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IV. 研究成果の刊行物・別刷

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 monocional antibody against mouse monocional 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.

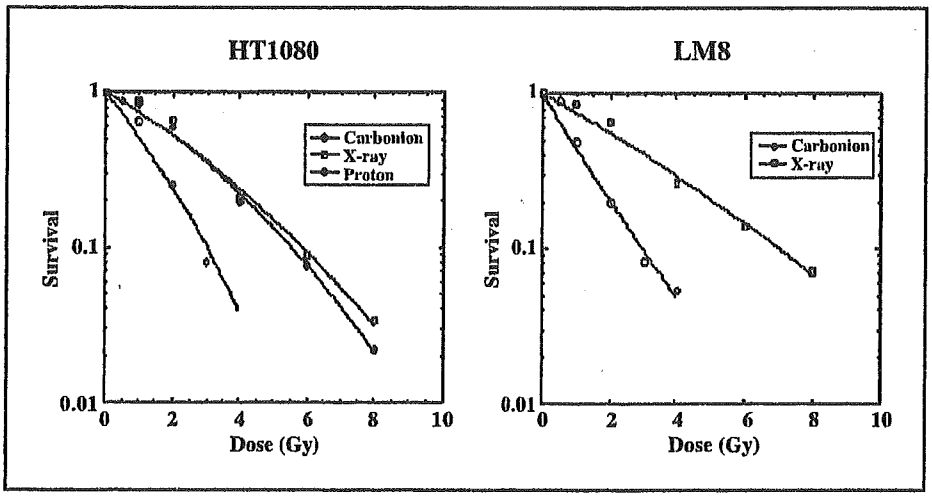

Figure 1. Clonogenic survival curves after photon, proton, or carbon beam irradiation for cancer cells. Surviving fractions against physical doses were plotted and fitted to surviving curves using the following linear-quadratic model: $SF = \exp(-\alpha D - \beta D^2)$, where SF is the surviving fraction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

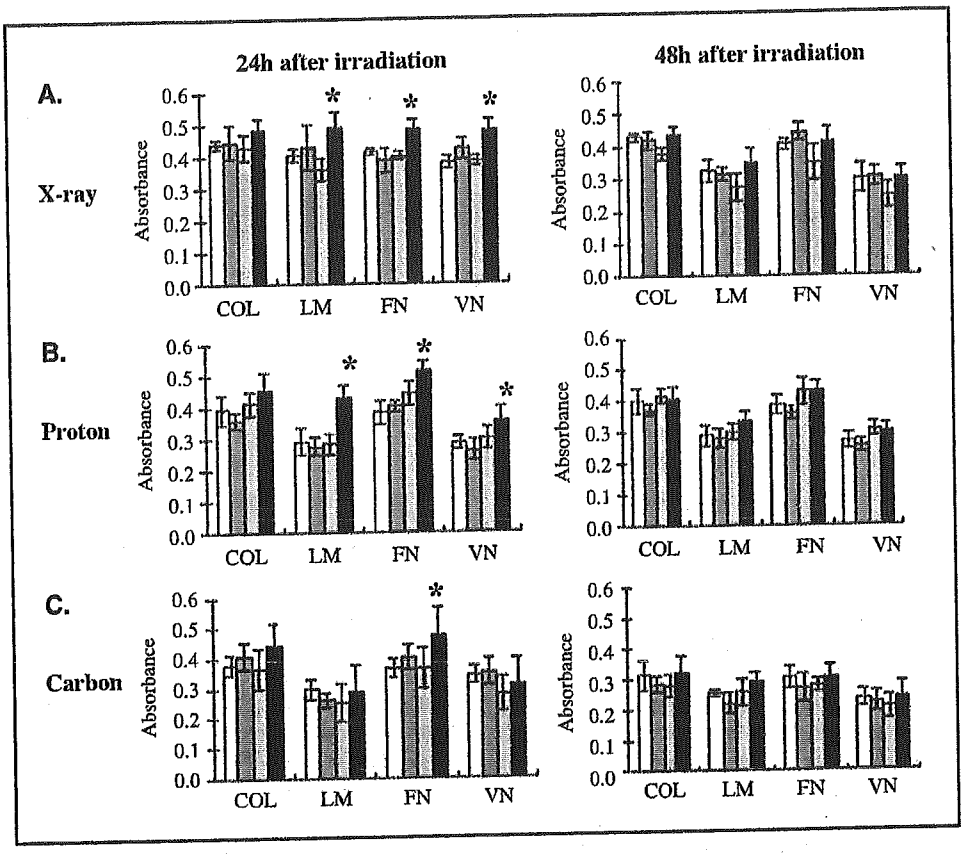

Figure 2. Effects of irradiation on adhesion of cancer cells to collagen (*COL*), laminin (*LM*), fibronectin (*FN*), or vitronectin (*VN*). *A*, cells were untreated or irradiated with photon beams. *White bar*, untreated control cells; *dark gray bar*, cells irradiated at 0.5 Gy; *light gray bar*, 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).

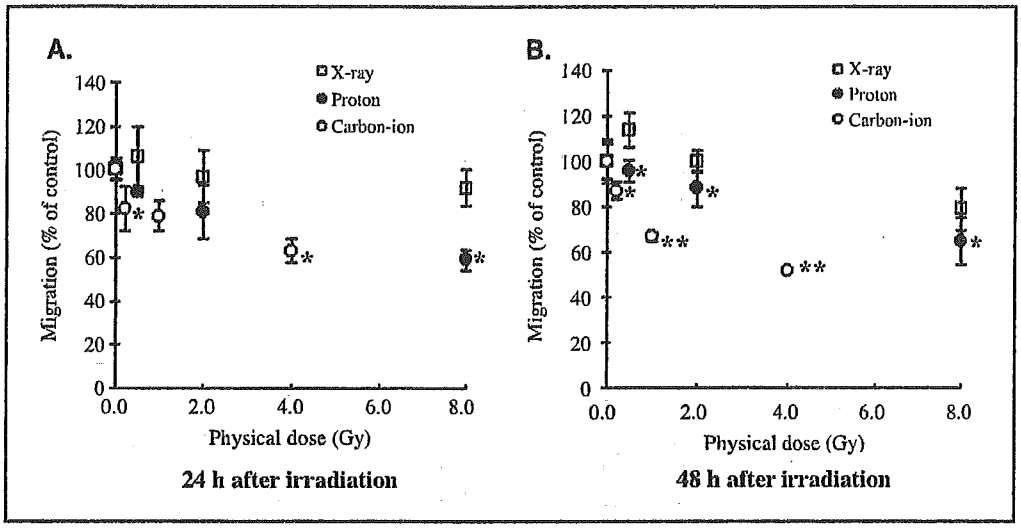

Figure 3. Effects of irradiation on cell migration of HT1080 cells. HT1080 cells were exposed to X-ray (□), proton (●), or carbon (○). *Vertical axis*, no. migrated cells (%control); *horizontal axis*, physical dose. *Points*, mean; *bars*, ± SD. *, $P < 0.05$; **, $P < 0.01$ (ANOVA, compared particle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

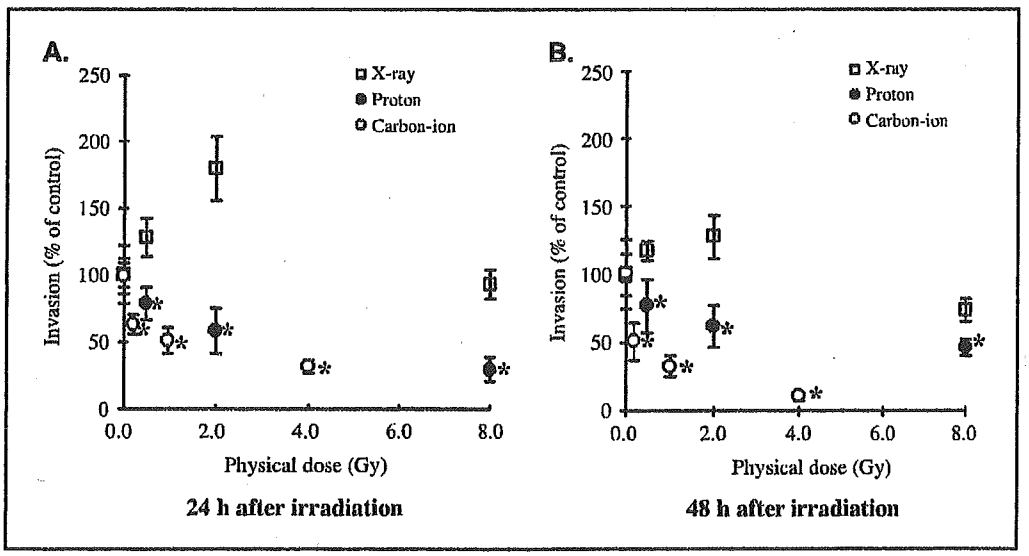

Figure 4. Effects of irradiation on cell invasion of HT1080 cells. HT1080 cells were irradiated with X-ray (□), proton (●), or carbon (○). *Vertical axis*, no. invaded cells (% control); *horizontal axis*, physical dose. *Points*, mean; *bars*, ± SD. *, $P < 0.01$ (ANOVA, compared particle beams with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

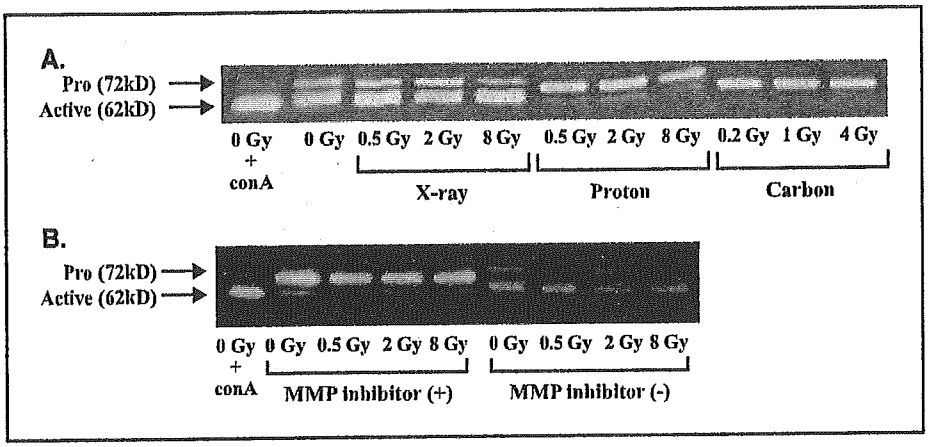

Figure 5. Effects of irradiation on MMP-2 activity of cancer cells. Supernatants from untreated or irradiated cells were collected 24 hours after irradiation and analyzed by zymography for pro form (72 kDa or active form of MMP-2 (62 kDa). Untreated samples in addition to concanavalin A (*conA*) were 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

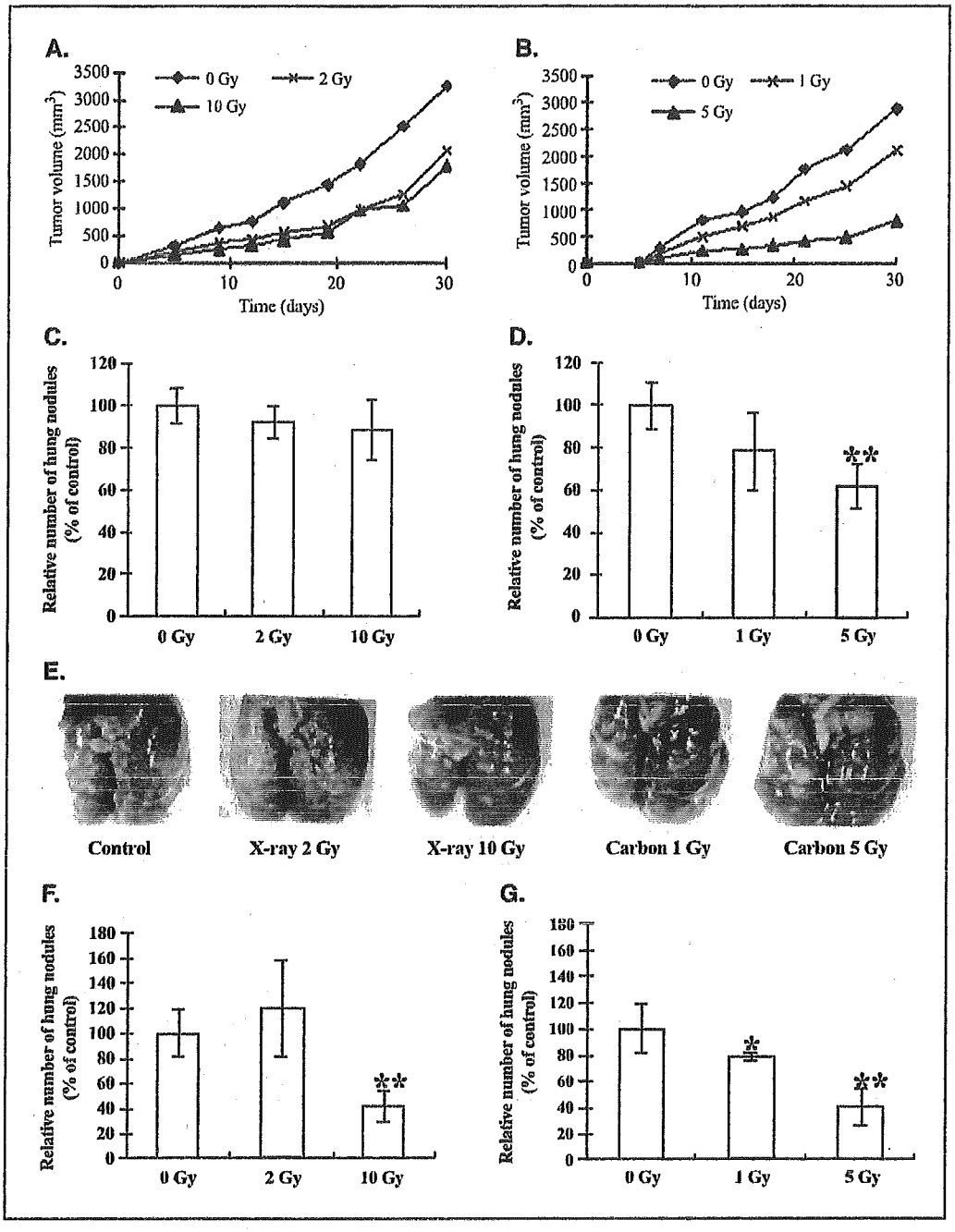

Figure 6. Metastatic capabilities of cancer cells irradiated with photon or carbon beam *in vivo*. Growth curves in subcutaneous tumors of LM8 osteosarcoma cells unirradiated, irradiated with X-ray (*A*), or carbon ion (*B*). The number of lung metastases treated with X-ray (*C*), or carbon ion (*D*) 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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ORIGINAL ARTICLE

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Radiation therapy for esophageal cancer: results of the patterns of Care Study in Japan 1995–1997

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Abstract
Purpose. To report the characteristics and treatment process of esophageal cancer patients treated with radiation therapy (RT) in Japan.
Methods and materials. The Patterns of Care Study (PCS) in Japan was conducted in 78 facilities nationwide including 40 academic (A) and 38 nonacademic (B) institutions using the original two-stage cluster sampling. Detailed information was accumulated on patients with cancer in the thoracic esophagus in stage I, II, or III who had received RT between 1995 and 1997.
Results. Of a total of 776 patients, 479 were treated in A institutions and 297 in B institutions. Median age was 67 years; 85.0% were men and 14.3% were women. Patients in stage I disease were more frequently identified in A institutions than in B (18.8% vs. 13.4%; $P = 0.001$). More than 99% had squamous cell carcinoma by histology, and the main tumors were often located in the midthoracic esophagus (62.2% of all patients). Pretreatment diagnostic modalities such as esophagram, endoscopy, endoscopic ultrasound, and computed tomography were done equally in A and B institutions. Chemotherapy was used for 39.7% of the patients. RT was combined with esophagectomy in the pre- or postoperative setting for 26.8% of the patients. Median fractionation and total external RT dose were 2 Gy and 60 Gy, respectively. Brachytherapy was more frequently used in A institutions than in B ($P = 0.001$).
Conclusions. This PCS study revealed the background and process of RT for esophageal cancer in Japan and also revealed the differences of the characteristics of patients and treatment procedures among two types of institutions.

Key words Patterns of Care Study · Esophageal cancer · Radiotherapy · International comparison

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Introduction

Esophageal cancer is a terrible disease, of which about 10000 patients die in Japan [1]. As the treatment approach, combined modalities with surgical resection, radiation therapy (RT), and chemotherapy (CT) have been investigated in some clinical trials, and many clinical experiences have been reported. According to randomized trial data showing the advantage of chemoradiation above RT alone [2,3], chemoradiation seems to have become the standard treatment for esophageal cancer. Brachytherapy has been used for esophageal cancer since the early half of the twentieth century. As high dose rate devices using iridium-192 became available, the efficacy and the complications of this modality were tested in the 1990s. Endoscopic ultrasound has been evaluated for its usefulness for evaluation of tumor extension of esophageal cancer and has become used in ordinary practice [4].

The first Patterns of Care Study (PCS) for esophageal cancer in the United States was conducted between 1995 and 1997, collecting data from 1992 to 1994 [5]. This study established the national benchmarks of RT of esophageal cancer in the United States and revealed the prevalence of chemoradiation. The most recent U.S. PCS for esophageal cancer has already been published and has confirmed

chemoradiation as the standard practice of this disease in the United States [6]. The PCS data format for esophageal cancer was imported to Japan from the United States courtesy of the American College of Radiology, and the first attempt of a PCS for esophageal cancer in Japan was carried out from 1996 to 1997, collecting data from 1992 to 1994 from 37 institutions [7–9]. Those results showed that the differences of personnel and equipment resources between academic and nonacademic institutions affect the process of esophageal cancer treatment. On the basis of these results, the following PCS study was planned extensively with the support of a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare (10-17, 14-6) in Japan.

The purpose of this study is to reveal patient characteristics and the treatment of esophageal cancer with radiation therapy in Japan from the point of view of institution strata.

Methods and materials

All the RT facilities, composed of more than 700 institutions in Japan, were divided into academic institutions (A), such as university hospitals or cancer centers, and nonacademic institutions (B). According to the Japanese facility master survey in 1990, A institutions were divided into A1, which treat 300 or more patients per year, and A2, which treat fewer than 300; B institutions are B1, which treat 120 or more patients per year, and B2, which treat fewer than 120. Two-stage cluster random samplings that included institutional sampling and patient sampling were conducted to select about 20 institutions in each facility cluster of A1–B2 and to accumulate esophageal cancer patients to investigate the data (Fig. 1) [10].

The PCS data format for esophageal cancer was customized to be input easily and directly using a portable personal computer, and training for collecting and imputting data was given for the members involved in this study.

The data were accumulated from 1998 to 2000 by extramural audits composed of radiation oncologists. They visited each selected institution and investigated the medical charts of the chosen patients, the RT reports, and imaging films including simulation graphics and lineac- or cobalt graphics. Although it was not easy to inspect all the medical records, the investigators tried to look up as many available data of the patients as possible. When they encountered unexpected problems dealing with the data, they placed a query on the Internet mailing list composed of all members of the group and administrative staffers. The problem was shared and discussed immediately online to solve it. Therefore, the uniformity and the accuracy of data entry were ensured.

Several criteria for selecting patients for this study were determined. Patients should have started RT between January 1, 1995 and December 31, 1997. Primary cancers of the thoracic esophagus were included. Tumor histology should be squamous cell carcinoma or adenocarcinoma or adenosquamous cell carcinoma. Thus, undifferentiated tumors, other rare tumors, or histology-undetermined cases

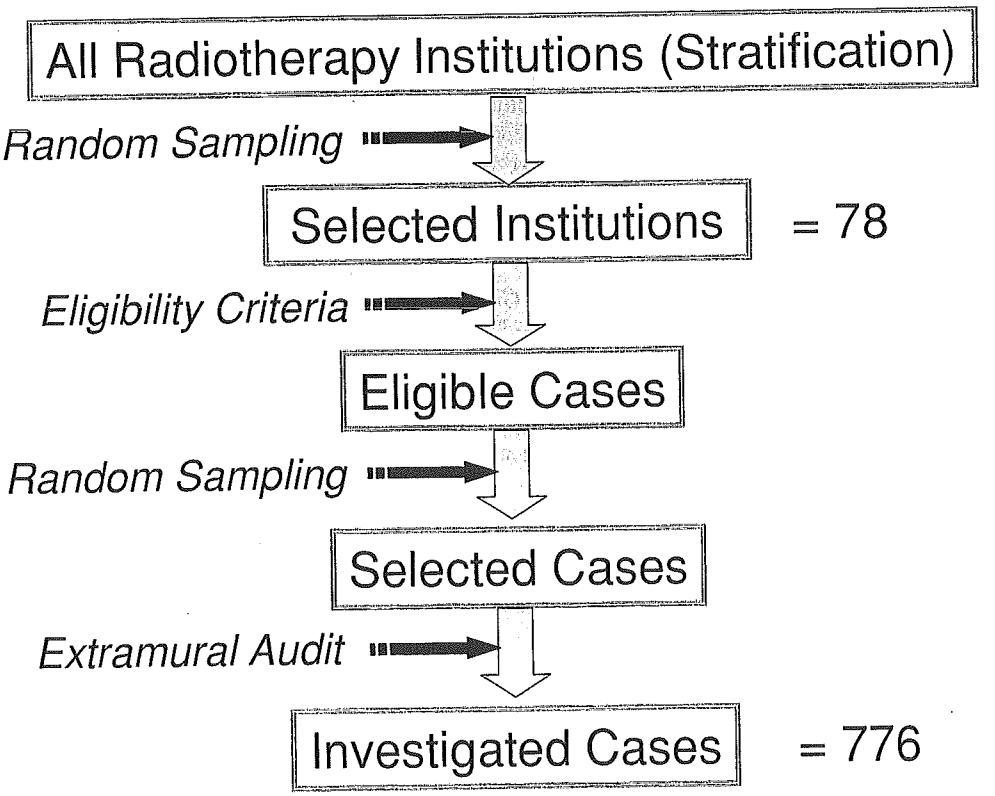

Fig. 1. The method of the Patterns of Care Study for esophageal cancer used in this study is briefly shown on this figure. Using two-stage cluster sampling, 78 radiation therapy institutions were randomly picked for investigation and a total of 776 eligible cases were investigated by extramural audit

were excluded. Patients with stage I, II, or III disease on Union Internationale Contra le Cancer (UICC) 1997 criteria were eligible. Therefore, patients with supraclavicular, neck, or abdominal lymph nodes metastases (M1a) and with distant metastases (M1b) were excluded. Depth of tumor invasion was evaluated by the "T" classification of the *Guide Lines for the Clinical and Pathologic Studies on Carcinoma of the Esophagus* (9th edition) [11]. In addition, restaging was performed on the modified 1983 American Joint Committee on Cancer (AJCC) staging system. This modified 1983 AJCC staging system, including evaluation of extraesophageal spread by computed tomography, was based on the clinical findings without surgical resection and is useful for evaluating the esophageal cancer patients who receive RT [12,13]. The staging system used in this study is as follows:

Stage I: lesion ≤5 cm in length, not obstructing and not circumferential
Stage II: lesion >5 cm in length or obstructing or circumferential
Stage III: evidence of extraesophageal spread

Patients with Karnofsky Performance Status (KPS) of 60 or more were eligible, and candidates should not have had other malignancies within 5 years before treatment. The PCS data in the United States used for comparison with this study are based on the reports from Coia et al. [5] and Suntharalingam et al. [6]. The chi-square test was used for comparison of patients and treatment factors.

Results

Detailed information was accumulated on 776 patients; 479 patients were treated in A institutions and 297 patients in B institutions. Numbers and percentages of the accumulated records of the patients on this study are shown in Table 1. Among all patients, 280 cases (36.1%) were treated in A1, 199 cases (25.6%) in A2, 204 cases (26.3%) in B1, and 93 cases (12.0%) in B2 institutions.

Workup

Patient and tumor characteristics in this study are shown in Table 2. The median age and minimums–maximum age range were 67 years and 31–92 years, respectively. Median ages of the patients who received surgery and those who did not were 63 and 70 years, respectively, and 85% of the patients were male. Median KPS value was 80; about three-quarters of the patients had a KPS greater than or equal to 80. Patients with good performance status were significantly more often observed in A institutions rather than in B institutions. Of the patients, 63% had the main lesion in the middle thoracic esophagus (Mt; upper half of the area between carina and esophagogastric junction). Almost all the patients (99.8%) had squamous cell carcinoma as shown by histology, and adenocarcinoma was found only in 2 patients. Although detailed T data were not available for 153 patients, 17.5% of the patients were classified as superficial cancer (Tis, 0.5%; T1a, 3.7%; T1b, 13.3%). Superficial cancer was more frequently treated in A institutions. According to the modified AJCC 1983 clinical stage, 18.8% of the patients were classified in stage I. The ratio of stage I was greater in A (21.8%) than in B (13.4%) institutions.

Table 1. Investigated institutions and patients with esophageal cancer in the Patterns of Care Study (PCS) in Japan, 1995–1997

Characteristic	No. of institutions	No. of patients (%)
Total no. of institutions	78	776
Academic (A)	40	479 (61.7%)
Treat ≥300/year (A1)	21	280 (36.1%)
Treat <300/year (A2)	19	199 (25.6%)
Nonacademic (B)	38	297 (38.3%)
Treat ≥120/year (B1)	19	204 (26.3%)
Treat <120/year (B2)	19	93 (12.0%)

For the diagnostic procedure, esophagrams and endoscopy were done for more than 90% of the patients (Table 3); 20.8% of patients received endoscopic ultrasound for evaluating their disease. No statistical significant differences were found between A and B institutions in using esophagram, endoscopy, endoscopic ultrasound, and computed tomography. According to tumor invasion depth, esophagram and endoscopic ultrasound were used for 73.9% and 45.5% of patients with T1a lesions, 85.4% and 44.3% of T1b, 97.7% and 21.3% of T2, 98.3% and 16.6% of T3, and 93.1% and 13.1% of T4, respectively. Among patients who were not treated with surgery, endoscopic ultrasound was used for 66.7% of T1a and 58.8% of T1b patients who received brachytherapy and for 42.9% of T1a and 41.4% of T1b patients who did not receive brachytherapy.

Treatment process

RT was delivered to all 776 patients. Surgery was done for 208 (28.4%) patients, and they received RT in the pre- or postoperative setting. Postoperative RT was more frequently used compared with preoperative RT. More patients received RT with surgery in A institutions than in B. Among 556 patients who did not receive surgery, more than half of them did not receive CT but were treated with RT alone. A combination of CT and RT (chemoradiation, CRT) was given to 39.7% of all patients, and the ratio did not differ between A and B institutions. More patients received CT in stage III disease than in stage I or II disease. Treatment processes including CT and surgery combined with RT in this study are shown in Table 4.

Agents used for CT were mainly 5-fluorouracil (5-FU) and cisplatin (Table 5). There were no significant differences between the ratio of use of cisplatin and 5-FU between A and B institutions. No patient was found to receive taxanes in this study.

Table 6 indicates the status of the implementation of RT on this study. External RT was delivered to most of the patients. Brachytherapy was applied to 94 patients, and only 6% did not receive external RT. Utilization of

Table 2. Patient and tumor characteristics of esophageal cancer (%), PCS Japan 1995–1997

Characteristic	Total: 776	Institutions		*P* value (A vs. B)
		A: 479	B: 297	
Age (years)				0.295
0–64	312 (40.2)	198 (41.3)	114 (38.4)	
65–74	260 (33.5)	161 (33.6)	99 (33.3)	
75–	204 (26.5)	120 (25.1)	84 (26.3)	
(Range)	31–92	31–90	43–92	
(Median)	67	67	68	
Sex				0.878
Male	659 (85.0)	405 (84.6)	254 (85.5)	
Female	111 (14.3)	69 (14.4)	42 (14.1)	
Unknown	6 (0.8)	5 (1.0)	1 (0.3)	
KPS				0.001
60	60 (7.9)	40 (8.5)	20 (7.0)	
70	126 (16.7)	60 (12.8)	66 (23.0)	
80	366 (48.4)	217 (46.3)	149 (51.9)	
90	181 (23.9)	131 (28.0)	50 (17.4)	
100	23 (3.0)	21 (4.5)	2 (0.7)	
Missing	20	10	10	
Histology				0.404
SqCC	761 (99.6)	477 (99.8)	284 (99.6)	
Adeno	2 (0.3)	1 (0.2)	1 (0.4)	
Adenosquamous	1 (0.1)	0 (0.0)	1 (0.1)	
Missing	12	1	11	
Tumor invasion (JSED 9th edn)*				
Tis	3 (0.5)	2 (0.5)	1 (0.4)	
T1a	23 (3.7)	18 (4.7)	5 (2.1)	
T1b	86 (13.3)	68 (17.7)	19 (7.9)	
T2	132 (21.2)	75 (19.5)	57 (23.8)	
T3	238 (38.1)	144 (36.5)	94 (39.2)	
T4	145 (23.2)	81 (21.1)	64 (26.7)	
Missing	153	96	57	
Clinical stage (AJCC 1983)				0.029
I	135 (18.8)	100 (21.8)	35 (13.4)	
II	227 (31.5)	145 (31.7)	82 (31.3)	
III	345 (47.9)	205 (44.8)	140 (53.4)	
Unknown	13 (1.8)	8 (1.7)	5 (1.9)	
Missing	56	21	35	
Main tumor location				0.048
Ut	91 (12.4)	68 (15.0)	23 (8.3)	
Mt	457 (62.2)	274 (60.2)	183 (65.8)	
Lt	164 (22.4)	102 (22.4)	62 (22.3)	
Others	21 (2.9)	11 (2.4)	10 (3.6)	
Missing	43	24	19	

SqCC, squamous cell carcinoma; Adeno, adenocarcinoma; adenosquamous, adenosquamous cell carcinoma; Ut, upper thoracic esophagus; Mt, middle thoracic esophagus; Lt, lower thoracic esophagus; KPS, Karnofsky Performance Status; JSED, Japanese Society for Esophageal Disease; AJCC, American Joint Committee on Cancer

* *P* value was not calculated because of many missing cases

Table 3. Percentage of patients undergoing evaluation procedures

	Total	Institutions		*P* value (A vs. B)
		A	B	
Esophagram	92.1%	92.1%	92.2%	0.473
Endoscopy	91.0%	92.2%	89.2%	0.371
Endoscopic ultrasound	20.9%	21.7%	19.7%	0.490
Computed tomography	84.7%	83.4%	86.9%	0.447

brachytherapy was significantly higher in A institutions than in B. For external RT, a high-energy (≥6 MV) machine was more frequently used in A institutions than in B. Although only 3.2% of the patients were treated with Co-60 in A institutions, 8.6% received this in B. All-fields irradiation on each treatment day was delivered to more than three-fourths of the patients. Most of the patients received RT in the supine position. Median total dose was 60 Gy and median fraction dose was 2 Gy, which did not differ between A and B institutions. More than half of the patients received 60 Gy or more with external RT. Hyperfractionation was applied to 22 patients.

Discussion

Because the investigation by the PCS is a retrospective procedure and is based on the information written on the

Table 4. Treatment used in the study overall by institution types and by stages (%)

	Total: 776	Institutions		Stages (AJCC 1983)		
		A: 479	B: 297	Stage I: 135	Stage II: 227	Stage III: 345
RT and CT (total)	308 (39.7)	190 (39.7)	118 (39.7)	36 (26.7)	84 (37.0)	177 (51.3)
Alone	192 (24.7)	103 (21.5)	89 (30.0)	18 (13.3)	43 (18.9)	123 (35.7)
With surgery	116 (14.9)	87 (18.2)	29 (9.8)	18 (13.3)	41 (18.1)	54 (15.7)
Pre-op	33 (4.3)	27 (5.6)	6 (2.0)	5 (3.7)	12 (5.3)	16 (4.6)
Post-op	71 (9.1)	50 (10.4)	21 (7.1)	12 (8.9)	25 (11.0)	32 (9.3)
Pre & Post-op	6 (0.8)	6 (1.3)	0 (0.0)	0 (0.0)	3 (1.3)	3 (0.9)
Unknown	6 (0.8)	4 (0.8)	2 (0.7)	1 (0.7)	1 (0.4)	4 (1.2)
RT without CT (total)	430 (55.4)	275 (57.4)	155 (52.2)	94 (69.6)	139 (61.2)	156 (45.2)
Alone	338 (43.6)	208 (43.4)	130 (43.8)	71 (52.6)	106 (46.7)	128 (37.1)
With Surgery	92 (11.9)	67 (14.0)	25 (8.4)	23 (17.0)	33 (14.5)	28 (8.1)
Pre-op	21 (2.7)	12 (2.5)	9 (3.0)	1 (0.7)	9 (4.0)	10 (2.9)
Post-op	64 (8.2)	49 (10.2)	15 (5.1)	22 (16.3)	23 (10.1)	13 (3.8)
Pre & Post-op	4 (0.5)	3 (0.6)	1 (0.3)	0 (0.0)	0 (0.0)	3 (0.9)
Unknown	3 (0.4)	3 (0.6)	0 (0.0)	0 (0.0)	1 (0.4)	2 (0.6)
Unknown and missing	38 (4.9)	14 (2.9)	24 (8.1)	5 (3.7)	4 (1.8)	12 (3.5)

RT, radio therapy; CT, chemotherapy; Pre-op, preoperative; Post-op, postoperative

Table 5. Agents used and timing of administration in patients who received chemotherapy

Agents used	Total	Institutions		*P* value (A vs. B)
		A	B	
5-FU	81.7%	80.8%	83.2%	0.9652
Cisplatin	83.0%	85.0%	79.8%	0.5051
Mitomycin	1.9%	0.0%	5.0%	0.0018

medical chart, a certain degree of error might be included and reliability may differ depending on the types of data. To resolve these points and to increase the reliability of the Japanese PCS, audits were exclusively by active radiation oncologists who received training for this investigation. An internet mailing list was actively used to solve unexpected problems. Furthermore, erroneous data were checked at the analysis center.

This study considered it important to compare the treatment process between A and B institutions, because one of the purposes of this PCS is to contribute to equalization of treatment opportunity for cancer patients. As the universal care system is established and covers most of diseases in Japan, and patients can choose any hospital regardless of their residence or their economic status, unequal distribution of medical resources means that care delivery can be unfair.

Although the median age of patients was the same in A and B institutions, distribution of KPS and clinical stage was not the same, and patients in good status and in early stage were more frequently treated in A than in B. Patient condition may affect the combination of surgery, whose rate was higher in A institutions. Utilization of evaluation methods was not different between A and B institutions, which indicates that pretreatment examination was performed almost equally regardless of institution strata in Japan. However, the use of high-energy external RT machines (≥6MV) was not the same in both institutions. A high-energy linear accelerator is desirable for mediastinal irradiation to reduce the excess dose to the normal lung, heart, or spinal cord, and the cobalt-60 machine may not be suitable for treating thoracic esophageal cancer. Of the patients treated in B institutions, 8.6% received RT with a cobalt-60 machine, but most patients in A institutions were treated by a high-energy machine of 6MV or higher. System replacement from a cobalt-60 machine to high-energy equipment should be done, especially in B institutions. Median external fractionation and total dose of RT were 2 Gy and 60 Gy, respectively, which did not differ between A and B. However, brachytherapy was more frequently used in A institutions than in B. Because the number of high dose rate (HDR) machines in Japan was increased from about 20 to 60 between 1995 and 1997, predominantly in the A institutions, they might have used HDR for the purpose of investigating the treatment of esophageal cancer.

Administration of CT was about 40% overall and did not differ between A and B. The ratio of use of CT increased with stage, but only half of the patients received CT, even in stage III. More than half the stage I patients were treated with RT without using CT. Some reports support that preoperative CRT is beneficial for local control and survival for esophageal cancer [4,14], but it was used for only 4.5% of the patients. One reason of less use of CT might be the high percentage of low KPS patients who are not eligible for CT. As some reports evaluating the efficacy and toxicities of CRT for esophageal cancer have been published from Japan [15,16], we should consider the transition and prevalence of CRT in the next study. The main agents used for CT were cisplatin and 5-FU in this study. The use of new agents such as taxanes should also be evaluated in the future.

Comparison between the countries is also available using the results of this study. It is important to investigate the differences of patient characteristics and treatment proce-

Table 6. Status of implementation of radiation therapy (RT)

	Total	Institutions		*P* value (A vs. B)
		A	B	
Combination of external RT and brachytherapy				0.001
External beam only	87.7%	82.4%	96.3%	
Brachytherapy only	0.8%	1.3%	0.0%	
Both	11.5%	16.3%	3.7%	
External-beam RT				
Machine energy				0.001
Co-60	5.3%	3.2%	8.6%	
≤4 MV	16.8%	10.0%	27.8%	
6 MV	12.5%	8.3%	19.2%	
10 MV	59.7%	69.2%	44.3%	
>10MV	5.8%	9.3%	0.0%	
All fields treated/fx				0.058
Yes	76.5%	77.4%	75.1%	
No	22.6%	21.1%	24.9%	
Unknown	0.9%	1.5%	0.0%	
Patient position				0.002
Supine	96.1%	97.5%	93.9%	
Prone	3.2%	1.5%	6.1%	
Other	0.6%	1.0%	0.0%	
Total dose (range)				0.029
<40 Gy	10.7%	11.0%	10.3%	
≥40–<50 Gy	15.3%	16.4%	13.4%	
≥50–<60 Gy	19.4%	20.8%	17.1%	
≥60 Gy	54.6%	51.8%	59.3%	
Total dose (median)	60 Gy	60 Gy	60 Gy	
Dose/fx (Range)				0.001
≤1.8 Gy	33.3%	33.3%	33.3%	
>1.8–≤2.0 Gy	58.1%	60.9%	53.3%	
>2.0–≤3.0 Gy	5.6%	3.2%	9.8%	
>3.0 Gy	0.0%	0.0%	0.0%	
Other	3.0%	2.6%	3.6%	
Dose/fx (median)	2.0 Gy	2.0 Gy	2.0 Gy	
Hyperfractionation	2.8%	2.5%	3.4%	
Brachytherapy				
Total	12.3%	17.6%	3.7%	0.001
HDR	12.0%	17.2%	3.7%	0.001
Total dose (range)	4–50 Gy	5–32 Gy	4–50 Gy	
Total dose (median)	12 Gy	14 Gy	10 Gy	
LDR	0.3%	0.4%	0.0%	

fx, fractions; HDR, high dose rate; LDR, low dose rate

dures for esophageal cancer [17]. More than 99% of the patients in this study had squamous cell carcinoma and only a few patients had adenocarcinoma, which was consistent with the reports of the Japanese Society for Esophageal Disease [18]. The ratio of adenocarcinoma in the United States was 49.6%. Although it is not easy to compare the ratio in the two countries because cancer located at the esophagogastric junction in Japan is likely to be classified as gastric cancer, the histological difference may influence treatment strategy. The use of CT was extremely different between the United States and Japan (89% vs. 39.7%). Considering the higher total RT dose was used in Japan compared to the United States (60 Gy vs. 50.4 Gy), it may be said that more efforts were anticipated in RT than in CT in Japan.

This study reveals the nationwide background and treatment process of esophageal cancer patients treated with RT in Japan. The results of this study will provide good information about RT for esophageal cancer and may contribute to evaluating institutional differences and time-dependent transitions of background and treatment strategies of this disease.

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Review Article

Patterns of Care Study in Japan

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Patterns of Care Study in Japan

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Background: The Patterns of Care Study (PCS), started in the 1970's, is a well-known study used for clinical quality assurance (QA) in radiation oncology in the United States. PCS has been introduced in Japan since 1996.
Methods: Three national PCS surveys have been performed by means of external audit to evaluate patterns of care for the patients with carcinoma of any of esophagus and cervix treated with radiation between 1992 and 1994, for those with carcinoma of any of esophagus, cervix, breast, lung and prostate between 1995 and 1997, and for those with any of the five disease sites between 1999 and 2001. In the first PCS, feasibility of the study was confirmed. In the second PCS, two-stage cluster sampling of institutions and patients was performed and national averages for the survey items were calculated as QA measures. In the third PCS, additional imaging data were collected. The Japan/USA PCS workshops were held at San Francisco in 2001 and at Tokyo in 2003.
Results: Significant variations in process and structure were observed according to institutional stratification. In academic institutions, external beam energy ≥6 MV for deep-seated tumors of esophagus, lung, prostate and cervix, and brachytherapy for those of cervix and esophagus were used more frequently. There was an average of less than one full-time equivalent radiation oncologist in most non-academic institutions. These variations influenced the outcomes. There were also significant differences between USA and Japan in various aspects, e.g. a difference in radiation dose of 20% for uterine cervix cancer patients. It is higher in the USA. The number of new cancer patients requiring radiation is increasing steeply (120 000 in 2000 and 170 000 in 2005). Based on PCS data, structural guidelines were published and distributed throughout Japan.
Conclusion: PCS is useful for establishing the clinical QA for radiation oncology as well as other specialties through detailed monitoring and evaluation of their structures, processes and outcomes.

Key words: Patterns of Care Study – process – radiation oncology – structure – outcome

INTRODUCTION

The idea of the Patterns of Care Study (PCS) for use in radiation oncology was developed in 1969 by Kramer and Herring (1). The National Cancer Institute funded the American College of Radiology (ACR) to perform PCS in 1974 (2). They applied Donabedian's model of quality assessment to PCS as shown in Fig. 1 (3). In this model, quality of care was evaluated by monitoring the structure, process and outcome of the actual treatment of patients and understanding the relationships among these three factors. Structure of all radiotherapy facilities in the United States was monitored by questionnaire in terms of size, equipment, personnel and location, resulting in the establishment of a facility master list (FML) that have been updated every 5 years. The process comprises actions to evaluate and treat patients. The items included in the process surveys were determined by adapting best current management practices and decision trees for target disease sites, in which radiation therapy plays an important role. Outcome includes results for patients such as survival or complication rates. PCS was performed every 4 or 5 years for the patients treated with radiation in 1974, 1978, 1983, 1989, 1994 and 1999. Outcome surveys were continually updated every 5 years for the same patients after the initial process and outcome surveys. Important evidence disclosed by the American PCS that even

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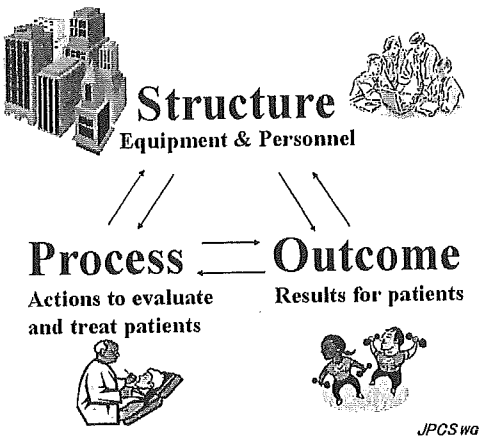

Figure 1. Donabedian's model of quality assessment for PCS (3).

Table 1. Important evidence disclosed by the American PCS that even elementary radiotherapy techniques are important

	Nationwide outcome (%)	
	Complication	Recurrence (survival)
AP–PA only→other technique (4)		
Prostate cancer	6→3	N/A
One field/day→ >one field/day (4)		
Cervix cancer	13.5→4.8	N/A
Appropriate dose selection (Gy) (6)		
Prostate cancer T3 (Ext. RT <55→ >70)	N/A	38→10
Appropriate beam selection (4)		
Prostate cancer T3 (<6 MV→ ≥6 MV)	N/A	31→19
Use of intracavitary irradiation (5)		
Cervix cancer	14→0	41→17 (37→70)

AP-PA, antero-posterior and postero-anterior directions; Ext. RT, external radiotherapy.

elementary radiotherapy techniques are important is shown in Table 1. For example, technical improvements resulting from changing anterior–posterior parallel opposed fields to other multiple fields for prostate cancer and from one field/day to >one field/day (4), and the utilization of brachytherapy for uterine cervix cancer (5) significantly reduced complication rates nationwide. In addition, appropriate dose selection for prostate cancer from 55 to 70 Gy (6) and beam selection from <6 to ≥6 MV (4) significantly reduced recurrence rates. Finally, the introduction of brachytherapy for uterine cervix cancer improved survival dramatically (5). For the improvement of nationwide outcomes for cancer patients, even these elementary and simple techniques must therefore be performed carefully and extraordinarily well. This is the basic concept of clinical quality assurance (QA) projects nationwide. PCS monitors accomplishments in terms of process and outcome within a specific time window.

When we introduced PCS in Japan, Dr G. E. Hanks, form chairman of the Department of Radiation Oncology at Fc Chase Cancer Center and Principal Investigator of the PC and Dr J. B. Owen, Director of PCS, ACR, supported us in a technical and academic aspects and have continued to encou age us (7–9). In this review article, we summarize the metho and most important achievements of PCS in Japan as well its significance and future goals.

PROGRESS OF JAPANESE PCS

First PCS (PCS92–94)

In 1996, we introduced PCS in Japan with partial support frc a Grant-in-Aid for Cancer Research Groups from the Minist of Health, Labor and Welfare in Japan (nos 8–27 and 8–29). V selected esophagus and cervix cancers as target disease sit because annual numbers of these patients exceeded 4000 i either cancer type according to a structure survey by the Jap Society of Therapeutic Radiology and Oncology (JASTR(We used the same database as that of American PCS, courte of ACR, which was installed on a personal computer. Since important characteristic of PCS is data collection by exter audit, we selected 37 radiotherapy facilities nationwide for c PCS survey and the audit was performed from July 19 to February 1998 by the author and detailed informati was collected for 561 patients with esophageal cancer (7 and 490 patients with cervix cancer (9). However, we could i use the original two-stage cluster sampling employed for American PCS because of budget limitations for the first tri

Second PCS (PCS95–97)

In 1998, PCS was fully supported by a Grant-in-Aid from Ministry of Health, Labor and Welfare in Japan (no. 10– (10). The PCS data format was determined and the relev computer software was developed for five disease sites, breast, esophagus, cervix, lung and prostate, through consen panel discussion to establish the best current management a guidelines. An external audit team of 23 radiation oncolog was recruited from 10 academic institutions. External aud were performed from September 1998 to January 2001 for participating institutes. We stratified radiotherapy facili nationwide into four categories according to the FML crea by JASTRO in 1995. This stratification was based on academic conditions and the annual number of patients trea with radiation in each institution, because academic inst tions require and have access to more resources for educat and training while the annual caseload also constitutes ess tial information related to structure. Patients with carcinom any of the five disease sites were randomly selected by me of two-stage cluster sampling consisting of sampling of ir tutions from the four institutional strata in the first stage sampling of patients from these institutions in the second st (Fig. 2). Detailed and accurate data for 4399 patients v carcinoma of any of the five sites were collected by au National averages (NAs) and regional averages of vari