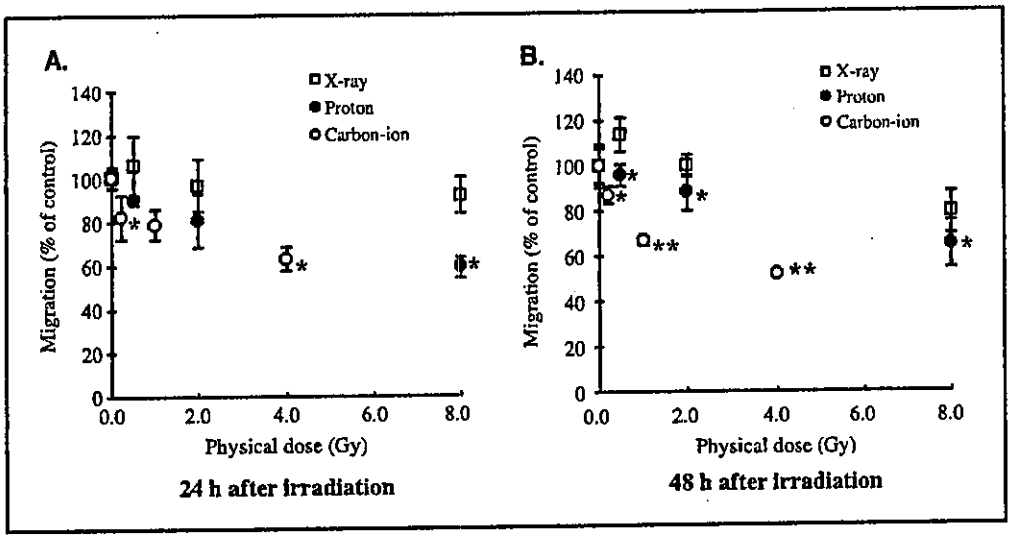

Figure 3. Effects of irradiation on cell migration of HT1080 cells. HT1080 cells were exposed to X-ray (□), proton (●), or carbon (○). *Vertical axis*, no. migrated cells (%control); *horizontal axis*, physical dose. *Points*, mean; *bars*, ± SD. *, $P < 0.05$; **, $P < 0.01$ (ANOVA, compared particle beams with X-ray at similar killing doses).

cell metastasis. To assess the effect of photon, proton, and carbon ion beams on cell motility, we examined the migration of malignant cells 24 and 48 hours after irradiation using chemotaxis assay. For proton as well as carbon ion irradiation, suppression of migration of irradiated cells became apparent at 24 and 48 hours after irradiation in a dose-dependent manner (Fig. 3*A* and *B*). On the other hand, an increase in migration was observed by lower dose (0.5 Gy) of X-ray irradiation at 48 hours after irradiation (Fig. 3*B*). At similar cell killing doses, proton or carbon particle irradiation, compared to X-rays, inhibited the migration (for carbon irradiation except 24 hours after 2 Gy, $P < 0.05$; At 24 hours after 8 Gy or at 48 hours after proton irradiation, $P < 0.05$).

We next focused on changes in the invasive capability of cancer cells after irradiation using the Matrigel invasion assay. Proton as well as carbon ion beam irradiation significantly reduced the invasion capabilities of irradiated cells (Fig. 4*A* and *B*). X-ray irradiation promoted cell invasion even at the dose levels below 2 Gy (Fig. 4*A* and *B*). Remarkably, invasive potentials of malignant cells were significantly increased by about 2-fold at 24 hours after X-ray irradiation at dose of 2 Gy (Fig. 4*A*). For cells irradiated with 8 Gy at 48 hours after X-ray irradiation, invasion capabilities were decreased as compared with untreated controls (Fig. 4*B*). Proton or carbon ion irradiation at comparable cell-killing doses resulted in significantly diminished invasion capabilities 24 or 48 hours after irradiation compared to that resulting from X-ray irradiation ($P < 0.01$).

Effects of Irradiation on MMP-2 Activity. The process of tumor cell invasion and metastasis requires the degradation of connective tissue associated with vascular basement membranes and interstitial connective tissue. Therefore, MMP-2 activity required for tumor invasion was examined by gelatin zymography. Gelatin zymography revealed that proton and carbon ion irradiation strongly inhibited MMP-2 activity of cancer cells in a dose-dependent manner (Fig. 5*A*). For cells irradiated with X-ray, MMP-2 activity were not changed compared with untreated controls (Fig. 5*A*).

The Influence of MMP Inhibitor on Photon-Enhanced Cell Invasion. Particle irradiation inhibited invasion capability due to an association with inhibition of MMP-2 activity. Therefore, we investigated whether the MMP inhibitor could

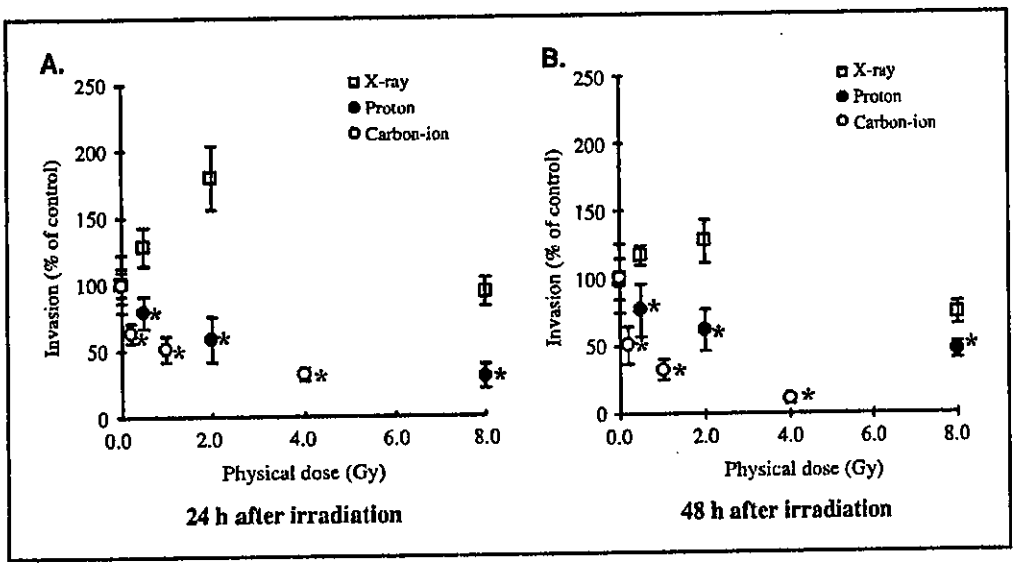

Figure 4. Effects of irradiation on cell invasion of HT1080 cells. HT1080 cells were irradiated with X-ray (□), proton (●), or carbon (○). *Vertical axis*, no. invaded cells (% control); *horizontal axis*, physical dose. *Points*, mean; *bars*, ± SD. *, $P < 0.01$ (ANOVA, compared particle beams with X-ray at comparable cell killing doses).

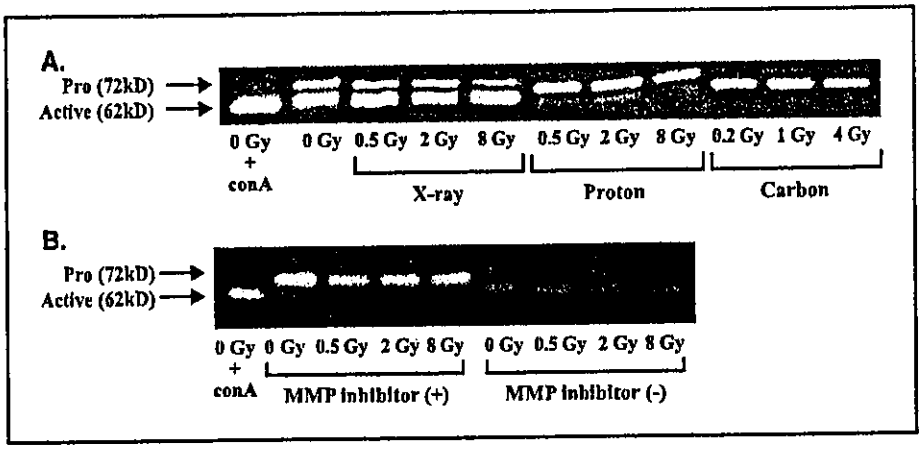

Figure 5. Effects of irradiation on MMP-2 activity of cancer cells. Supernatants from untreated or irradiated cells were collected 24 hours after irradiation and analyzed by zymography for pro form (72 kDa or active form of MMP-2 (62 kDa). Untreated samples in addition to concanavalin A (*conA*) were used for positive control. *A*, cells were untreated or irradiated with X-ray, proton, or carbon ion. *B*, cells were untreated or irradiated with X-ray in the presence or absence of the MMP inhibitor. MMP inhibitor (25 μmol/L) was added in condition medium just after irradiation.

prevent photon-induced increase in invasive potential. The addition of the MMP inhibitor GM6001 to the upper well resulted in a marked reduction of photon-induced invasion (Table 2). Furthermore, gelatin zymography showed that administration of GM6001 at concentration of 25 μmol/L reduced the active form of MMP-2 (Fig. 5*B*).

The Effect of Irradiated Cells on Metastatic Capabilities *In vivo*. To investigate whether irradiated cells affect metastastic capabilities *in vivo*, LM8 osteosarcoma cells irradiated with X-ray or carbon ion were injected s.c. into right thighs or i.v. into tail vein of mice. In s.c. tumor, the tumor volumes obtained from irradiated cells were decreased as compared with those of untreated cells (Fig. 6*A* and *B*). There was no difference in the volume of tumors from cells treated with X-ray at doses between 2 and 10 Gy (Fig. 6*A*). However, for cells irradiated with carbon ion, tumor volume was decreased dose dependently (Fig. 6*B*). Pulmonary metastases of cancer cells irradiated with X-ray did not changed in comparison with those of untreated controls (Fig. 6*C*), whereas treatment with carbon ion reduced the number of lung metastases in a dose-dependent manner (Fig. 6*D*).

For mice inoculated i.v., X-ray irradiation resulted in a 1.2-fold increase in the number of metastatic lung nodules in mice as compared to mice injected with untreated cells (Fig. 6*E* and *F*). However, a significant suppression of lung metastases was observed in cells irradiated with carbon ion (Fig. 6*E* and *G*).

Table 2. The influence of MMP inhibitor on photon-enhanced cell invasion

	0 Gy	0.5 Gy	2 Gy	8 Gy
Control media	100 ± 10	117 ± 7	107 ± 8	93 ± 10
+25 μmol/L MMP inhibitor	80 ± 7	87 ± 11	76 ± 11	58 ± 5
Each treated versus control, *P*	<0.05	<0.05	<0.05	<0.05

NOTE: Data were calculated with reference to untreated controls defined as a percentage scale.

Discussion

Metastasis brings about the greatest threat to the survival and quality of life for cancer patients. The ultimate goal of cancer therapy is to treat the primary tumor and any underlying metastases. Particle radiotherapy such as proton and carbon ion has established its efficacy by demonstrating superb results (23–28). The advantage of particle beams over photon is superior distribution of radiation dose due to the physical characteristics, which makes it possible to spare normal tissues close to the target. However, the effects of particle beams on metastatic potential of cancer cells are not yet well understood. We hypothesized that particle beams might inhibit metastatic potential for ion beam-specific biological effects and first focused on the *in vitro* models including adhesion, migration, invasion, and the expression level or activity of molecules related to metastasis such as αVβ3, β1 integrin, and MMP-2.

Various factors are related to metastatic potentials. Changes in integrin expression level are likely to affect cell adhesion closely linked cell functions. X-ray, proton, and carbon ion irradiation of more than 4 Gy was seen to increase significantly cell adhesion capability to extracellular matrix significantly. Cordes et al. (29, 30) showed that radiation-induced increase in adhesion capacity could be modulated by radiation-induced increase in β1 integrin expression. However, our findings showed that the expression levels of β1 integrin were increased (≥4 Gy irradiation) but did not show significant differences among X-ray, proton, and carbon ion irradiation. The reason for these discrepant results may be that the use of flow cytometry does not enable us to detect β1 integrin affinity but only the expression level of β1 integrin. Integrin affinity for extracellular matrix can be regulated by intracellular signals such as the Ras–, R-Ras– and Rap1-GTPases (31, 32). β1 Integrin transduces biochemical signals from the extracellular environment, especially with respect to cell survival. It seems likely that radiation (≥4 Gy) may activate β1 integrin affinity and thus leading radiation-induced (≥4 Gy) increase in adhesion capacity due to cell survival.

Cell migration and invasion are fundamental components of tumor cell metastasis. Wild-Bode et al. (9) reported that sublethal dose of X-ray irradiation induced the expression levels of the αVβ3 integrin of glioblastoma and led to enhancement of cell migration. We confirmed that X-ray irradiation promotes cell migration capabilities concomitant with the up-regulation of αVβ3 integrin at lower dose level. However, our study showed that both proton and carbon ion irradiation significantly decreased cell migration

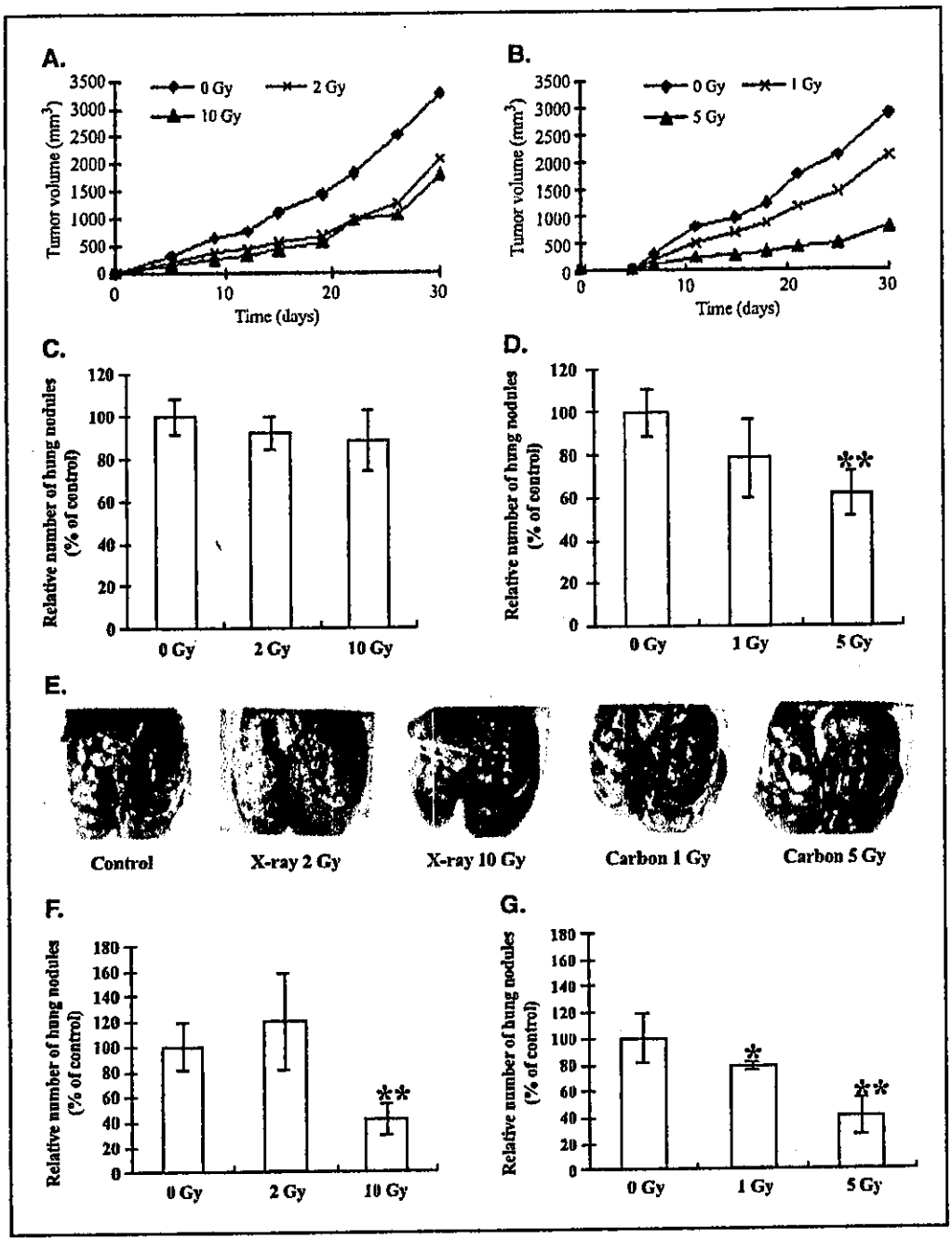

Figure 6. Metastatic capabilities of cancer cells irradiated with photon or carbon beam *in vivo*. Growth curves in subcutaneous tumors of LM8 osteosarcoma cells unirradiated, irradiated with X-ray (*A*), or carbon ion (*B*). The number of lung metastases treated with X-ray (*C*), or carbon ion (*D*) in subcutaneous tumor. Representative of lung metastasis for mice injected intravenously (*E*). Therapeutic effects of cells irradiated with X-ray (*F*) or carbon ion (*G*) on experimental pulmonary metastasis from mice inoculated intravenously. *Columns*, mean; *bars*, ± SD. *, $P < 0.05$ and **, $P < 0.01$ (Student's *t* test versus the untreated group).

and invasion capabilities in a dose-dependent manner. Many studies have shown that MMP-2 plays a critical role in tumor invasion. There have been many reports on the enhancement of MMP-2 activity by X-ray irradiation (9, 12, 33, 34). One of the mechanisms of this enhancement is that the activation of wild-type p53 by photon irradiation and the resulting increase in MMP-2, which can promote radiation-induced metastasis. Bian and Sun (35) reported that the 5′ flanking region of the *MMP-2* gene contains a perfect p53 binding sequence and that the binding of wild-type p53, but not mutant p53, to this site up-regulates *MMP-2* gene expressions. In a previous study, for HT1080 cells expressed wild-type p53, γ-ray irradiation with doses from 4 to 15 Gy up-regulated this expression (36). Our study showed that MMP-2 was strongly inhibited by carbon ion and proton irradiation. Therefore, invasion capabilities of irradiated cells were significantly suppressed by particle beams. Furthermore, we confirmed that MMP inhibitor blocked the photon-enhanced invasion of cancer cells. Our results concur with Wild-Bode's report that administration of *o*-phenantroline that is one of the MMP inhibitors significantly inhibited photon-induced invasiveness. Asakawa et al. (37) showed that p53-dependent radiation-induced growth inhibition of SAS tongue carcinoma cells transplanted into nude mice was observed following X-ray irradiation but not carbon ion irradiation. Our finding suggests that particle beam irradiation is not affected by p53 status.

The phenomena underlying the suppression of metastatic capability by particle irradiation *in vitro* were studied further by

investigating metastatic potentials of cancer cells irradiated with carbon ion or photon beams *in vivo*. For mice inoculated s.c. or i.v., treatment with carbon ion reduced the number of lung metastases in a dose-dependent manner as compared with untreated controls. For several experimental tumors, inadequate X-ray radiation resulted in an increase in metastasis (38). One possible explanation for this increase is that radiation-induced DNA changes increase the metastatic potential of cancer cells (39). Our data suggest that carbon ion irradiation induced DNA changes which suppressed the metastatic capabilities of tumor cells, leading to suppression of pulmonary metastases *in vivo*. This may have been caused by carbon ion irradiation producing a higher proportion of double-strand DNA breaks than does X-ray irradiation.

In this study, the focus was to elucidate the effects of particle beam on metastatic potential of cancer cells. However, little is known about the basic radiobiological effects of particle beam except for the end point of cell survival, especially about the effects on metastatic capabilities. To date, a few groups have reported on the effects of particle beams on cell functions associated with metastatic capabilities. Our group showed that carbon ion irradiation inhibited MMP-2 activity and down-regulated αVβ3 integrin, thus leading to inhibition of *in vitro* angiogenesis (17). Ando et al. (40) reported that the induction by carbon ion irradiation of vascular endothelial growth factor that plays an important role in tumor growth and metastasis. However, lung carcinoma cells irradiated with carbon ion induced vascular endothelial growth factor mRNA expression and increased protein levels dose dependently. Particle therapy still has much room to be studied for optimum use in clinical oncology compared with conventional photon beam treatment. Further intensive studies are also necessary to elucidate the relevant molecular mechanisms specifically related to particle irradiation. In future experiments, other carcinoma cell lines will be examined to confirm that this phenomenon is not specific to one cell line.

The phenomena we observed in this study have two significant impacts on the clinic. First, with advent of recent high precise modality such as intensive modulated radiation therapy, radiation oncologists have been focusing on making the radiation field as small as possible to the clinical target volume. There may be a risk that excellent local controls can be hampered by later increase of distant metastasis. Then, we need individualized radiation field based on such biological behavior of each cancer cell. Second, particles such as proton and carbon may have totally different mechanism of action on cell migration and invasion, because these functions were significantly inhibited even at lower doses of particle. These significant differences in cell functions may be caused by differences in biological mechanisms between particle and electromagnetic wave but cell-killing effect concerning cell survival evaluated with colony formation assay of proton are similar to that of photon.

Photon radiation therapy should be asked with some caution. Lower photon irradiation promotes cell migration and invasive capabilities. However, metastatic capabilities of cancer cells irradiated with 8 Gy of photon beams did not changed in comparison with those of untreated controls. The clinical implications reported by Wild-Bode et al. (9) are that alterations in the fractionation of radiotherapy for human glioblastoma multiform may need to be considered and that inhibitors of migration and invasion may prevent irradiation-induced dissemination of glioma cells from the target volume of irradiation when given during radiotherapy. In addition to these implications, we suggest that not only dose escalation that can eradicate tumors is needed but also examination of the individual radiation field margins, by considering cell migration and identifying microscopic diseases by means of molecular imaging is needed.

To summarize, our study found that particle irradiation decreased cell migration and invasion in a dose-dependent manner and strongly inhibited MMP-2 activity *in vitro*. *In vivo*, treatment with carbon ion reduced the number of lung metastases in a dose-dependent manner. On the other hand, lower X-ray irradiation facilitated cell migration and invasion concomitant with up-regulation of αVβ3 integrin *in vitro*.

In conclusion, these data suggests that particle irradiation suppresses metastatic potential even at lower dose whereas photon irradiation promotes cell migration and invasive capabilities at lower dose level. These findings provide preclinical rationales that particle radiotherapy may be superior to conventional photon beam therapy in possible preventive effects on metastases of irradiated malignant tumor cells.

Acknowledgments

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References

1. Giancotti FG, Ruoslahti E. Integrin signaling. Science 1999;285:1028–32.
2. Danen EH, Yamada KM. Fibronectin, integrins, and growth control. J Cell Physiol 2001;189:1–13.
3. Saga S, Chen WT, Yamada KM. Enhanced fibronectin receptor expression in Rous sarcoma virus-induced tumors. Cancer Res 1988;48:5510–3.
4. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992;69:11–25.
5. Ruoslahti E. The Walter Herbert Lecture. Control of cell motility and tumour invasion by extracellular matrix interactions. Br J Cancer 1992;66:239–42.
6. Felding-Habermann B, Cheresh DA. Vitronectin and its receptors. Curr Opin Cell Biol 1993;5:864–8.
7. Deryugina EI, Luo GX, Reisfeld RA, Bourdon MA, Strongin A. Tumor cell invasion through matrigel is regulated by activated matrix metalloproteinase-2. Anticancer Res 1997;17:3201–10.
8. Brooks PC, Stromblad S, Sanders LC, et al. Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin α v β 3. Cell 1996;85:683–93.
9. Wild-Bode C, Weller M, Rimner A, Dichgans J, Wick W. Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. Cancer Res 2001;61:2744–50.
10. Sonveaux P, Brouet A, Havaux X, et al. Irradiation-induced angiogenesis through the up-regulation of the nitric oxide pathway: implications for tumor radiotherapy. Cancer Res 2003;63:1012–9.