Herein, we report a novel utilization of cancer cell microencapsulation that acts as an effective seed of liver metastasis in a rat model. Transplantation of *ex vivo* precultured 300 μm cancer microcapsules, formed from three different human pancreatic cancer cells, into the liver of nude rats via a portal vein resulted in efficient and stabile production of liver metastases.

Materials and Methods

Cell Lines

Three human pancreatic cancer cell lines (SUIT-2, AsPC-1, and BxPC-3) were used. SUIT-2 cells were generously provided by Dr. Iwamura (Miyazaki Medical College, Miyazaki, Japan; ref. 22). AsPC-1 and BxPC-3 were obtained from the American Type Culture Collection (Bethesda, MD). SUIT-2 was maintained in DMEM (Sigma-Aldrich, Taufkirchen, Germany) containing 5% fetal bovine serum (Sigma-Aldrich). AsPC-1 and BxPC-3 cultured in RPMI 1640 (Sigma-Aldrich) supplemented with 10% fetal bovine serum.

Cancer Cell Encapsulation to Form Artificial Cancer Cell Aggregates

Cancer microcapsules were engineered by conventional coaxial airflow methods (23). The size of the cancer microcapsules was targeted to ~300 μm because capsule sizes >100 μm were assumed to be beneficial for physical trapping in the peripheral portal vein in the liver, whereas those <300 μm are technically difficult to produce.

SUIT-2 cancer cell pellets were suspended in a 1.5% solution of potassium alginate (Kimica Corp., Tokyo, Japan) and the density was adjusted to ~1×10^7 cells/mL. AsPC-1 and BxPC-3 cancer cell pellets were encapsulated in the same manner, except that Matrigel (BD Biosciences, Bedford, MA) in a 25% (vol/vol) was added and the cell density was increased to ~2×10^7 cells/mL, because the proliferation of these cell lines was slow in pure alginate alone. The cell-alginate mixture was extruded through a 31-gauge needle at 5.0 mL/min and sheared by airflow, resulting in the formation of droplets having a diameter of 300 μm. The alginate droplets were allowed to directly fall into a cationic solution of 1.1% $CaCl_2$, promoting gel formation. The calcium alginate beads were chemically cross-linked with 0.05% (wt/vol) poly-L-lysine in 0.9% NaCl for 3 minutes. The capsules were recoated with 0.03% (wt/vol) alginate in 0.9% NaCl for 4 minutes. Finally, the remaining alginate core was dissolved with 1.6% (wt/vol) sodium citrate for 6 minutes.

In vitro Culture of Cancer Microcapsules: Capsule Burst, Histology and Cell Proliferation

Cancer microcapsules were incubated *in vitro* for several days at 37°C in 5% CO_2 to ensure that they were fully viable at the time of administration to rats. The time when >10% of cancer microcapsules burst was defined as the bursting day for each cell line. Two or 3 days before the bursting day was assumed to be the optimal time for portal injection. The histology of cancer microcapsules at the optimal day for portal injection was observed by embedding cancer microcapsules in optimum cutting temperature compound (Diagnostic Division, Miles, Inc., Elkhart, IN) and frozen sections were stained with H&E. To analyze the cell number included in each capsule at the optimal day for portal injection, microcapsules were sampled, enzymatically digested, and cells were counted using a hematocytometer.

Injection of Cancer Microcapsules in Nude Rats

Nude rats (male F344/NJcl-mu rats), 6 weeks of age with a weight of 100 to 125 g (Clea Japan, Tokyo, Japan), were employed. The rats were anesthetized by i.p. injection of pentobarbital, a midline incision was made, and the portal vein was exteriorized and linearized, thus enabling the insertion of a heparinized 20-gauge catheter (Terumo, Tokyo, Japan). A catheter was inserted at the very distal part of the mesenteric vein, near the cecum, and advanced 4 cm towards the liver and the tip of catheter was placed at the major trunk of the portal vein, with the point 5 to 8 mm near the liver hilum. The cancer microcapsules suspended in 1 mL saline were injected manually at ~0.1 mL/s and flushed with 0.5 mL of saline. The site where the catheter was inserted was ligated for hemostasis with 5-0 nylon sutures. Ligation of this point never caused intestinal necrosis because collateral vessel networks are well formed in the rat.

All animal experiments were done with the approval of the Animal Research Committee of the University of Tsukuba. Animals were maintained in a barrier facility on HEPA-filtered racks and fed with autoclaved laboratory rodent chow.

Liver Metastases Production by Portal Vein Injection of Cancer Microcapsules or Single Cell Suspension

To produce liver metastases in nude rats by injection of cancer microcapsules via the portal vein, 3,000 cancer microcapsules for each rat were administered: 12, 6, and 6 nude rats were employed for SUIT-2, AsPC-1, and BxPC-3 microcapsules, respectively. The same number of single cells included in 3,000 cancer microcapsules (2.1×10^6 for SUIT-2 and 4.5×10^6 for AsPC-1 and BxPC-3) were also injected via the portal vein. In order to inject a homogeneous single cell suspension, excluding cell aggregates or clumps, cell solutions were passed through a mesh strainer with a 40 μm pore size prior to administration. Six nude rats were employed for single cell injection of SUIT-2, AsPC-1, and BxPC-3, respectively. We assumed that the appropriate metastatic extent for evaluating the procedure would be ~10% to 20%. All rats were sacrificed at different times depending on the cell lines (SUIT-2 at 4 weeks, AsPC-1 at 6 weeks, and BxPC-3 at 5 weeks) with the intent of obtaining metastases with a suitable extent.

Evaluation of Sacrificed Nude Rats, Injected Cancer Microcapsules, or Single Cancer Cells

Incidence of liver metastases. To assess the potential for liver metastases, the incidence of liver metastases was evaluated macroscopically. The rate of liver metastases was defined as the number of rats positive for liver metastases divided by the number of experiments. Formalin-fixed, paraffin-embedded sections were subjected to microscopic examination.

Undesired metastases to sites other than the liver. To evaluate whether metastases occurred only in the liver, other organs and areas, i.e., peritoneal cavity, injection site, and lungs were carefully examined macroscopically. Any suspicious lesion was removed and subjected to histologic analysis.

Numerical evaluation of the extent of metastatic liver nodules: volumetric examination. In order to quantify the objective extent of liver metastases, a numeric calculation was employed. The metastatic extent was defined by the following formula: metastatic extent (%) = (metastatic volume / volume of entire liver) × 100. Formalin-fixed livers were divided into four lobes (left, middle, right, and caudate) and each lobe was then cut to a thickness of 2 mm. Next, the area of metastatic nodules in all serial sections was measured using image-processing software WinROOF (Mitani Corporation, Fukui, Japan). The metastatic volume and total volume of the liver were calculated by integration.

Variation of the Extent of Liver Metastases by Injecting Different Numbers of Cancer Microcapsules

To determine whether the extent of liver metastases varied according to the number of cancer microcapsules injected, various numbers of SUIT-2 microcapsules were injected. Five, 12, 7, and 8 nude rats were injected with 6,000, 3,000, 1,000, and 333 microcapsules, respectively, and both the incidence of liver metastases and the extent of tumor volume affected were calculated.

In vivo Sequential Observation of Cancer Microcapsule–Derived Liver Metastases

In order to assess the development of liver metastases from cancer microcapsules, livers were extracted from nude rats at days 3, 7, or 28 after portal injection of 3,000 SUIT-2 microcapsules. Livers were cut into serial 2 mm sections and stained with H&E to determine (*a*) distribution of cancer microcapsule, (*b*) status of microcapsules, i.e., whether capsules were ruptured or unruptured, and (*c*) distribution and size of liver metastases.

Pathophysiology of Liver Metastases: Cancer Microcapsules in Rats and Single Cell Injection in Mouse

The pathophysiology of liver metastases in nude rats generated by cancer microcapsules and those derived with conventional methods, i.e., injection

of single cells into the spleen in nude mouse were assessed. The following variables were evaluated: (*a*) macroscopic location of metastatic nodules, (*b*) microscopic histopathology of tumors, (*c*) desmoplastic reaction, and (*d*) neovascularization. Cancer microcapsule–derived liver metastases were assessed on day 28 in nude rats injected with 3,000 SUIT-2 microcapsules. Because single cell injection to the spleen of nude rats never generated liver metastases in our hands, nude mice were employed. Liver metastases derived from single cells were assessed 28 days after splenic injection of 2.1×10^6 cells/50 μL of SUIT-2 cells in nude mice. The macroscopic location and histopathology were analyzed using representative H&E stained slides. The extent of the desmoplastic reaction was compared by evaluation of collagen fibers visualized by Masson trichrome staining. Neovascularization was evaluated by the microvessel count (MVC) method as reported by Weidner et al., with minor modifications (24). Representative sections were stained immunohistochemically with anti–von Willebrand factor antibody (polyclonal rabbit anti-human factor VIII–related antigen; Dako Corporation, Santa Barbara, CA). The number of von Willebrand factor–positive vessels were counted and the average counts of five selected hotspots, i.e., the highest neovascularization areas in high power (×200) fields, were recorded as the MVC for each case (25).

Assessment of the Efficacy of Anticancer Drugs Using the Present Liver Metastases Rat Model

To investigate whether the rat liver metastases model was useful in evaluating the effect of anticancer drugs, 15 nude rats portally injected with 3,000 SUIT-2 microcapsules were randomly subdivided into three groups (five animals per group) on day 7. The first group was treated with gemcitabine (Gemzar, Eli Lilly, Indianapolis, IN) administrated via the dorsal vein at 80 mg/kg twice a week for 3 weeks (26). The second group was treated with irinotecan (Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan) at a dose of 60 mg/kg twice a week for 3 weeks (10). The third control group received 0.5 mL saline solution twice a week for 3 weeks. All rats were sacrificed on day 28 and the extent of metastases was determined as described above.

Statistical Analyses

Differences in the metastatic rate between cancer microcapsules and single cell suspensions were analyzed using Fisher's exact test. Variations in the extent of liver metastases by injecting different numbers of cancer microcapsules were compared by one-way ANOVA. A $P < 0.05$ was considered statistically significant. Statistical calculations were done with the StatView software package (version 5.0, Abacus Concepts, Inc., Berkeley, CA).

Results

In vitro Culture of Cancer Microcapsules: Capsule Burst, Histology, and Cell Proliferation

The size of cancer microcapsules engineered in the present study was quite uniform (Fig. 1*A*). The average diameter of 100 randomly sampled microcapsules of SUIT-2, AsPC-1, and BxPC-3 microcapsules was 305 ± 39, 298 ± 21, and 362 ± 35 μm, respectively. Sequential *in vitro* observation of microcapsules showed that the cancer cells in microcapsules proliferated well and formed spheroids at days 5 to 7. Histologic observation of cancer

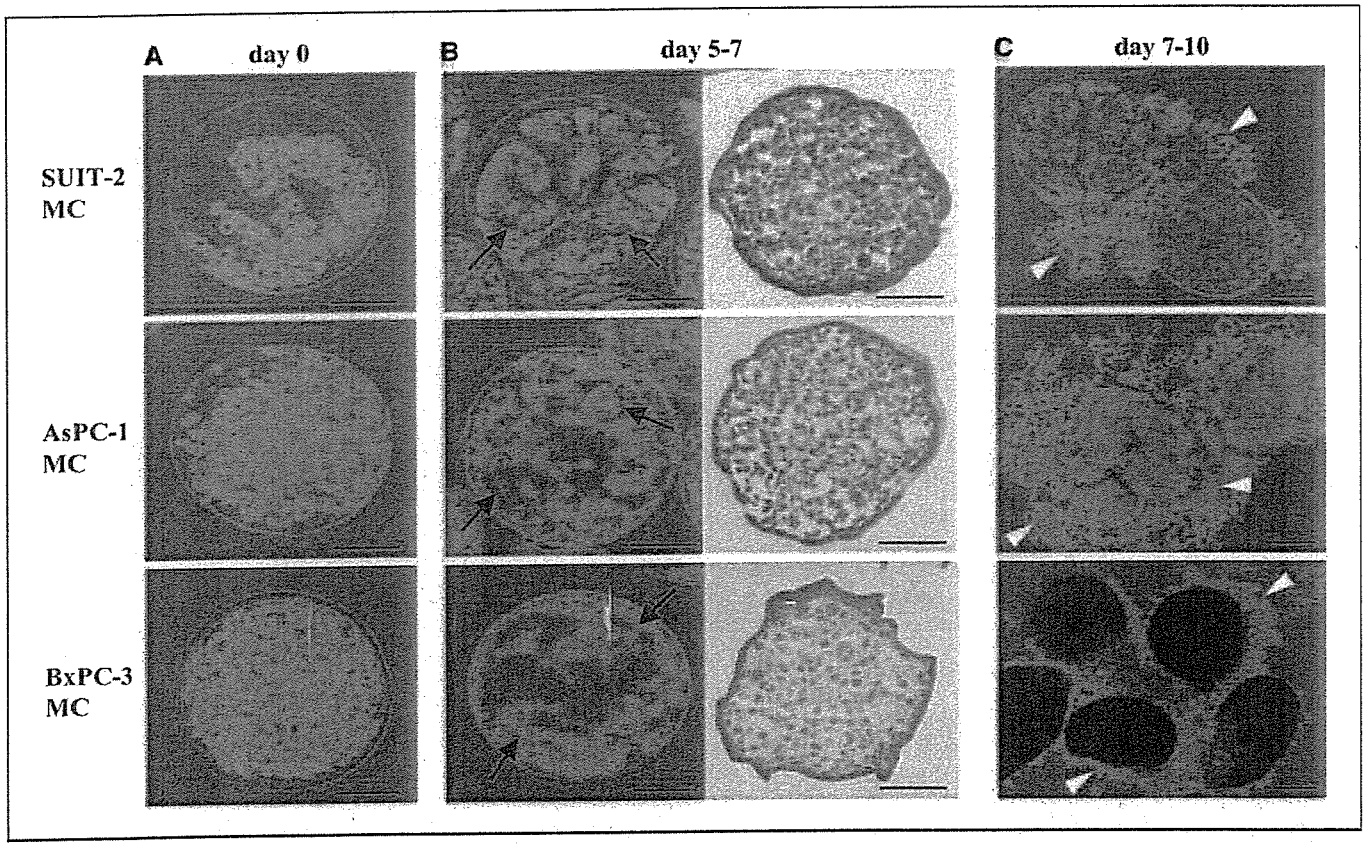

Figure 1. *In vitro* culture of cancer microcapsules: sequential observation and histology. Human pancreatic cells were encased in 300 μm alginate microcapsules using coaxial airflow (see Materials and Methods). *A*, representative pictures of cancer microcapsule from SUIT-2, AsPC-1, and BxPC-3 cells at day 0, immediately after production. *B*, after 5 to 7 days of *in vitro* incubation, cancer microcapsules were filled with proliferated cells forming spheroids (*arrows*). Histologic observation of frozen section stained with H&E of cancer microcapsules at the optimal day of portal injection showed three-dimensional proliferation of cancer cells. *C*, cancer microcapsules began to burst at days 7 to 10. Cancer cells, extruded from ruptured capsules continued to proliferate (*white arrowheads*). *Bars*, 100 μm.

microcapsules showed that all the cancer microcapsules proliferated in a three-dimensional manner (Fig. 1*B*). Further *in vitro* culture resulted in bursting of the outer layer of cancer microcapsules, and cancer cells were finally deviated outward and continued to grow in flasks (Fig. 1*C*). Although the speed of cell proliferation in cancer microcapsules differed between the three cell lines, all three cancer microcapsules burst nonetheless. The bursting times of SUIT-2, AsPC-1, and BxPC-3 microcapsules were on days 7, 7, and 10, respectively. From these results, the optimal day for portal injection of microcapsules from SUIT-2, AsPC-1, and BxPC-3 was assumed to be on days 5, 5, and 7, respectively. The average number of cells that were encased in one cancer microcapsule, at the optimal day for portal injection, was 718 cells for SUIT-2, 1,530 for AsPC-1, and 1,640 for BxPC-3.

Assessment of Liver Metastases Production in Nude Rats, Injected Cancer Microcapsules, or Single Cancer Cells

The metastatic potential to the liver of these three cell lines, by either single cell injection into the spleen and/or orthotopic implantation, has been reported to be different. SUIT-2 provides constant overt liver metastases (7). AsPC-1 is known to generate liver metastases, however, the extent of metastases is variable (7, 27). BxPC-3 does not cause liver metastases as reported by several authors (7, 17). Injection of three cancer microcapsules formed from different cell lines resulted in the successful production of overt liver metastases in all cases (Fig. 2).

Rate of liver metastases. The rate of liver metastases in rats injected with SUIT-2 microcapsules, AsPC-1 microcapsules, and BxPC-3 microcapsules was 100% (12 of 12), 100% (6 of 6), and 83% (5 of 6), respectively. In contrast, no liver metastases were formed by the injection of nonencapsulated single cancer cells (Table 1). Macroscopic observation and hepatic cross-sections showed that metastatic nodules tended to precipitate at peripheral hepatic margins (Fig. 2).

Metastases at organs other than the liver. No metastatic lesions other than in the liver were seen, and peritoneal, injection site, or pulmonary dissemination was not observed. However, occasional skin incisional wound metastases was seen in two rats that were injected with 3,000 SUIT-2 microcapsules (Table 1).

Numerical evaluation of the metastatic liver nodules: volumetric analyses. The metastatic extent to the liver produced by the injection of SUIT-2, AsPC-1, and BxPC-3 microcapsules was 14.6 (15.9/107.1 cm^3) ± 7.0%, 9.7 (11.4/113.2 cm^3) ± 5.8%, 15.0 (19.1/111.4 cm^3) ± 12.5%, respectively. The macroscopic extent of liver metastases is known to be larger than the calculated tumor volume. Our previous study showed that the tumor volume of clinically massive liver metastases remains ~10% to 30% when calculated by computed volumetry (28).

Control of the Extent of Liver Metastases by Injection of Varying Numbers of Cancer Microcapsules

The rate of liver metastases in rats injected with 6,000, 3,000, 1,000, and 333 microcapsules was 100% (5 of 5), 100% (12 of 12), 86% (6 of 7), and 50% (4 of 8), respectively. The extent of metastases in rats injected with 6,000, 1,000, and 333 microcapsules was 29.5 (46.0/146.2 cm^3) ± 13.1%, 1.9 (1.8/96.5 cm^3) ± 1.9%, 0.2 (0.2/88.4 cm^3) ± 0.3%, respectively (Fig. 3).

In vivo Sequential Observation of Liver Metastases Derived from Cancer Microcapsules

Distribution of cancer microcapsules at 3 days after portal injection in liver showed that two-thirds were lodged in the small peripheral (20–50 μm) portal veins, although one-third were trapped in the central wide (200–500 μm) portal vein (Fig. 4*a1*). It should be noted that 300 μm of microcapsules reached peripheral regions more than initially expected because the diameter of Glisson's sheath or the portal vein neighboring cancer microcapsules lodged were ~20 to 50 μm in diameter (Fig. 4*a2*). A total of 175 cancer microcapsules were observed in 10 representative slices on day 3, 35% (62 of 175) of which were ruptured. The proportion of ruptured microcapsules increased to 70% (88 of 128) at day 7 and 100% at day 28. Although intact cancer microcapsules should also be involved in the formation of metastatic foci, all cancer microcapsules at day 28 were assumed to be ruptured because almost all were buried in tumor nodules. Sequential

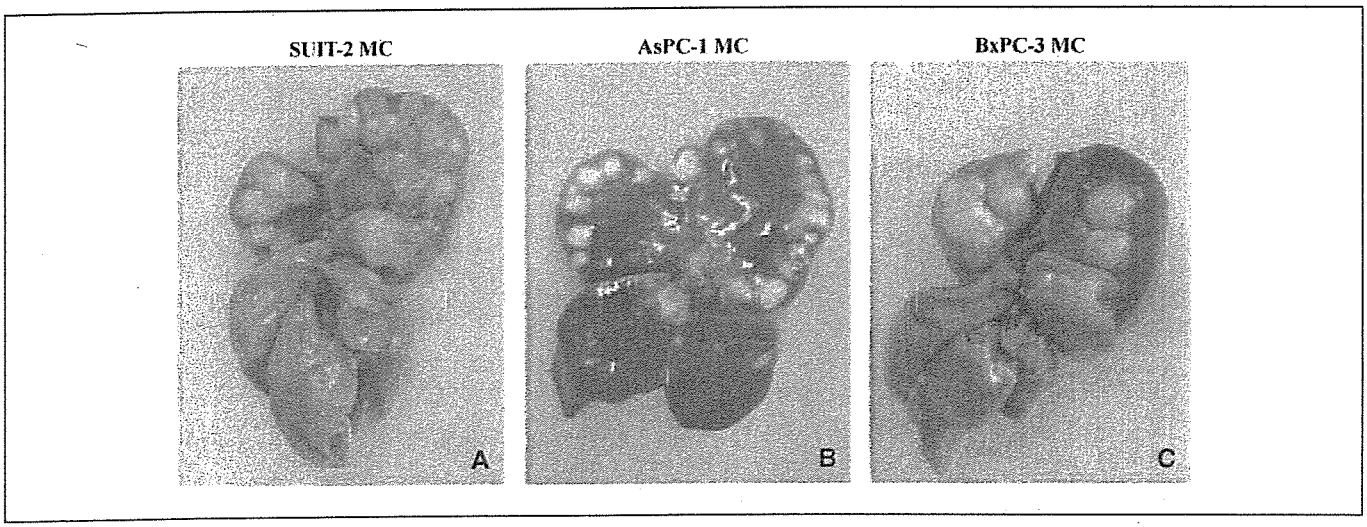

Figure 2. Overt liver metastases in nude rats by injection of three different human pancreatic cancer microcapsules via the portal vein. Representative gross appearance of rat livers at 28 days after injection of SUIT-2 microcapsules (*A*), 42 days after injection of AsPC-1 microcapsules (*B*), and 35 days after injection of BxPC-3 microcapsules (*C*). The hepatic metastatic extent produced by injection of SUIT-2, AsPC-1, and BxPC-3 microcapsules was 14.6 (15.9/107.1 cm^3) ± 7.0%, 9.7 (11.4/113.2 cm^3) ± 5.8%, and 15.0 (19.1/111.4 cm^3) ± 12.5%, respectively. Note that metastatic nodules, especially of AsPC-1 microcapsules, tended to precipitate at the peripheral regions of the liver.

Table 1. Liver metastasis take rate in rats by different injection form of cancer cells: cancer microcapsule versus single cells

	Cancer microcapsule*					Single cells†		
	n	Liver (rate %)	Peritoneum	Injection site	Lung	Wound	*n*	Liver	Others
SUIT-2	12	12 (100)	0	0	0	2	6	0	0
AsPC-1	6	6 (100)	0	0	0	0	6	0	0
BxPC-3	6	5 (83)	0	0	0	0	6	0	0

*Three thousand microcapsules with a 300 μm diameter were injected.
†A single cell suspension of 2.1 to 4.5 × 10^6 cancer cells were injected.

analysis of metastases revealed that cancer cells gradually extruded from the outer layer of the microcapsules at day 3, which could not be recognized macroscopically (Fig. 4*a2–3*). At day 7, metastatic foci developed to 0.5 to 2 mm, accounting for 6% of the sectional area (Fig. 4*b1*). Tumor growth was equally achieved with cancer microcapsules in both peripheral (Fig. 4*b2*) and central regions (Fig. 4*b3*). At day 28, overt liver metastases occupied 53% of the sectional area (Fig. 4*c1*) and were diffusely distributed from the peripheral to proximal regions (Fig. 4*c2–3*).

Pathophysiology of Liver Metastases: Cancer Microcapsules in Rats and Single Cell Injection in Mouse

Using both methods, metastatic foci were mainly distributed in the peripheral one-third of the hepatic hilum, i.e., marginal area of the liver (Fig. 5*a1* and *b1*). Microscopic observation of liver metastases from cancer microcapsules showed glandular formation around remnant microcapsules (Fig. 5*a2*). Single cell–derived liver metastases showed medullary proliferation of cancer cells

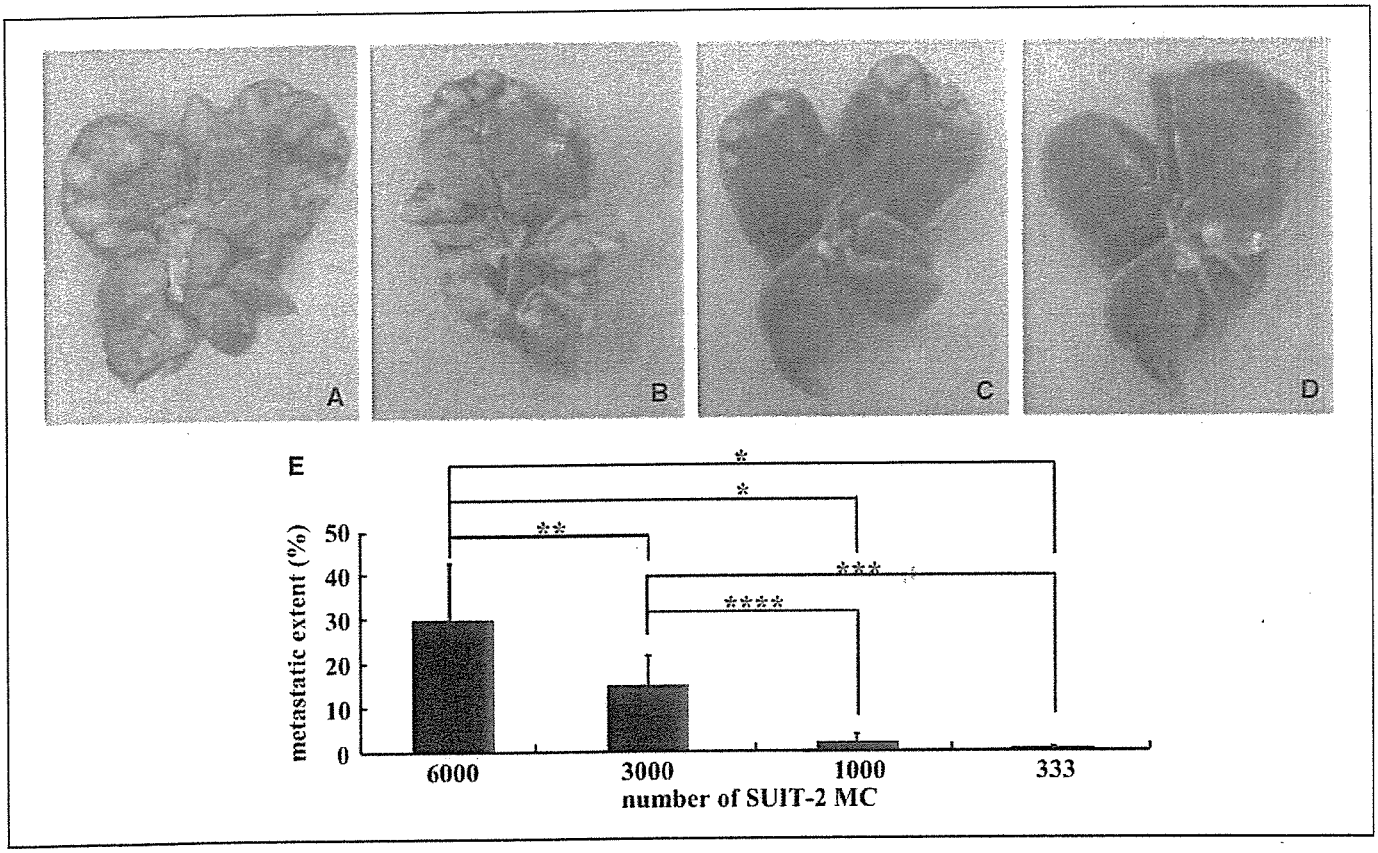

Figure 3. Intentional control of the extent of liver metastases by injecting different numbers of cancer microcapsules. Livers from nude rats 28 days after administration of 6,000 (*A*), 3,000 (*B*), 1,000 (*C*), and 333 (*D*) SUIT-2 microcapsules. *E*, the extent of liver metastases was numerically calculated (29.5% for 6,000 microcapsules, 14.6% for 3,000 microcapsules, 1.9% for 1,000 microcapsules, and 0.2% for 333 microcapsules), statistically demonstrating that the extent of liver metastases could be intentionally controlled by injecting different numbers of cancer microcapsules (*, $P < 0.0001$; **, $P = 0.0031$; ***, $P = 0.0009$; ****, $P = 0.049$).

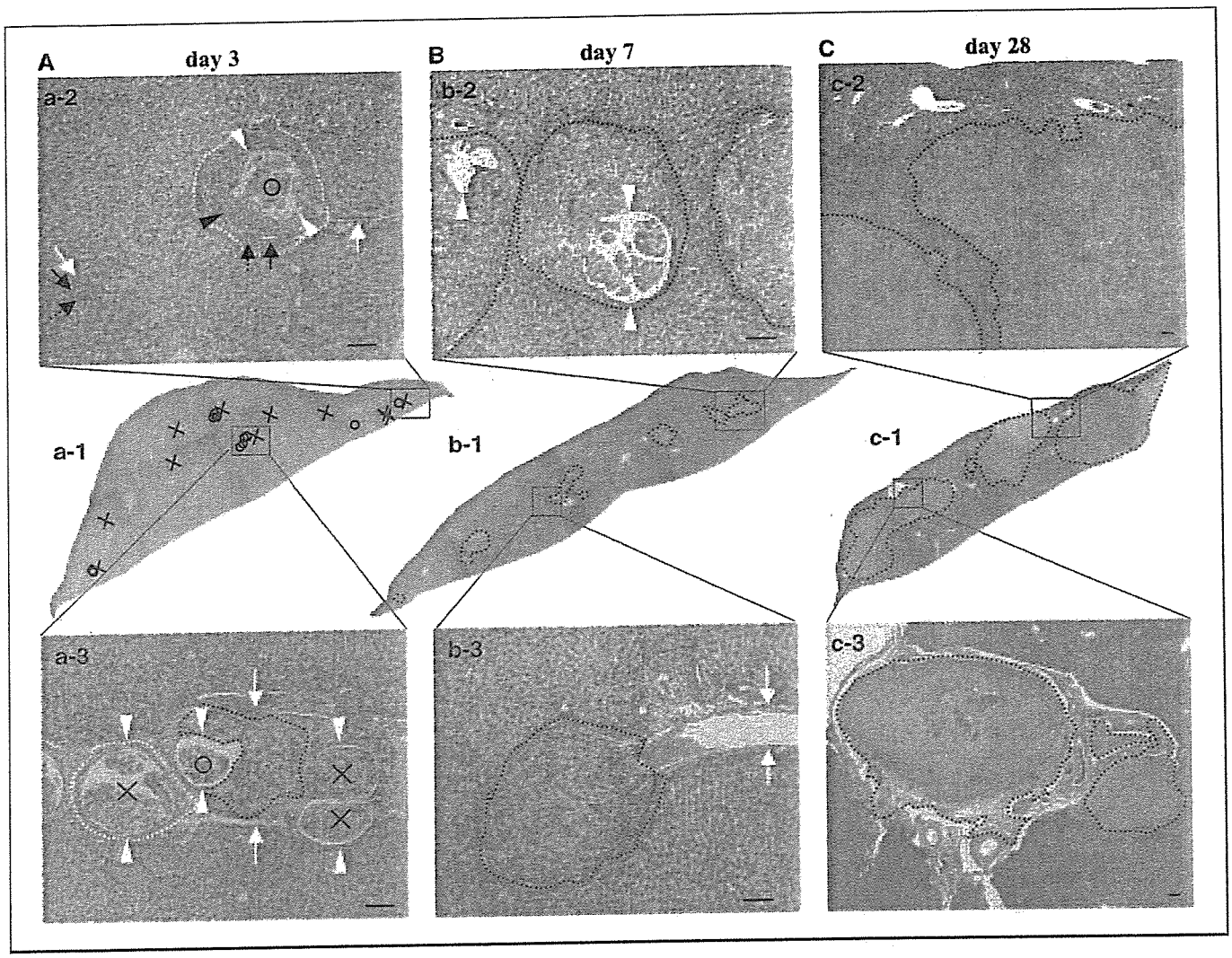

Figure 4. Sequential *in vivo* observation of liver metastases generated from cancer microcapsules. Representative pictures of rat liver, at day 3 (*A*), day 7 (*B*), and day 28 (*C*) after portal injection of 3,000 SUIT-2 microcapsules. *a1,* distribution of cancer microcapsules were marked on loupe observation (O, ruptured; ×, unruptured). Two-thirds of the microcapsules were lodged in peripheral small (20–50 μm) portal veins, although one-third were trapped in the central wide (200–400 μm) portal vein. *a2,* microscopic observation of the peripheral region on day 3, including a ruptured capsule. Outer layers of microcapsule (*white arrowheads*) and extruded cancer cell proliferation (*black arrowheads*). Fibroblastic proliferation around cancer microcapsules, a hallmark of the host reaction to foreign bodies, was sometimes observed at day 3 (*white dotted line*). It should be noted that 300 μm microcapsules reached regions much more peripheral than expected, because the diameter of the portal vein in Glisson's sheath (*white arrow*) neighboring lodged cancer microcapsules were ~20 to 50 μm in diameter. Peripheral bile ducts (*black arrow*), hepatic arteries (*black dotted arrows*). *a3,* microscopic observation of the central region at day 3. Four microcapsules, one marked with an "O" was ruptured and three marked with an "×" were unruptured and trapped in the large portal vein (400 μm), on both sides (*white arrows*). Extruded cancer cells beginning to proliferate (*black dotted line*). *b1,* loupe observation at day 7. Recognizable metastatic foci of 0.5 to 2.0 mm accounting for a sectional area of 6% (*surrounded by a dotted line*). *b2,* microscopic observation of peripheral regions showed noteworthy proliferation of cancer cells (*black dotted line*) around microcapsules (*white arrowheads*). *b3,* microscopic observation of the central region showed metastatic foci at the tip of a 120 μm portal vein (*two white arrows*). *c1,* loupe observation at day 28. Liver metastatic foci, which occupy a sectional area of 53%, were diffusely observed from the proximal to the peripheral area of the liver. *c2,* microscopic observation of the peripheral region and (*c3*) the central region showed that cancer microcapsules and fibrotic proliferation were surpassed by cancer cells and could not be recognized in mature metastatic foci.

with a cellularity of >90% and no glandular formation (Fig. 5*b2*). The desmoplastic reaction, proliferation of interstitial collagens, visualized by Masson trichrome staining was more evident in liver metastases originating from cancer microcapsules (Fig. 5*a3* and *b3*). The desmoplastic reaction was heterogeneously distributed in the liver metastases from cancer microcapsules. Dense collagen proliferations predominantly distributed the surrounding areas of cancer microcapsule remnants, presumably due to a foreign body reaction to the extracellular components of the microcapsules. Regarding neovascularity, the MVC of cancer microcapsules and single cell–derived metastases were 30.4 ± 7.0, and 62.8 ± 14.4, respectively.

Application of Liver Metastases Model for Evaluation of Anticancer Drug Efficacy

The extent of metastases in rats treated with gemcitabine, irinotecan, or saline was 0.52 (0.44/82.2 cm^3) ± 0.72%, 0.12 (0.1/80.6 cm^3) ± 0.16%, and 23.7 (26.6/106.4 cm^3) ± 11.3%, respectively. Objective evaluation for anticancer drug efficacy was possible because undesired metastases to organs other than liver was not observed.

Discussion

The present cancer microcapsule method led to the successful and efficient production of liver metastasis in rats. The mechanism

for successful production of liver metastases may be explained by the following considerations. First, cancer cells are forcibly trapped (mechanically embolized) because the microcapsules were large enough not to pass through the liver. Secondly, the cancer cells delivered to the implantation sites were fully viable. Lastly, embolization of cancer microcapsules to the peripheral portal vein mediates local ischemia, releasing cytokines and/or growth factors.

Cancer cell implantation in the liver is believed to occur at the sinusoids, and the principal mechanism is binding between cell surface adhesion molecules of single cancer cells and receptor molecules on hepatic endothelial cells (29). We assumed that an additional factor likely to be equally important is the mechanical entrapment of cancer cell clumps at the peripheral portal vein. The diameter of the portal vein of nude rats at the liver hilum is ~1 mm and gradually narrows to 20 to 50 μm before going to the periphery of the liver. Sequentially, the peripheral portal vein shifts to the liver sinusoids, i.e., the space between hepatocytes, the diameter of which is 7 μm. The size of a single cancer cell is ~8.3 to 47 μm (30), and a previous study has shown that large cancer cells are advantageous in liver implantation (31). Once cancer cells form aggregates, the size might increase to ~30 to 50 μm (32). Larger aggregates, compared with cell suspensions, are known to be more effective in both implantation and survival to form gross tumor colonies after i.v. injection (33, 34). In order to improve the ratio of cell aggregates, previous experimental models

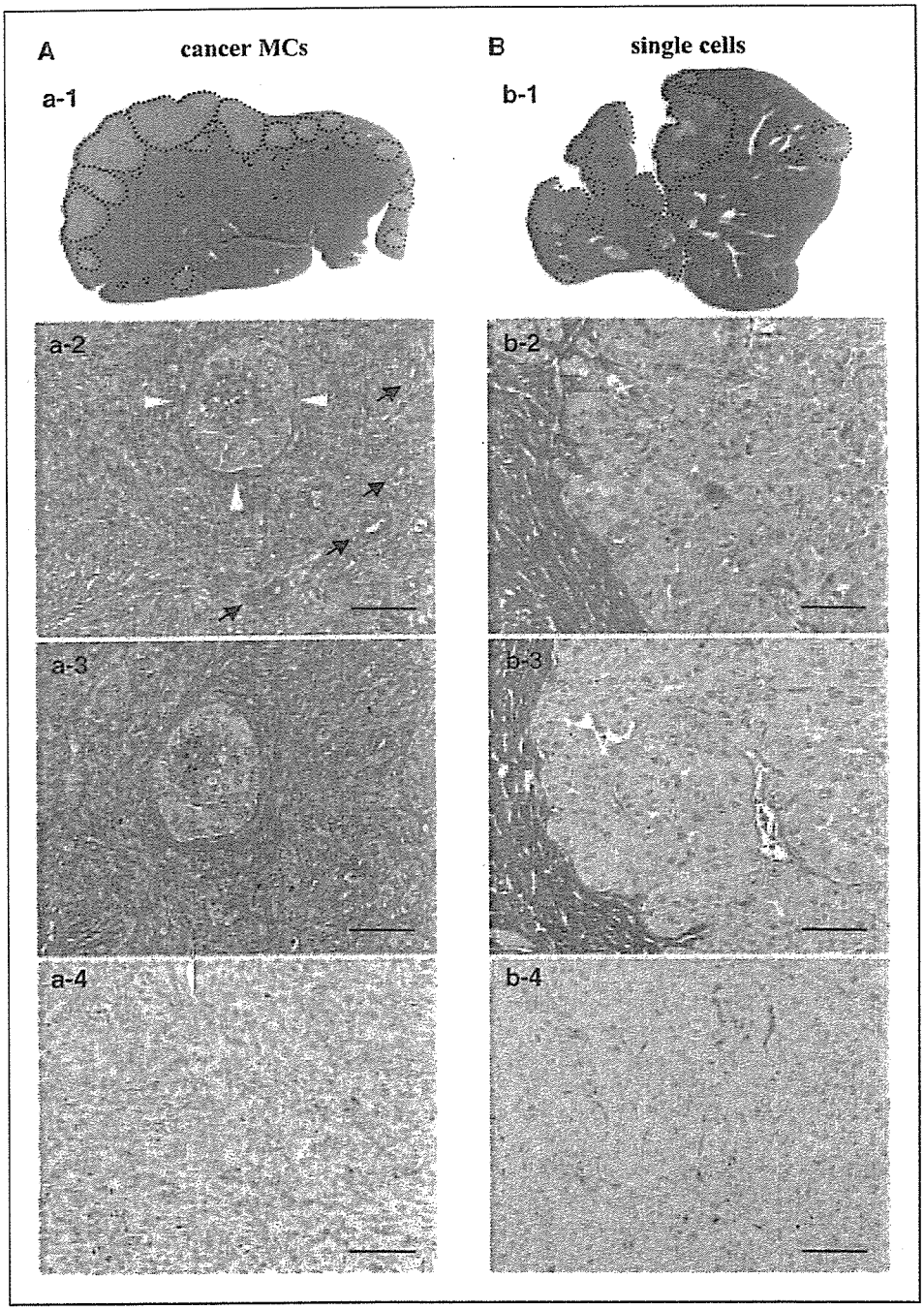

Figure 5. Pathophysiologic assessment of liver metastases generated by cancer microcapsules in rats versus single cell injection in mice. Histopathologic and immunohistochemical findings of SUIT-2 liver metastases, 28 days after administration of 3,000 cancer microcapsules in a nude rat (*A*) and single cell suspension (2.1×10^6 cells) in a nude mouse (*B*). *a1* and *b1*, macroscopic image stained with H&E shows that metastatic nodules (*surrounded by a dotted line*) tend to form at the peripheral margin of the liver in both methods. *a2*, microscopic observation of cancer microcapsule–derived liver metastases showed glandular formation (*arrows*) around remnant microcapsule (*white arrowheads*), indicating a histologic presentation similar to that of primary pancreatic cancer. *b2*, microscopic observation of liver metastases derived from single cells showed that medullary proliferation of cancer cells with a cellularity of >90% with no glandular formation that is not histologically similar to primary pancreatic cancer. *a3* and *b3*, proliferation of interstitial collagens, visualized by Masson trichrome staining, was more evident in liver metastases derived from cancer microcapsules than in those from single cell suspensions. *a4* and *b4*, the extent of neovascularity was evaluated by the MVC method using anti–factor VIII antibodies. The MVC of cancer microcapsule and single cell–derived metastases was 30.4 ± 7.0, and 62.8 ± 14.4, respectively. Liver metastases with cancer microcapsules were less vascularized with respect to those derived using a single cell suspension. *Bars*, 100 μm.

have employed an *in vitro* rotary cell culture system (35–37). In these models, however, the size and number of cancer cells included in these aggregates varies greatly. Furthermore, these cell aggregates are physically fragile and are easily damaged by transplantation before arriving to potential implantation sites. As a result, the frequency of liver metastases by this method ranges from 20% to 40% even under optimal conditions (36, 38). Moreover, only a highly limited number of cell lines are capable of forming aggregates in this *in vitro* system, greatly limiting their utility. We used uniform cancer microcapsules with a diameter of 300 μm, which resulted in 100% of the injected cancer microcapsules becoming trapped in the peripheral portal vein, and thus, never pass through the liver sinusoids to the hepatic vein.

A second advantage of cancer microcapsules may be their capacity to deliver viable cancer cells to implantation sites. When suspensions of cancer cells are injected via the portal vein or spleen in animals, the cells are rapidly attacked by the host immune system and also suffer from hemodynamic forces (39). In fact, although the majority of injected cancer cells were found to be arrested in the liver sinusoid several minutes after injection, the vast majority of injected cancer cells were disseminated or no longer viable at 24 hours. Finally, only 1% of injected cancer cells survive in the liver, and further progression to form metastatic foci is even less probable (40, 41). Although observation by intravital video microscopy showed that melanoma cells can survive in the liver (>36% even at day 13; ref. 42), it seems reasonable to assume that, in general, relatively few cells are the seeds of metastases. In our cancer microcapsules, cells were preincubated *ex vivo* until the logarithmic growth phase and were effectively protected by the outer layer of the microcapsule throughout the processes of initial administration, delivery, embolization, and growth before bursting. Physical protection by the microcapsule may beneficial, although this may not be necessary in all cell types, increasing the ratio of viable cells in liver and therefore aiding in the formation of metastases.

The third mechanism that may explain the success of the present liver metastatic model may be related to liver ischemia, which induces the release of cytokines and/or growth factors, simultaneously possess the potential to stimulate cancer cell growth (43–45). It is quite reasonable to assume that once cancer microcapsules are embolized, more peripheral liver parenchyma will be included in ischemic environments. There is also clinical evidence that hepatic pedicle clamping during liver surgery causes liver ischemia and mediates the release of cytokines such as tumor necrosis factor α, interleukin 1β, and other growth factors, accelerating cancer growth (46–48). Together with these results, we assume that local ischemia of transplanted sites of the liver might contribute, at least in part, to the successful production of overt metastases.

One question that arises is whether the pathophysiology of liver metastases generated by the present cancer microcapsule method is equivalent to that of widely used liver metastases generated by single cell injection. It should be highlighted that liver metastasis using the cancer microcapsule method has only been tested in rats and successful liver metastasis by single cell injection using certain special cells (SUIT-2, in the present report) was observed only in mice; therefore, histopathologic comparisons were made using different species. Regarding the region of liver metastases production, this method is initially expected to provide artificially proximal tumors, located in the proximity of the wide portal vein which has a diameter of 300 μm. In reality, however, the regions of cancer microcapsules embolized were distributed more peripherally than expected, resulting in the precipitation of metastatic nodules in the marginal area of the liver. The fact that 300 μm of cancer microcapsules were found at the 20 to 50 μm peripheral portal vein shows the considerable plasticity of the portal vein. There were some cancer microcapsules that were likely to have been trapped in the central area, where the diameter of the portal veins ranges from 200 to 400 μm. Metastatic formation in the central region, however, was not a phenomenon specific to the cancer microcapsule method because they were also observed following single cell injection. Regarding the region of liver metastases, therefore, cancer microcapsules confer a similar hepatic distribution in rats to that of conventional single cell injection in mice.

Almost invariably, the histopathology of tumors in animal models is quite different from that of primary, clinical cancer specimens. Pancreatic cancer is well-known for its hypovascular nature and extensive desmoplastic reaction. Tumors in single cell–derived liver metastases in mice usually show endocrine tumor–like growth with an expansive growth pattern, without the formation of glands. Given this, it was unexpected that tumors generated by cancer microcapsules in rats formed glands around fibroblasts. Because cancer cells are known to be heavily affected by surrounding fibroblasts and infiltrating hematopoietic cells (49), the presence of a foreign body reaction to cancer microcapsules also seemed to be beneficial in mimicking the histopathology of primary pancreatic cancer. Together with the unique characteristics of the present animal model, such as stable production of liver metastases and the presence of metastases only in liver, this presents immense advantages in evaluating the effect of therapeutics aimed at controlling liver metastases. In fact, the effectiveness of commonly used anticancer chemotherapeutic agents can be evaluated in an objective and quantitative manner.

Although we succeeded in producing consistent liver metastases in rats using cell lines with little or no metastatic potential (AsPC-1, BxPC-3), it is unknown if the cancer microcapsule method will be applicable to all cell lines and will always produce overt liver metastases. The necessary conditions for liver metastases in the present microcapsule system are that cancer cells have two capabilities. The first is a growth potential that is powerful enough to burst the outer layer of the microcapsule, whereas the second is the ability to proliferate in liver parenchyma after being extruded from the ruptured microcapsule. The first condition may be enhanced by the unique application of the present method, i.e., the ability of coencapsulation with different cells or substances. Matrigel, an extracellular matrix that contains several growth factors and cytokines, was used as a "burst-supporting" agent in the present study, although coculture with various growth factors, cytokines, extracellular matrix components, and fibroblasts might also augment cell proliferation and capsule burst. Regarding the second condition, some cancer cells have never been reported to proliferate in liver even after direct intrahepatic injection, indicating that the liver is not an appropriate soil for some cell lines (18, 50). Application of the present microcapsule system to those cell lines might not generate liver metastases, even if they have the capacity to rupture the outer layer of microcapsules.

In conclusion, we succeeded in producing consistent overt liver metastases in nude rats using cancer microcapsules with a diameter of 300 μm, whereas the administration of single cancer cells never produced liver metastases in rats. Although the

advantage of this microcapsule method has been shown here in rats, this method may be applicable for larger animals such as rabbits, dogs, and pigs. In smaller animals such as mice, liver metastasis production was possible with the single cell injection method only if we used certain cancer cell lines. The present cancer microcapsule method may be useful for obtaining liver metastases in mice, especially for cell lines that will not form liver metastases with conventional methods. It should be noted, however, that technical improvements such as the production of smaller (<100 μm) cancer microcapsules and better surgical skill in injecting cancer microcapsules to the narrow portal vein, and especially the hemostasis step after injection, are necessary for applying the present method in mice. Even though the present microcapsule system is artificial, this may nonetheless provide information for understanding the mechanism of clinical liver metastases, highlighting the importance of anatomical-mechanical entrapment. We believe that the present cancer microcapsule method could contribute to the development of new anticancer therapeutics by providing consistent tumor growth in animal models.

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総　説　　特　集：放射線治療最前線－放射線治療のEBM－

非小細胞肺癌

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Current Status of Radiation Therapy
－Evidence-based Medicine (EBM) of Radiation Therapy－

Non-Small Cell Lung Cancer (NSCLC)

Minako Sumi

The goal of radiation therapy for non-small cell lung cancer (NSCLC) is to improve the survival rate of patients without increasing treatment-related toxicity and to improve patients' quality of life. Several prospective randomized trials have demonstrated a survival advantage in combined modality treatment over radiotherapy or chemotherapy alone when a cisplatin-based chemotherapy regimen is utilized in the treatment plan. Combined modality treatment of cisplatin-based chemotherapy and radiotherapy is standard treatment for selected patients such as those with better performance status with locally or regionally advanced lung cancer including T3-T4 or N2-N3.

Determining the contribution of new agents in combined modality treatment will require carefully designed and conducted clinical trials.

High-dose involved field radiation therapy using 3D-conformal radiation therapy potentially enables the use of higher doses than standard radiation therapy, because less normal tissue is irradiated, and may improve local control and survival. The combination of radiotherapy with chemotherapy and dose escalation using 3D-conformal radiation therapy is also a possibility in unresectable NSCLC.

In surgery cases, the results of several Phase III trials of cisplatin-based preoperative chemotherapy have suggested survival improvement. But the concept needs to be tested in a larger Phase III trial.

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緒　言

放射線治療は手術・化学療法とともに腫瘍治療の一環として，放射線生物学・物理学の研究および治療技術・装置開発を重ねている．その特徴としては，①機能・形態の温存，②治療対象部位の制限が少ない，③合併症を有する患者や高齢者などでも適応可能であり対象の制限が少ない，などが挙げられる．しかし，これらの特徴は局所制御率のさらなる向上と有害反応のさらなる軽減があってこそ，臨床においてその有用性を発揮すると考える．その方法論として放射線生物学の進歩は分割照射方法・放射線増感剤や防護剤・chemoradiationの研究により成果を上げ，一方で放射線物理(工)学は小線源治療・粒子線治療・三次元照射の開発および進歩をもたらした．

本稿では最近の非小細胞肺癌放射線治療に関する知見として，非切除例に関する化学療法の併用と三次元放射線治療および，外科療法と放射線治療の併用のevidence-based medicine (EBM)について述べる．

肺癌の疫学

悪性新生物は1981年以降死因の第一位を占め[1]，平成12年には死亡数295,399人，総死亡の30.7%となっている．悪性新生物による死亡をその部位別にみると，平成11年においては男性では肺癌が最多であり(21.6%)，次いで胃癌(18.6%)，肝癌(13.4%)の順であった．女性では胃癌が最多であり(15.6%)，次いで肺癌(12.4%)，結腸癌(9.9%)の順となっている．従来多かった胃癌や子宮癌の死亡数が減少傾向にあるのに対し，肺癌の罹患率は増加しており特に高齢者においても罹患率が高いことが注目されている．

放射線治療と化学療法の併用

従来小細胞肺癌に比較し，非小細胞肺癌では化学療法に対する感受性が低いと考えられた時期もあったが，生存率の向上への化学療法の寄与を検討する目的で臨床試験が多数施行された．1995年にNon-small Cell Lung Cancer Col-

laborative Group により発表されたmeta-analysisの結果では[2]，cisplatinを含む併用化学療法は生存期間の中央値を10.3カ月より12カ月へ延長させることが示された．この分析では，化学療法は2年生存率を4%延長させ，死亡のリスクを13%減少させると報告された．この結果をうけ化学療法の非小細胞肺癌への応用が加速された(Table 1)．American Society of Clinical Oncologyにより1997年発表された診療ガイドラインでは[3]，よいPS(ECOG/Zubrod 0-2)の切除不能局所進行非小細胞肺癌に対しては，プラチナ製剤を含む多剤併用化学療法と放射線治療の併用が推奨された．

放射線治療と化学療法併用のメカニズムとしては，1) spatial cooperation(ある治療で作用を受けない他の治療では作用を受けること)，2)toxicity independence(治療の毒性が重複しないこと)，3)enhancement of tumor response (cisplatin等で示されている放射線増感作用)，4)protection of normal tissue(放射線の正常組織に対する影響が増強しないこと)などが挙げられている[4]．併用の方法としては，化学療法に引き続き放射線治療を施行する方法 (sequential)，放射線治療と化学療法を同時に併用する方法(concurrent)，抗癌剤を増感剤として使用する方法 (sensitizing)が検討されている．

化学療法と放射線治療の併用は予後の改善に有用であることが臨床試験で示されているが[5)-8)]，予後の改善と有害事象の軽減は引き続き臨床試験の課題となっている．予後因子としてはKPS，体重減少，N因子などが挙げられているが，従来の臨床試験の対象は良好なKPSや若年者などが選択されていることが多い．よって，今後増加が見込まれる高齢者や予後不良因子を有する患者に対する適切な治療方法の確立も，今後の臨床研究の重要な課題である．

分割照射方法

照射スケジュールの検討に重要な意味をもつ一回線量と，照射回数，照射期間の検討においては，通常分割照射standard fractionation(1.8〜2.0Gy/回，1日1回，週5日)と過分割照射hyperfractionation(1.1〜1.2Gy/回，1日2回以上6時間以上の間隔で照射，週5日)，加速分割照射accelerated fractionation(1.5Gy/回，1日2回以上6時間以上の間隔で照射，週5日など)が使用される．

Hyperfractionationは，遅発性有害事象の増加を避けつつ，総線量を増加することにより局所制御の向上をめざす方法として検討されてきた．この際，急性期の有害事象は増加することとなり，その軽減が重要な課題となっている．accelerated fractionationは，治療期間の短縮を目指した照射スケジュールであり，小細胞癌においてはその有用性が評価されているが非小細胞肺癌でも検討課題となっている．

通常分割照射における至適線量決定のための臨床試験としては，RTOG 73-01 (Phase III)があり，40 vs. 50 vs. 60Gyの3群で比較試験が施行され，局所制御率はおのおの48%，65%，61%であり，生存期間中央値もそれぞれ7カ月，9カ月，10カ月であった．3年生存率はおのおの6%，10%，16%と60Gyが最良であったが，5年生存率ではいずれも6%となり，差は認められなかった[9]．多分割照射の臨床試験としてはRTOG 81-08 (Phase I/II)があり，1.2Gyで1日2回照射を施行し総線量の増加による治療成績の改善を60 vs. 63 vs. 69.6Gyの3群で比較する臨床試験が施行された．いずれも治療に耐え得たことで，つづくRTOG 83-11 (Phase I/II)では79.2Gyまで総線量が増加された．長期経過観察の結果，5年生存率は5グループで6〜8%であったが，favorable performance subsetでの5年生存率は60Gy，63Gy，69.6Gy，74.4Gy，79.2Gyのおのおので12%，8%，10%，10%，9%となり，69.6Gyを推奨線量とした[10]．この結論は，RTOG 88-08 (Phase III)として，通常分割60Gy±化学療法と多分割照射69.6Gyの比較試験へと発展していくこととなった．

加速照射の方法には，いわゆるconcomitant boostとよばれる照射方法と，continuous hyperfractionated accelerated radiotherapy (CHART)がある．Saundersらの比較試験では，2年生存率が60Gy/30分割の通常分割照射(n = 225)では20%であったのに対し，CHART (n = 338)では29%に上昇し生存期間の延長が示されたと報告した[11]．しかし急性期の有害事象中とくに高度の食道炎が問題であり，通常分割照射で3%であったのに対しCHARTでは19%に上っていた．CHARTは，放射線治療計画の進歩によるリスク臓器の線量の軽減など，治療方法の改善により有害事象の軽減が可能となれば，有力な治療方法となる可能性がある．

現在さまざまな化学療法と放射線治療の併用による臨床試験が行われているが，RTOG 9410は，放射線治療のスケジュールとタイミングに関する比較試験である．化学療法後に通常分割60Gyを行う(sequential)異時併用(SEQ)と，化学療法と放射線治療を同時併用(concurrent)するが放射線治療のスケジュールを通常分割照射60Gy (CON-QD)と多分割照射69.6Gy (CON-BID)とした，3方法の比較試験が施行された．RTOG 9410では，生存期間の中央値はCON-QDが17カ月と最もよいものの(CON-BIDで16カ月，SEQで14.6カ月)，照射野内再発までの期間はSEQに比較し有意にCON-BIDで長い(p = 0.007)と報告された[12]．急性期の有害事象では，Grade 3以上の非血液毒性がCON-BIDで63%と，CON-QDの50% (p = 0.011)やSEQでの31% (p < 0.001)に比較し高いことが注目された．本試験は，2002年に長期予後に関する経過観察の結果が発表予定であるが，今後の非小細胞肺癌の放射線治療の標準化に影響を与える可能性がある．

三次元放射線治療計画

三次元放射線治療 (Three-dimensional conformal radiotherapy; 3D-CRT)は，放射線腫瘍医の追及する治療計

Table 1 化学療法を併用した非小細胞肺癌の臨床試験の成績

Authors	Number of Patients	Dose	Timing	Chemotherapy	Median Survival	2yr Survival	3yr Survival	Long Survival
Dillman[5]	77	60 Gy/30 Fr		None	9.6 mo	13%	10%	6%/7yr
CALGB8433	78	60 Gy/30 Fr	Sequential	Cisplatin+Vinblastin	13.7 mo	26%	24%	13%/7yr
Sause[16]	152	60 Gy/30 Fr		None	11.4 mo	21%	11%	5%/5yr
RTOG8808 &	149	60 Gy/30 Fr	Sequential	Cisplatin+Vinblastin	13.2 mo	32%	17%	8%/5yr
ECOG4588	149	69.6Gy/58 Fr (1日2回)		None	12 mo	24%	14%	6%/5yr
Furuse[8] JCOG9202N	158	56Gy/28 Fr	Sequential	Mitomycin+Vindesine +Cisplatin	13.3 mo	27.4	14.7%	8.9%/5yr
	156	56Gy/28 Fr	Concurrent	Mitomycin+Vindesine +Cisplatin	16.5 mo	34.6	22.3%	15.8%/5yr
Komaki[12]		60 Gy/30 Fr	Sequential	Cisplatin+Vinblastin	14.6 mo			
RTOG9410	592	60 Gy/30 Fr	Concurrent	Cisplatin+Vinblastin	17 mo			
		69.6Gy/58 Fr (1日2回)	Concurrent	Cisplatin+VP-16	16 mo			

画をCTやMRI，PETなどの放射線診断学と治療装置に関するテクノロジーの進歩が支え，実現した治療方法といえよう．その応用と成果は重要臓器に囲まれた，従来の二次元放射線治療では正常組織の有害反応ゆえに放射線治療にとり困難の多かった領域，脳腫瘍・頭頸部腫瘍や前立腺癌などの治療で成果が報告され，諸臓器の治療でその応用が進行している．

3D-CRTとは永田らによれば[13]，“薄い間隔で撮像された複数のCT画像に基づいて，正確なターゲット領域(CTV・PTV)とリスク臓器(organs at risk; OAR)の幾何学的配置を決定し，それらを画像処理した種々の三次元画像を用いたうえで，適切な三次元線量計算に基づく正確な放射線治療計画”としている．従来の放射線治療が“照射方向と照射野辺縁の設定をしてからターゲット内の線量分布を確認する”のに対し，“ターゲットと関連正常臓器の輪郭を設定してから，計算された三次元画像を利用することによって，照射方向や照射門数を決定する”ように，治療計画は大きな変化を遂げた．さらに，強度変調放射線治療(Intensity-Modulated Radiotherapy; IMRT)では“ターゲットの内部の詳細な照射線量と各種関連リスク臓器の詳細な容積線量を定義(prescribe)した後に，治療計画装置によって最適な照射方法を決定する”こととなり，望ましい線量分布の実現が治療計画装置の進歩により可能となりつつある．

3D-CRTは，ターゲットへの線量の集中を可能とし有害反応の軽減をもたらし得るが，総線量の増加により局所制御率の向上が望み得る領域においては局所制御率をも期待させることとなった．3D-CRTには日本で開発された原体照射や，定位放射線照射，non-coplanar固定多門三次元照射，本邦で開発された歳差運動照射，アメリカで開発されたCyber-knifeなどがある．

非小細胞肺癌に対する放射線治療の新しい展開としては，3D-CRTを利用した臨床試験が施行されている．3D-CRTの特徴であるターゲットの形状に則した照射野の設定やターゲットの形状に則した線量分布の設定による周囲正常組織の線量の軽減は，非小細胞肺癌放射線治療における病巣に対する線量分布と適正化と肺や食道の有害反応の軽減とをもたらし得る．放射線治療にかかわるターゲットの決定においてはICRU Report 62に従い対象を決定していくが(Table 2)，その容積はGTV < CTV < ITV < PTVの順に大きくなる(Fig. 1)．対象とする疾患やその組織型・分化度，臨床病期などを考慮した設定が不可欠である．ターゲットの決定において重要な役割を果たすのは画像診断であり，CTやMRI，PETにとどまらずMolecular ImagingやFunctional Imagingの応用で腫瘍の浸潤・残存範囲や正常組織の機能を考慮した治療計画の可能性が実現されている．

非小細胞肺癌の放射線治療においては

Gross Tumor Volume (GTV)

= CT上の異常領域，腫瘍と考えられる領域

Clinical Tumor Volume (CTV)

= GTV + 顕微鏡的浸潤領域 + 肺門や縦隔リンパ節領域

Internal Target Volume (ITV) = CTV + IM (呼吸性移動)

Planning Target Volume (PTV) = ITV + SM

という治療領域の設定がなされている．Dillmanらは肺癌放射線治療のretrospective quality control reviewにおいて，23%でターゲットが充分含まれていなかったと報告しており[14]，3D-CRTのプロセスにおける照射野設定の適正化は治療成績に影響し得ると考える．

GTVに対する総線量増加によるメリットは，RTOG 8311などの臨床試験により一定の患者群で報告されているが[15),16)]，ここで問題となったのが高線量群における高度な肺臓炎の発生による生存率への影響であった．従来の照射方法によるGTVに対する70Gy以上の照射は正常組織の線量を考慮すると不適切とする報告もあり[17),18)]，3D-CRTを応用した総線量増加が期待されている[18),19)] (Table 3)．

GrahamらはV20(the percent volume of the total lung exceeding 20Gy)が，肺臓炎の単独での最良の指標であると報告している[20]．Grade 2 以上の肺臓炎発生はV20とよく相関しており，V20が22%未満の症例では発症はなく，V20が22～31%であった症例のGrade 2 は 7%(Grade 3 は 0%)であり，V20が32～40%ではGrade 2 は13%(Grade 3 は 5%)と増加し，V20が40%を超えるとGrade 2 は36%(Grade 3 は23%)に達したとしている．肺臓炎の重篤度とV20が相関するとも報告しており，多変量解析においてV20は肺臓炎のsingle best predictorであると結論づけた．

RTOG 9311 (Phase I/II)は総線量増加を行う多施設共同臨床試験である[21]．この臨床試験は最初の肺癌に関する 3D-CRTの多施設臨床試験であり，従来の臨床試験に比較し特異な点がいくつかある．V20で層別化しており，肺の照射容積を低減し肺臓炎のリスクを軽減するために予防的リンパ節照射をしない臨床試験となっている．RTOG 9311は通常分割照射であり，総治療期間が延長している点も注目すべきであろう．腫瘍によっては総治療期間の増加によりtumor control probabilityが低下することが示唆されており[22)-24)]，非小細胞肺癌症例ではRTOGによる臨床試験の解析結果よりunfavorable clinical feature (i.e. KPS < 90, weight loss > 5%, N3 stage)がない症例において，局所制御と生存率の低下が頭頸部癌同様およそ1.5%/日となる可能性が指摘されている[15),25)]．Preliminary resultsでは肺の急性有害反応は生じておらず，食道のGrade 3 の遅発性有害反応が 2/94，肺のGrade 3 の遅発性有害反応が 3/93で生じたとされている[21)]．本試験においては，RTOG 9410で問題となった非血液毒性の解決に 3D-CRTが有用である可能性が期待されている．この臨床試験につづき，RTOG L-0117 (Phase I/II)では，3D-CRTを応用したdose escalationと同時併用化学療法の臨床試験が開始されている．アメリカの肺癌放射線治療においては 3D-RTPが根治治療症例の多くで施行されており，本邦においても普及が進んでいるが，3D-RTPを用いた放射線治療を応用した総線量増加や化学療法との併用に関しては，今後多くの臨床試験による治療成績の検証が待たれるところである．

Table 2　放射線治療にかかわるターゲットの決定

GTV	Gross tumor volume	画像や触診で確認できる肉眼的腫瘍体積
CTV	Clinical target volume	臨床標的体積 = GTV + 顕微鏡的進展範囲
ITV	Internal target volume	CTV + IM
PTV	Planning target volume	計画標的体積 = ITV + SM

IM: internal margin = 呼吸移動や腸管のガスによる影響など体内臓器の移動にかかわる
SM: set up margin = 毎回の治療における設定誤差にかかわる

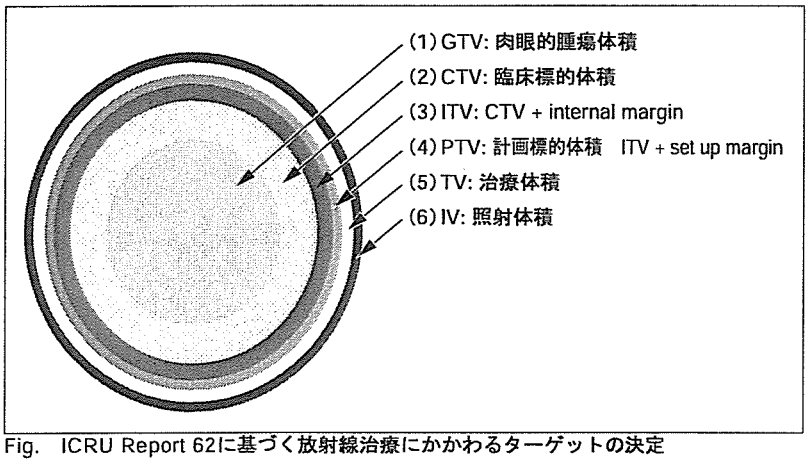

Fig.　ICRU Report 62に基づく放射線治療にかかわるターゲットの決定

外科療法と放射線治療の併用

非小細胞癌の術後再発の80%が遠隔転移であるとされ，1970年代以降多くの化学療法や放射線治療を用いた臨床試験が行われてきた[26)](Table 4)．化学療法に関しては，1995年にmeta-analysisが報告され，プラチナ製剤を含む化学療法ではhazard ratioが0.87で 5 年生存率としては 5%の改善効果が期待されるとされた[2)]．1991年にEastern Cooperative Oncology Groupにより開始されたintergroup trialは，術後照射と術後照射+化学療法を生存期間と局所制御率において比較した臨床試験であった[27)]．生存期間の中央値は術後照射群で38.8カ月，術後照射＋化学療法群で37.9カ月であり，照射野内再発も術後照射群で13%，術後照射＋化学療法群で12%と有意差を認めなかった．1998年に発表されたPORT meta-analysisでは[28)]，術後照射はN0-1 症例においては有害事象が問題であり早期の完全切除例においてはdetrimental(有害)であると報告した．N2 症例に対してはその役割が明らかでなく，さらなる検討が必要と結論づけた．今後，切除症例に対する放射線治療の役割は，局所および遠隔転移の制御とともに有害事象の軽減を課題として臨床試験が計画される必要がある．

術前治療としての集学的治療には，微小遠隔転移の制御や腫瘍のdebulkingによる切除率の向上，外科切除時の腫瘍細胞の播種や散布防止などが目的とされる．小規模臨床試験では術前治療群で良好な成績が報告されているが[29),30)]，これらの試験では対照群の予後が不良であることや症例数が少ないことが問題とされており，いまだcontroversialである．縦隔鏡などで組織学的確診を得たN2 症例を対象に臨床試験が進行中であり，充分な第III相試験の結果の蓄積が必要と考える．

Table 3 3D-CRT を用いた非小細胞肺癌の臨床試験の成績

Institution	Number of Patients	Dose	Median Survival	1yr Survival	2yr Survival	3yr Survival
University of Michigan[32]	88	> 60 Gy	15 mo	—	37%	15%
University of Chicago[33]	37	60-70 Gy	19.5 mo	75%	37%	—
Memorial Sloan Kettering[34]	45	64-72 Gy	16 mo	—	33%	—
Washington University[35]	126	60-74 Gy	21.5 mo	57%	43%	29%

Table 4 非小細胞肺癌の術後照射に関する臨床試験の成績

Authors Institution		Number of Patients	Dose	Median Survival	2yr Survival	3yr Survival	5yr Survival
術後照射							
Keller[27]	Surgery⇒RT	242	50.4Gy	38.8 mo		52%	39%
	Surgery⇒RT+CTx	246	50.4Gy	37.9 mo		50%	33%
PORT Meta-Analysis	Surgery	1072			55%		
Trialists Group[28]	Surgery⇒RT	1056	30-60Gy		48%		
Neoadjuvant chemotherapy (clinical IIIA)							
Roth[30]	Surgery	32	None	14 mo		19%	15%
MD Anderson*	CTx⇒Surgery	28	None	21 mo		43%	36%
Rosell[29]	Surgery⇒RT	30	50Gy	8 mo			
Barcelona**	CTx⇒Surgery⇒RT	30	50Gy	26 mo			

*MD Anderson: 化学療法はcyclophosphamide + etoposide + cisplatin
**Barcelona: 化学療法はmitomycin + ifosfamide + cisplatin

まとめ

肺癌に対する標準的治療の確立には，局所制御率と生存期間の向上および有害事象の軽減を図るための工夫が必要である．化学療法や有害事象対策の新しい薬剤を含めた臨床試験が，現在数多く施行されている．放射線治療の最適化には患者固定や呼吸同期，治療計画，総線量および分割照射の検討が寄与すると考えられるが，対象に合わせた治療方法の検討として，高齢者やPSの低い症例の検討も今後ますます重要となる．

肺癌の放射線治療に関しては，三次元治療計画の普及や強度変調放射線治療の導入などが予想される．問題点を解決していくには，よくデザインされた臨床試験が必要であり，放射線治療の技術の発展とその普及とともに，治療の安全性や品質管理がより重要となってくると考えられる．

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医療実態調査研究による放射線治療施設の基準化（案）の検証

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VERIFICATION OF JASTRO STRUCTURE GUIDELINE FOR RADIATION THERAY BY THE PATTERNS OF CARE STUDY

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はじめに

急増するがん患者に対して質の高い放射線治療を全国どこでも提供できるようにするため，日本版ブルーブックともいうべき放射線治療施設の構造の基準化案（以下，基準化案）が，厚生労働省がん研究助成金阿部班（8-27）にて阿部光幸先生らによりまとめられた[1]．この基準化案は米国ブルーブック[2]を参考にし，阿部班の班員，研究協力者らによる共同作業を経て決定された．この基準は理論的に考案され，今後のわが国の放射線腫瘍学の理想像も盛り込む形で決定された．当初より，装備，人員不足等のわが国の放射線治療を取り巻く厳しい現実と乖離した基準ではないかとの批判もあった．一方，米国の臨床的精度管理QAプログラムである医療実態調査研究Patterns of Care Study（PCS）[3]が，同研究班と同池田班（8-28）の支援により全国の放射線治療施設の協力を得てわが国に初導入され，引き続く同井上班（10-17）にて本格的に施行された[4]．このPCSで集積されてきたデータは現実のものであり，この段階で基準化案を検証することが可能となった．

本報告では，日本放射線腫瘍学会の平成11・12年度研究調査として，基準化案をPCSにより集積した全国放射線施設の具体的治療症例のデータ，当該施設の人員，装備のデータを用いて妥当性を検証することを目的とする．詳細な解析結果については既に第一報で報告している[5]ので，本報告ではPCSデータとの関連とPCSにて推定した放射線治療患者数の将来需要の中で，基準化案に残されている課題を考案する．

対象と方法

1995年日本放射線腫瘍学会定期構造調査[6]にて集積された556施設のデータから，厚生労働省がん研究助成金阿部班（8-27），池田班（8-29），井上班（10-17）にてPCSの訪問調査Auditが施行された106施設の構造のうち装備に関するデータを抽出した．人員に関してはPCS Audit時にその施設の実質的人員数（FTE: full time equivalent）を調査した．基準化案においては年間患者数により，A，B，Cの3施設に層別化している（Table 1）．PCS調査106施設は，年間300例以上治療しているA施設層：45施設，150例以上300例未満のB施設層：33施設，150例未満のC施設層：28施設に分類された．A施設層が備えるべき基準をA基準，同様にB施設層，C施設層が備えるべき基準をそれぞれB基準，C基準としている．基準案には，設置面積，物理士数も詳細に定められている[1]．しかし1995年の日本放射線腫瘍学会定期構造調査の調査項目には含まれていないので，すべての項目を適用すると本研究自体が成立しない．したがってTable

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Table 1 Stratification of institutions by JASTRO structure guideline.

Strata	No. patients	No. institutions
A	≥300/year	45
B	≥150, <300/year	33
C	<150/year	25
Total		106

Table 2 Summary of JASTRO structure guideline.

Summary of guideline	A.	B.	C
Equipment			
· Linac/betatron/microtron	+	+	or ^{60}Co
· CT or X-ray simulator	+	+	+
· RALS/interstitial RT system	+	+	-
· Dosemeter, Water phantom	+	+	+
Personnel			
· Radiation Oncologist	≥3,	≥2,	≥1
· Radiation Technologist	≥4,	≥2,	≥2
Special treatment			
· TBI, SRT, IORT, 3DCRT Brachytherapy, Hyperthermia	5,	≥3,	not required

Table 3 Stratification of institutions used in Japanese PCS.

	Strata	No. patients	No. institutions
A1	Univ/CC	≥300/year	70
A2	Univ/CC	<300/year	51
B1	Other	≥120/year	187
B2	Other	<120/year	248
Total			556

Univ: university hospital, CC: cancer center

2に示す主要な装備，人員，特殊治療法についてのみの各施設層基準の充足率を算出した[5]．先ず装備として加速器の必要性を定めているが，C基準では^{60}Co装置も許容している．すべての施設層でCTまたはX線シミュレータを必要としている．密封小線源治療はA基準，B基準で要求している．線量計，水ファントムはすべてで必要としている．人員については，放射線腫瘍医をそれぞれ3名以上，2名以上，1名以上必要としている．技師は4名以上，2名以上，2名以上必要としている．全身照射（TBI），定位照射，術中照射，三次元照射，密封小線源治療，温熱療法の特殊治療はA基準，B基準では，それぞれ5つ，3つ以上施行可能とし，C基準では要望してない．

一方，厚生労働省がん研究助成金阿部班（8-27），岡池田班（8-29），井上班（10-17）PCSでは，基準化案策定に先行して異なる層別化を用いてきた（Table 3）．大学病院・がんセンターをいわゆる欧米のAcademic institutionと捉え*A*施設とし，それ以外の国公立病院をNon-academic institutionと捉え*B*施設とした[7]（註：以降，PCSの施設層をイタリック体で示す）．それぞれ年間症例数300例，120例でさらに層別化し，*A1*，*A2*，*B1*，*B2*の4施設層とした．この層別化は前者*A*施設では放射線腫瘍医，診療放射線技師の教育（＝再生産）を行わなければならないため，装備，人員の面で多めの資源を必要とすると判断されたためである．またPCSでは全国の放射線治療施設を施設と症例の2段階クラスタサンプリングを用いて効率的に抽出するため統計学的に妥当な層別化を必要とした．さらに得られた調査結果と構造との関連を分析するためにも構造の層別化が必要であった．1995年時点で*A1*，*A2*，*B1*，*B2*施設層に全国の70施設，51施設，187施設，248施設が分類された．基準化案では症例数負荷により3施設層に分類されており，A施設層には国全体の2割弱，B施設層には3割強，C施設層には5割弱の患者数が分類される．

PCSへの調査施設に対して過去10年間の患者実数の推移を1999年末にアンケート調査した[8]．これに施設層内総施設数による補正をおこない，全国年間放射線治療数を推定した．増加率より今後の需要を推定し[8,9]，基準化案での施設層患者数の今後10年の推移をシミュレーションした．

結　果

基準化案充足率

基準化案が，現実にどの程度充足されていたかを，Fig. 1に示す．A施設のA基準は7.7%，B施設のB基準は5%，C施設のC基準は25%でのみ充足されていた．それぞれ1ランク下のB基準はA施設：40%，B施設：C基準50%，C施設：C基準以下75%で充足されていた[5]．

厚生労働省がん研究助成金PCSの構造と基準化案充足率および診療過程

PCS施設層による構造（装備，人員）の概要と基準化案

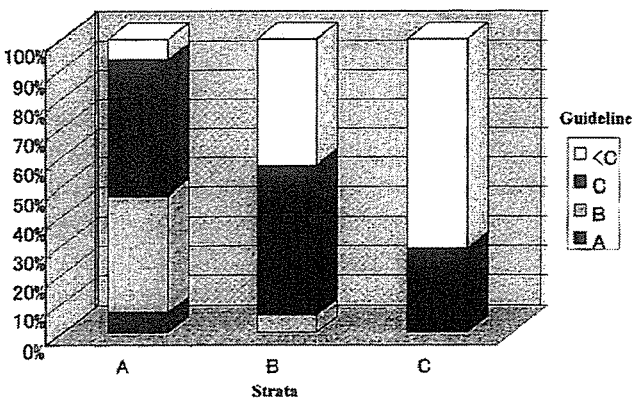

Fig. 1 Compliance rate of JASTRO structure guideline for the stratified institutions[5].

Table 4 Structure by statification of institution in PCS.

		PCS Strata			
		A1	*A2*	*B1*	*B2*
Linac (Mean)*		1.75	1.35	1.05	0.78
CT simulator (%)*		57	40	22	5
HDR RALS (%)*		71	73	42	4
Rad. Onc. (FTE median)#		2.4	1.1	0.6	0.2
Rad. Technologist#		4	2	1.7	1
Annual No. patients (mean)*		510	275	220	75
JASTRO structure guideline[5]					
Compliance Guideline	A	7.7	0	0	0
	B	51.3	10.5	4	0
	C	41	47.4	56	21.7
	(<C	0	42.1	40	78.3)

*1995 JASTRO Structure Survey, #1996-2000 PCS Audit, [5]JASTRO 10: 249-250, 1998.

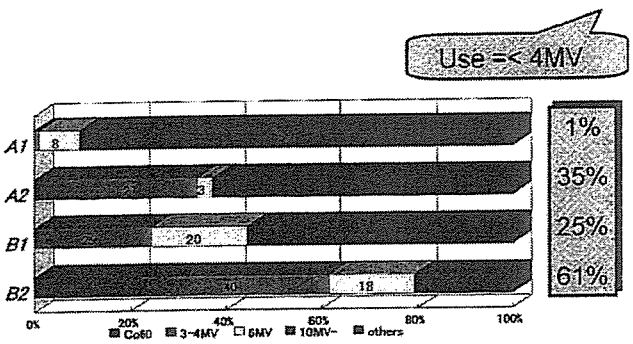

Fig. 2 Utilization of external beam energy for esophageal cancer patients without surgery group (n=556) in PCS according to stratification of institutions[10] (With permission of Shinohara Shyuppan Shinsha, Co. Ltd.).

の充足率をTable 4に示す．装備として，Linacの平均設置台数は，*A1*，*A2*，*B1*，*B2*施設層では，それぞれ1.75台，1.35台，1.05台，0.78台であった．CT simulatorは57%，40%，22%，5%に，HDR RALSは71%，73%，42%，4%に設置されていた．人員として，放射線腫瘍医数FTE中央値は，2.4人，1.1人，0.6人，0.2人であった．放射線治療技師数FTE中央値は，4人，2人，1.7人，1人であった．年間患者数平均値は，510人，275人，220人，75人であった．

基準化案充足率は*A1*施設のA基準：7.7%，*A2*施設のA基準：0%，*B1*施設のB基準：4%，*B2*施設のB基準：0%であった．一ランク下のB基準充足率は，*A1*施設：51.3%，*A2*施設：10.5%であった．*B1*施設のC基準充足率：56%，*B2*施設のC基準充足率：21.7%であった．

PCS施設層別の診療過程の一例をFig. 2に示す．井上班（10-17）で集積された胸部食道癌非手術例（n=556）への外部放射線治療のビームエネルギー分布は，不適切な4 MV以下が，*A1*，*A2*，*B1*，*B2*施設層で1%，35%，25%，61%に使用されていた[10]．

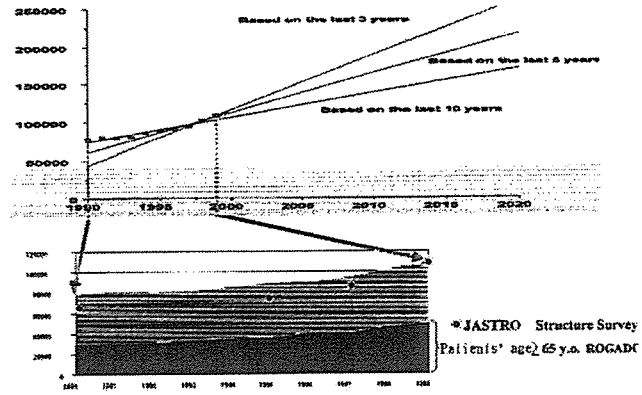

Fig. 3 Estimated annual number of cancer patients treated with radiation in Japan[8),9)].

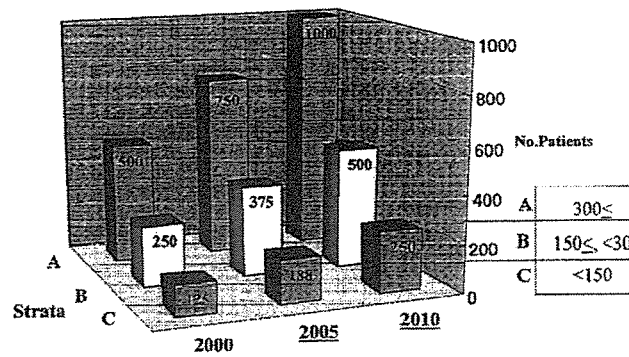

Fig. 4 Simulation of annual number of cancer patients treated with radiation according to JASTRO structure guideline.

日本における放射線治療患者数の推定と施設層別シミュレーション

過去10年間でわが国の放射線治療患者数は1.4倍に増加していた（Fig. 3）[9]．PCS施設層すべてにおいて増加していた．特にB2施設層での増加率が顕著であった．今後の増加率の推定は，幾つかのシミュレーションが可能であるが，単純な傾向として最近になる程，増加率が急峻である．最近3年のデータで外挿すると今後10年で放射治療患者は現在の1.9～2倍になると推定された[9]．

この増加率を基準化案の施設層に適用すると，Fig. 4に示す如く，今後5年～10年という短い期間で，各施設は基準化案の1ランク上の施設層に移行すると推定された．

考　案

基準化案の充足率は，現状ではA施設，B施設ともに10%以下と低く，各施設層は1ランク下の基準を半数が満たしているに過ぎなかった．C施設では1/4が基準を満たしていた．これは低コストで必要最低限の装備を広く設置してき

た従来のわが国の経緯から考えれば，当然といえる結果である．一方，見逃されがちな重要な所見は，本基準化案ならびに厚生労働省がん研究助成金PCSによる施設層別化は構造の充実度を装備，人員の両面で見事に識別しており，患者負荷総数およびAcademic institution，Non-academic institutionという分類の仕方が構造問題を考察する上で十分機能していることを示唆している．装備に関しては，PCSの*A1*施設では2台弱のLinacがあり，ビームエネルギー選択の自由度が確保されていることを示していた．*A2*以下では1台以下であり，特に*B2*施設では2割強がまだ^{60}Coを使用していることが示唆された．今後，機器更新に伴い多エネルギー装置の設置は必須となろう．CT simulatorは*A*施設層で4割以上，HDR RALSは7割以上設置されているが，*B1*施設では2割，4割と普及率が低かった．*B2*施設ではわずか5%，4%の普及率であった．さらに人員に関して顕著な所見は，*B*施設層で放射線腫瘍医が1名確保されておらず，*B1*施設：0.6FTEにおいては診断業務との兼任，*B2*施設：0.2FTEにおいては週一回の非常勤勤務の実態が明らかであった．Non-academic institutionである*B*施設層においては大学からの関連病院への人員派遣体制等のシステムをも視野にいれたPCS層別化が現実的な分類と考える．*A*施設層と*B*施設層では装備，人員の供給に顕著な差が観察された．

これらの構造の差が，胸部食道癌非手術例に対するビームエネルギーの選択や子宮頸癌腔内照射適応率の差[10]などの診療過程に顕著な影響を及ぼしていた．このことは施設層ごとに診療の質Quality of Careが明らかに異なっていることを示唆している．装備，人員を含む構造とこれらの診療過程および治療結果をすべてモニタし，問題を明らかにし，解決するための具体的データを提供するのがPCSの最終目標である．

PCSにより推定される将来需要の増加を基準化案の施設層に適用すると今後5年～10年という短い期間で，B施設層はA施設層に，C施設層はB施設層に移行すると推定された．現在は1ランク下の基準を満たしているにすぎないので，現状容認の場合，元B施設が本来のB施設基準を，元C施設がC施設基準を満たすように時間をかけて進化していくという見方もできる．ただA施設には上限を設けていなかったので，たとえば「A'」施設として600例以上の場合には現実にはA基準が適用され，「A"」施設として1,000例以上の場合には欧米並みの新たな基準が必要になるかも知れない．この患者数の増加は放射線腫瘍学の発展にとっては追い風であり，患者に対して地域格差なく，質の高い放射線治療を提供できるように構造を戦略的に改善させて行かなくてはならない．その意味で基準化案ならびにPCSは重要な役割を担っている．

物理士供給問題は本検証においては避けたが，昨今の高精度放射線治療の臨床適用において若い放射線腫瘍医ならびに診療放射線技師による自己犠牲的な作業には限界がある．今後のわが国独自の研究開発を含めて，これらの重要作業を担える有能な医用物理士の教育，育成ならびに病院内での地位確保は，放射線腫瘍学の将来にとって重要課題である．全国の教育機関での大きな流れとして，従来の診療放射線技師を育成する医療短期大学が4年制化し，大学院前期，後期課程も続々と認可されている．形の上では若い医用物理学修士MS，医用物理学博士PhDを教育できる体制が整ってきた（ただ過渡期特有の，細部での不整合が存在している）．これらの教育研究機関とも密接に連携を取りながら，臨床現場でのニーズを敏感に捕捉できる有能な医用物理士を学会挙げて教育育成する体制を構築することも重要であろう．病院における地位確保対策として，たとえば線量計算に対する医師との責任分担を欧米のように法制化することも一つの案であろう．

結　語

1. 日本放射線腫瘍学会の基準化案の施設層別基準充足率は，現状では1ランク下あるいはそれ以下であった．
2. 施設層別に基準化案充足率に比例関係が観察された．PCS施設層別でも構造（装備，人員）の成熟度に比例関係と診療過程の顕著な違いが観察された．
3. 患者数増加率から，今後5～10年で各施設共に基準化案の1ランク上の施設層へ移行すると推定される．
4. 年間患者数600例以上のA施設でさらに上位ランクの新たな基準が必要である．

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CLINICAL INVESTIGATION **Lung**

THE PATTERNS OF CARE STUDY AND REGIONAL CANCER REGISTRY FOR NON–SMALL-CELL LUNG CANCER IN JAPAN

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Purpose: We examined whether the data registered in the Japanese Patterns of Care Study (PCS) for patients with non–small-cell lung cancer (NSCLC) represent the actual situation of radiotherapy in Japan. The Osaka Cancer Registry (OCR) data, forming the largest database of a regional cancer registry in Japan, were adopted for use as a benchmark against the national condition.

Patients and Methods: We examined 906 patients of the PCS treated between 1995 and 1997 and 845 patients of the OCR registered between 1988 and 1992. The investigation was made by descriptive statistical methods to measure age, stage, combined treatments, type of treated hospitals, and prognosis. Furthermore, the national averages (NAs) of the PCS process (PCS NA) were also calculated to compensate for the imbalance in the PCS data sampling.

Results: The mean age was 67.3 ± 10.1 in PCS and 64.4 ± 11.0 in OCR ($p < 0.001$), 67.2 in PCS NA. The male ratio was 84.2% in PCS and 84.0% in OCR ($p = 0.411$), 84.1% in PCS NA. The ratio of the patients at the localized stage was 24.2% in PCS and 15.6% in OCR ($p = 0.001$), 21.1% in PCS NA. The ratio of surgery combined was 24.2% in PCS and 28.9% in OCR ($p = 0.026$), 25.3% in PCS NA. The ratio of chemotherapy combined was 50.1% in PCS and 67.5% in OCR ($p = 0.001$), 47.4% in PCS NA. Because the definitions of institution classification and period of prognostic inquiry were different between the two databases, the 3-year survival rates were calculated for reference. In the nonsurgery group, it was 20.3% in PCS and 11.3% in OCR ($p = 0.001$), and in the surgery group it was 52.5% in PCS and 42.2% in OCR ($p = 0.057$).

Conclusions: Ages in the two databases were inconsistent. Sex distributions were consistent. Surgery and chemotherapy were more frequently performed for the OCR patients, and more patients at more advanced stages were also observed in OCR. The PCS NAs of sex, stage, and ratio of surgery combined were at the midpoints between those of PCS and OCR. The survival rate of NSCLC patients in the OCR was significantly inferior to that in the PCS. The follow-up rate of the PCS was lower than that of the OCR. The general features of PCS data showed similarity to OCR data, and the results of the PCS NAs suggested the effectiveness of this method to adjust the sampling imbalance in PCS. © 2003 Elsevier Inc.

Patterns of Care Study, Osaka Cancer Registry, Non–small-cell lung cancer, Radiation therapy.

INTRODUCTION

The Japanese Patterns of Care Study (PCS) was started in 1996 and is based on a modification of the PCS protocol of the United States (1). The purpose of the PCS is to determine the current status of radiotherapy and improve its quality in Japan. The purpose of this study was to examine whether the PCS data actually represent the national situa-

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