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Inhibitory Effects of Transforming Growth Factor-β1 Pretreatment on Experimental Pulmonary Metastasis of MCS-1 Chinese Hamster Mesenchymal Chondrosarcoma Cells

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FUJISAWA N., SATO N.L. and MOTOYAMA T.-I. *Inhibitory Effects of Transforming Growth Factor-β1 Pretreatment on Experimental Pulmonary Metastasis of MCS-1 Chinese Hamster Mesenchymal Chondrosarcoma Cells.* Tohoku J. Exp. Med., 1999, **187** (3), 203-213 —— Recent studies have suggested that transforming growth factor(TGF)-β1 acts as a multifunctional regulator of cell growth, and also modifies tumor progression and metastasis. In the present study, we investigated the effects of TGF-β1 on the proliferation and experimental pulmonary metastasis of MCS-1. MCS-1 are undifferentiated type cloned tumor cells established from a mesenchymal chondrosarcoma which spontaneously occurred in the soft tissue of a female Chinese hamster. MCS-1 cells were pretreated with TGF-β1 (0, 0.05, 0.5, 2, 10 ng/ml) for 72 hours in a medium containing 1% fetal bovine serum, then tested for in vitro growth by the MTT method, in vivo growth by subcutaneous inoculation into athymic nude mice (1×10^6 cells/mouse) and experimental pulmonary metastasis by injection into the lateral teil vein of athymic nude mice (5×10^4 cells/mouse). TGF-β1 significantly inhibited in vitro growth of MCS-1, depending on its concentrations, and also experimental metastasis with maximal inhibition at 0.5 or 2 ng/ml treatment compared to untreated controls. TGF-β1, however, was ineffective for in vivo subcutaneous growth of MCS-1. These results indicated that TGF-β1 might be an inhibitor of metastasis of mesenchymal chondrosarcomas including other types of non-epitherial cartilage or bone formation tumors. ———— experimental metastasis; TGF-β1; mesenchymal chondrosarcoma; Chinese hamster

In the treatment of malignant tumors, inhibition of metastasis is most important and also a difficult problem for both surgeon and oncologist. Metastasis is completed through many processes in a complex manner (Fidler 1990), and

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experimental models for each process have been required. Heterogeneity in the metastatic potential and behavior indicating the metastasizing route and organ specificity was seen not only in cell lines established from many types or origins of tumors but also in clones from the same parent line (metastatic variant theory) (Fidler 1978, 1990; Nicolson and Dulski 1986).

Human mesenchymal chondrosarcoma is a rare malignant tumor of bone or soft tissue, and inclines to metastasize to the lungs. Prognosis is usually poor. As far as we know, there have been many case reports of human mesenchymal chondrosarcoma (Salvadoret al. 1971; Steiner et al. 1973; Shimo-Oku et al. 1980; Nakashima et al. 1986; Sans et al. 1996), but very few experimental reports.

MCS-1 is one of the clonal cell lines established from its parent culture cells derived from a mesenchymal chondrosarcoma which spontaneously occurred in the cheek pouch of a female Chinese hamster (Fujisawa et al. 1991). MCS-1 exibits an undifferentiated sarcoma cell phenotype, with no tendency to differentiate to cartilage, but has a strong potential to metastasize to the lungs of athymic nude mice when inoculated into the teil vein (Fujisawa et al. 1995). This experimental system which uses MCS-1 cells can be a useful model for the later stage of intravenously disseminated pulmonary metastasis of human mesenchymal chondrosarcoma.

Transforming growth factor (TGF)-β1 is widely distributed in tissues (Thompson et al. 1989). This peptide is produced by both normal and malignant cells, and acts as a multifunctional cell regulator. Recent reports have suggested that TGF-β1 regulates tumor invasion and metastasis, and might enhance the metastatic potential (Welch et al. 1990; Mooradian et al. 1992; Wright and Huang 1996).

In the present experiment, we investigated the effects of TGF-β1 on the growth and experimental metastatic potential to the lungs of MCS-1 cells in athymic nude mice.

Materials and Methods

Animals

Specific pathogen free 7-week-old male BALB/c athymic nude mice were purchased from Charles River Japan (CRJ), Inc. (Kanagawa). A total of 80 mice were used for the present study in accordance with the guidelines of the animal experimentation of Niigata University. The mice were housed in plastic cages (CRJ, W143$\times$D293$\times$H148 mm, 3 or 4 mice/cage) with bedding (CRJ, White wood flakes) and kept in a lamina air flow rack and given a cubic diet, CE-2 (CLEA Japan, Inc., Tokyo) and water ad libitum. All feeding materials were sterilized in a autoclave. Constant temperature (23 ± 2°C) and humidity (40 to 70%) were maintained. The air in the room was exchanged 18 times per hour and illuminated with daylight fluorescent lamps for 12 hours (6 : 00 a.m. to 6 : 00 p.m.) a day.

Tumor cells

MCS-1 cells were used. This cell line was cloned by single cell culture from the parent line established from a mesenchymal chondrosarcoma found in the soft tissue (cheek pouch) of an adult female Chinese hamster (Fujisawa et al. 1991). MCS-1 cells were characterized as undifferentiated mesenchymal phenotype which have a strong potential to metastasize to the lungs when introduced into the circulation by injection into the lateral teil vein of athymic nude mice (Fujisawa et al. 1995).

Cell culture and TGF-β1 treatment

MCS-1 cells were routinely maintained in Eagle's minimal essential medium (MEM) (Nissui, Tokyo) supplemented with 10% fetal bovine serum (FBS) (GIBCO, Rockville, MD, USA) and the medium was changed every other day. Porcine platelet derived TGF-β1 was purchased from R & D Systems (Minneapolis, MN, USA). All cultures were incubated at 37°C in a humidified atmosphere at 5% CO_2 in air. In order to minimize the undesirable effects of the serum, the concentration of FBS in the medium was gradually reduced to 1%. In short, the cells were plated in MEM containing 5% FBS and cultured at 37°C for 24 hours. After that the medium was changed to MEM containing 1% FBS and cultured again for 24 hours. Thereafter, MCS-1 cells were treated with TGF-β1 in MEM containing 1% FBS for 72 hours. The medium was changed every 24 hours during treatments. Throughout the experiments, the cells were treated in the same manner.

In vitro growth experiment

MCS-1 cells were plated in a 96-well microplate (IWAKI GLASS, Chiba) and each well contained 1×10^4 cells in 100 μliter of MEM containing 5% FBS. After 48 hours, the cells were incubated with TGF-β1 (0, 0.05, 0.5, 2, 10 ng/ml) in MEM containing 1% FBS for 72 hours. The number of viable cells was measured by the MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) method (Twentyman and Luscombe 1987) with a cell proliferation kit (Boehringer Mannheim, Mannheim, Germany) and a micro plate reader (BIO-RAD, Richmond, CA, USA). In vitro growth assay was performed in duplicate and the mean absorbance was calculated for 12 wells/group.

Experimental pulmonary metastasis

MCS-1 cells were plated 1.5×10^5/dish in 60 mm plastic petri dishes (IWAKI GLASS, Chiba). Two dishes per group were used. After the treatment with TGF-β1, the cells were detached by means of 0.25% trypsin in PBS solution and pelleted by centrifugation in MEM supplemented with 1% FBS. Cells from two dishes were added and resuspended in the above medium at 2.5×10^5 cells/ml.

Viability was determined by trypan blue dye exclusion test, and the rate of cell clump formation was counted by using a hemocytometer. Only cell suspensions with viabilities over 95% and cell clump (consisting of 2 or 3 cells) formation rates below 10% were used for inoculation. Seven mice/group received 5×10^4 cells/0.2 ml intravenously through the lateral teil vein. The mice were killed by over-dose anesthetization with ether on the 25th day post-inoculation, and their lungs were removed. Each metastatic nodule in non-fixed lung was separately removed with hafted needles with the aid of a dissecting microscope, and the number of nodules larger than 0.5 mm in diameter was counted with a vernier caliper. This experiment was performed in duplicate (except for TGF-β1 10 ng/ml treatment in Exp. 2).

In vivo growth experiment (tumorigenicity)

MCS-1 cells (1×10^6/0.2 ml) were pretreated with TGF-β1 in the same manner as described in the metastasis assay and were subcutaneously inoculated into the dorsal region of the athymic nude mice (3 mice/group). Up to the 19th day postinoculation, the width (W) and length (L) of solid tumors were mesured twice a week with a caliper, and the tumor volume (V) was estimated by the formula: $V\ (mm^3)=(W^2\times L)/2$.

Statistical analyses

Statistical analyses were done by one-way layout analysis of variance and Dunnet's procedure for multiple comparisons with a control.

Results

Effects of TGF-β1 on MCS-1 cell growth in vitro

Table 1 indicates the mean absorbance of each group of two experiments after treatment with TGF-β1 for 72 hours. TGF-β1 significantly inhibited the growth of MCS-1 cells compared with untreated controls ($p<0.001$ by one-way layout

Table 1. *Effects of TGF-β 1 on in vitro growth of MCS-1*

TGF-β1 (ng/ml)	Absorbance	
	Experiment 1[a]	Experiment 2[a]
0 (Control)	0.222±0.025	0.238±0.023
0.05	0.209±0.028	0.165±0.018[b]
0.5	0.172±0.018[b]	0.153±0.010[b]
2	0.171±0.024[b]	0.149±0.010[b]
10	0.138±0.011[b]	0.144±0.008[b]

Data are expressed as the mean±S.D. [a]$p<0.01$ by one-way layout analysis of variance performed to compare the values for each experiment. [b]$p<0.01$ with Dunnet's procedure for multiple comparisons with a control.

analysis of variance). In Exp. 1, the inhibition rates were 16% at 0.05 ng, 23% at 0.5 ng, 23% at 2 ng and 38% at 10 ng/ml. They were statistically significant except a case of 0.05 ng/ml concentration according to Dunnet's procedure for multiple comparisons with the control ($p<0.01$). In Exp. 2, inhibition rates were 31% at 0.05 ng, 36% at 0.5 ng, 37% at 2 ng and 39% at 10 ng/ml. They were all statistically significant ($p<0.01$). The growth inhibitory effects of TGF-β1 were dose-dependent and the maximal inhibition was observed at a concentration of 10 ng/ml in both experiments.

Effects of TGF-β1 on experimental pulmonary metastasis of MCS-1

Untreated MCS-1 cells made larger number of metastatic nodules in the lungs of the athymic nude mice than TGF-β1 treated MCS-1 cells (Fig. 1). Pretreatment with TGF-β1 significantly inhibited experimental pulmonary metastasis of MCS-1 cells in both Exp. 1 and 2 ($p<0.01$) (Fig. 2). The mean number of MCS-1 metastatic nodules (and inhibition rate) treated with TGF-β1 at 0, 0.05, 0.5, 2 and 10 ng/ml were 366, 185 (49.5%), 67 (81.7%), 107 (70.8%) and 94 (74.3%), respectively in Exp. 1, and those treated with TGF-β1 at 0, 0.05, 0.5 and 2 ng/ml were 200, 72 (64.0%), 66 (67.0%) and 39 (80.5%), respectively, in Exp. 2. The maximal inhibition rate was observed when MCS-1 cells were treated with 0.5 ng/ml TGF-β1 in Exp. 1 and 2 ng/ml in Exp. 2.

Effects of TGF-β1 on MCS-1 cell growth in vivo

The mean sizes (mm^3) of subcutaneous solid tumors in each group 19 days after inoculation of MCS-1 cells into athymic nude mice were 2405 in control, 2485

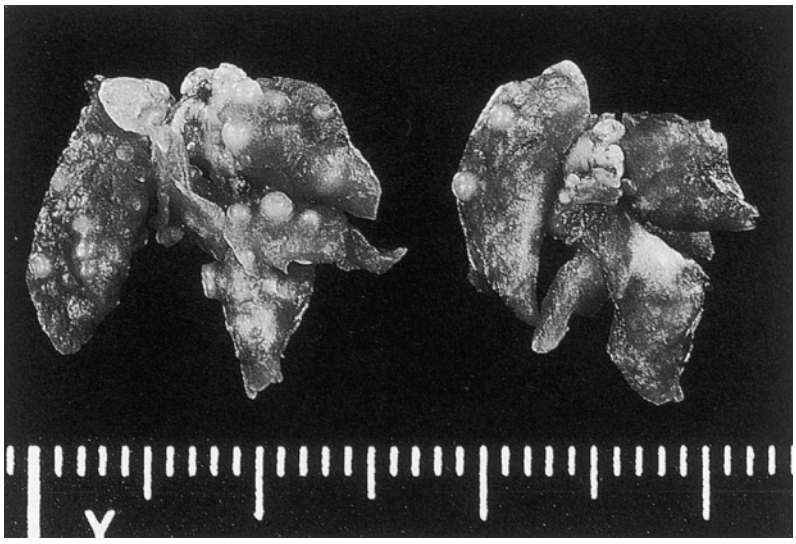

Fig. 1. Macroscopic findings of representative lungs of BALB/c athymic nude mice from an experiment to show colonization by MCS-1 5×10^4 cells/mouse in TGF-β1 2 ng/ml pretreatment (right) and in controls (left). Lungs were removed 25 days post-inoculation and fixed. TGF-β1-treated cells make less metastatic nodules (white or light gray colored) than untreated control.

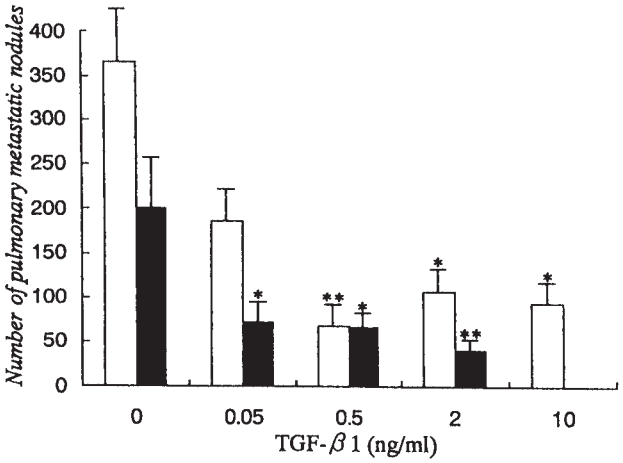

Fig. 2. Effects of TGF-β1 on experimental MCS-1 pulmonary metastasis. Columns (□, Experiment 1; ■, Experiment 2) are expressed as the mean number of metastatic nodules, and error bars are expressed as S.E.M. $p<0.01$ by one-way layout analysis of variance performed to compare the values for each experiment. $^{*}p<0.05$ and $^{**}p<0.01$ by Dunnet's procedure for multiple comparisons with each control. Seven BALB/c athymic nude mice/group were used for each experiment.

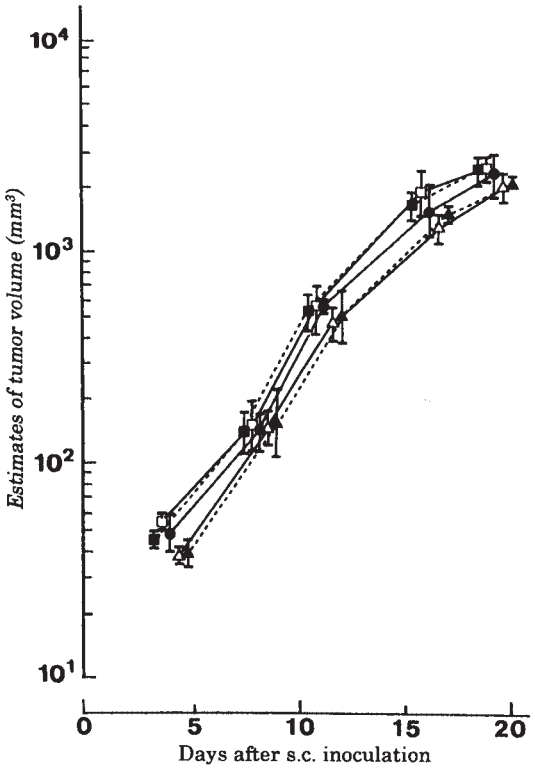

Fig. 3. In vivo growth of MCS-1 after subcutaneous (s.c.) injection into BALB/c athymic nude mice. The growth of cells treated with TGF-β1 was not significantly different at any concentrations of TGF-β1 compared with the untreated controls. Tumor volume was estimated by the formula: tumor volume (mm^3), $(a^2 \times b)/2$; a = width (mm), b = length (mm). Values are the mean ± S.E.M. obtained from 3 mice. ●—●, Control; ■—■, 0.05 ng; □—□, 0.5 ng; ▲—▲, 2 ng; △—△, 10 ng/ml.

at 0.05 ng, 2486 at 0.5 ng, 2207 at 2 ng and 2180 at 10 ng/ml of TGF-β1. No significant difference between TGF-β1 pretreated groups and the untreated control group was seen in the in vivo growth rate or the mean size of solid tumors (Fig. 3). Similar tumorigenicity was seen in all groups.

Discussion

TGF-β1 was identified as a cartilage inducing factor, CIF-A (Seyedin et al. 1986) which induced cartilagenous differentiation of undifferentiated mesenchymal cells (Seyedin et al. 1985) and stimulated type ll collagen synthesis of cultured periosteal cells (Izumi et al. 1992). TGF-β1 is also closely related to tumor growth and metastasis (Nicolson 1993). TGF-β1 is considered to have an effect on the differentiation, growth and metastasis of MCS-1 undifferentiated type sarcoma cells derived from mesenchymal chondrosarcoma. TGF-β1 inhibits proliferation of many kinds of malignant cells as well as almost all normal cells (Roberts et al. 1985). But some tumor cell lines such as those of human breast cancer, human gastric carcinoma and mouse prostate cancer do not respond to the growth inhibitory activity of TGF-β1 (Arrick et al. 1990; Ito et al. 1992; Sehgal et al. 1996). On the other hand, TGF-β1 stimulates the growth of other types of tumor such as Swarm rat chondrosarcoma (Guerne and Lotz 1991) and highly metastasizing human adenocarcinoma of the sigmoid colon (Hoefer and Anderer 1995).

TGF-β1 is now categorized as a bifunctional regulator of cell growth, and it has different effects depending upon the cell type or culture conditions in vitro (Roberts et al. 1985). In fact, the growth of vascular smooth muscle cells (Majack 1987) and poorly differentiated rat prostate cancer cells (Morton and Barrack 1995) are inhibited at low cell density but conversely enhanced or not changed at high cell density by TGF-β1.

In tumor metastatic behavior, a significant correlation between TGF-β1 gene expression or protein synthesis and metastatic potential was observed in invasive human breast cancer (Walker et al. 1994), H-ras transformed mouse fibrosarcoma (Spearman et al. 1994) and mouse prostate cancer (Sehgal et al. 1996). Pretreatment with TGF-β1 stimulates the experimental potential of rat MTLn3 mammary adenocarcinoma cells to metastasize to the lungs by intravenous injection into syngeneic rats (Welch et al. 1990). It has been suggested that the enhanced metastatic potential of MTLn3 is due to the induction of high invasive activity by TGF-β1 which stimulates the collagenolytic activities of matrix metalloproteinases (MMPs). MMPs such as gelatinase A (MMP-2) and gelatinase B (MMP-9) destroy the basement membrane barrier and the tumor cells can invade and penetrate more easily. Furthermore, it has been suggested that TGF-β1 enhances the invasion of human breast cancer cells by the induction of urokinase-type plasminogen activator which indirectly activates MMPs (Arnoletti et al. 1995). These reports support the idea that TGF-β1 is inclined to assist tumor

invasion and metastasis.

In the present study, however, pretreatment with TGF-β1 (0.05–10 ng/ml) for 72 hours significantly reduced the number of metastatic nodules of MCS-1 cells in the lungs of athymic nude mice. Although in vitro growth of MCS-1 was dose-dependently inhibited, no effect of TGF-β1 was observed on the in vivo growth of subcutaneously injected MCS-1 cells. These results suggest that the inhibitory effect of TGF-β1 on metastasis of MCS-1 is not due to a change in the growth potential of MCS-1 cells in lungs.

Hoefer and Anderer (1995) reported that the in vitro growth of human adenocarcinoma cells of the sigmoid colon was increased by TGF-β1 in the lung metastatic subline derived from xenotransplant into athymic nude mice, but not in its parent line. But the development of subcutaneously inoculated primary tumors into athymic nude mice and distant lung metastases from both parent line and metastatic subline, could be suppressed by treatment of mice with anti-TGF-β1 antibody. They therefore assumed that the metastatic potential of tumor cells is independent of TGF-β-mediated growth-regulatory effects in vitro. Many peptide growth factors including TGF-β1 have been identified as multifunctional cell regulators unrelated to the control of growth (Sporn and Roberts 1988). We think that the inhibitory effects of TGF-β1 on experimental metastasis of MCS-1 cells are independent of its growth suppression in vitro, and TGF-β1 blocks a certain step in the serial process of metastasis after infiltration of cells into circulation. Only a few studies showing that TGF-β1 inhibited tumor growth directly in vivo and metastasis indirectly have yet been reported. Subcutaneous or peritumoral injection of TGF-β1 inhibited the growth of A549 human lung tumor cells in athymic nude mice (Twardzik et al. 1989), and pretreatment with TGF-β1 reduced the invasion of human follicular thyroid cancer cells (Hoelting et al. 1994) and that of U-138MG human glioblastoma cells (Paulus et al. 1995).

In the present study, we have shown the first case in which pretreatment with TGF-β1 significantly inhibited experimental pulmonary metastasis by using the established mesenchymal chondrosarcoma cell line MCS-1. We performed an immunohistochemical study on type ll collagen formation in TGF-β1-treated and -untreated MCS-1 cells in culture because we thought that the mechanism of metastasis inhibition by TGF-β1 might be based on the induction of cell differentiation by which MCS-1 cells could alter their malignant metastasizing phenotype, but no induction of type ll collagen by TGF-β1 was observed (Data not shown).

Bereta et al. (1992) reported that TGF-β1 inhibited adhesion of mouse P815 mastocytoma cells to endotherial cells. We supposed that TGF-β1 might prevent MCS-1 cells from adhering to microvascular endothelium because of the changed expression of the cell surface adhesion molecules. As another mechanism of inhibition of metastasis, it was supposed that TGF-β1 might alter the responsiveness of MCS-1 cells to autocrine and/or paracrine growth factors secreted by

surrounding cells in the target organ.

It remains to be confirmed whether the inhibitory effects of TGF-β1 on tumor metastasis is specific for mesenchymal chondrosarcoma derived cells such as MCS-1, or general for all types of cartilaginous tumor cells. Furthemore, to clarify the mechanism of inhibition of metastasis is very important in making new choices for the clinical application of TGF-β1.

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References

1) Arnoletti, J.P., Albo, D., Granick, M.S., Solomon, M.P., Castiglioni, A., Rothman, V.L. & Tuszynski, G.P. (1995) Thrombospondin and transforming growth factor-beta 1 increase expression of urokinase-type plasminogen activator and plasminogen activator inhibitor-1 in human MDA-MB-231 breast cancer cells. *Cancer*, **76**, 998–1005.
2) Arrick, B.A., Korc, M. & Derynck, R. (1990) Differential regulation of expression of three transforming growth factor β species in human breast cancer cell lines by estradiol. *Cancer Res.*, **50**, 299–303.
3) Bereta, J., Bereta, M., Coffman, F.D., Cohen, S. & Cohen, M.C. (1992) Inhibition of basal and tumor necrosis factor-enhanced binding of murine tumor cells to murine endothelium by transforming growth factor-β1. *J. Immunol.*, **148**, 2932–2940.
4) Fidler, I.J. (1978) General considerations for studies of experimental cancer metastasis. In: *Methods in cancer research*, edited by H. Bush, Academic Press, New York, pp. 399–439.
5) Fidler, I.J. (1990) Critical factors in the biology of human cancer metastasis: Twenty-eighth G.H.A., Clowes memorial award lecture. *Cancer Res.*, **50**, 6130–6138.
6) Fujisawa, N., Sato, N.L. & Motoyama, T.-I. (1991) Establishment of clonal cell lines, with or without cartilage phenotypes, from a hamster mesenchymal chondrosarcoma. *Lab. Anim. Sci.*, **41**, 590–595.
7) Fujisawa, N., Sato, N.L. & Motoyama, T.-I. (1995) Experimental pulmonary metastasis of Chinese hamster mesenchymal chondrosarcoma cell lines. *Exp. Anim.*, **43**, 761–764. (in Japanese with English abstract)
8) Guerne, P.-A. & Lotz, M. (1991) Interleukin-6 and transforming growth factor-β synergistically stimulate chondrosarcoma cell proliferation. *J. Cell. Physiol.*, **149**, 117–124.
9) Hoefer, M. & Anderer, F.A. (1995) Anti-(transforming growth factor β) antibodies with predifined specificity inhibit metastasis of highly tumorigenic human xenotransplants in nu/nu mice. *Cancer Immunol. Immunother.*, **41**, 302–308.
10) Hoelting, T., Zielke, A., Siperstein, A.E., Clark, O.H. & Duh, Q.-Y. (1994) Aberrations of growth factor control in metastatic follicular thyroid cancer in vitro. *Clin. Exp. Metastasis*, **12**, 315–323.
11) Ito, M., Yasui, W., Kyo, E., Yokozaki, H., Nakayama, H., Ito, H. & Tahara, E. (1992) Growth inhibition of transforming growth factor β on human gastric carcinoma cells: Receptor and postreceptor signaling. *Cancer Res.*, **52**, 295–300.
12) Izumi, T., Scully, S.P., Heydemann, A. & Bolander, M.E. (1992) Transforming

growth factor β1 stimulates type II collagen expression in cultured periosteum-derived cells. *J. Bone Miner. Res.*, **7**, 115–121.

13) Majack, R.A. (1987) Beta-type transforming growth factor specifies organizational behavior in vascular smooth muscle cell cultures. *J. Cell Biol.*, **105**, 465–471.
14) Mooradian, D.L., McCarthy, J.B., Komanduri, K.V. & Furcht, L.T. (1992) Effects of transforming growth factor-β1 on human pulmonary adenocarcinoma cell adhesion, motility, and invasion in vitro. *J. Natl. Cancer Inst.*, **84**, 523–527.
15) Morton, D.M. & Barrack, E.R. (1995) Modulation of transforming growth factorβ1 effects on prostate cancer cell proliferation by growth factors and extracellular matrix. *Cancer Res.*, **55**, 2596–2602.
16) Nakashima, Y., Unni, K.K., Shives, T.C., Swee, R.G. & Dahlin, D.C. (1986) Mesenchymal chondrosarcoma of bone and soft tissue: A review of 111 cases. *Cancer*, **57**, 2444–2453.
17) Nicolson, G.L. & Dulski, K.M. (1986) Organ specificity of metastatic tumor colonization is related to organ-selective growth properties of malignant cells. *Int. J. Cancer*, **38**, 289–294.
18) Nicolson, G.L. (1993) Paracrine and autocrine growth mechanisms in tumor metastasis to specific sites with particular emphasis on brain and lung metastasis. *Cancer Metastasis Rev.*, **12**, 325–343.
19) Paulus, W., Baur, I., Huettner, C., Schmauer, B., Roggendorf, W., Schlingensiepen, K.H. & Brysch, W. (1995) Effects of transforming growth factor β1 on collagen synthesis, integrin expression, adhesion and invasion of glioma cells. *J. Neuropathol. Exp. Neurol.*, **54**, 236–244.
20) Roberts, A.B., Anzano, M.A., Wakefield, L.M., Roche, N.S., Stern, D.F. & Sporn, M.B. (1985) Type β transforming growth factor: A bifunctional regulator of cellular growth. *Proc. Natl. Acad. Sci. USA*, **82**, 119–123.
21) Salvador, A.H., Beabout, J.W. & Dahlin, D.C. (1971) Mesenchymal chondrosarcoma: Observations on 30 new cases. *Cancer*, **28**, 605–615.
22) Sans, M., Nubiola, D., Alejo, M., D'az, F., Anglada, A., Autonell, J. & Brugus, J. (1996) Mesenchymal chondrosarcoma of the foot, an unusual location: Case report and review of the literature. *Med. Pediatr. Oncol.*, **26**, 139–142.
23) Sehgal, I., Baley, P.A. & Thompson, T.C. (1996) Transforming growth factor β1 stimulates contrasting responses in metastatic versus primary mouse prostate cancer-derived cell lines in vitro. *Cancer Res.*, **56**, 3359–3365.
24) Seyedin, S.M., Thomas, T.C., Thompson, A.Y., Rosen, D.M. & Piez, K.A. (1985) Purification and characterization of two cartilage-inducing factors from bovine demineralized bone. *Proc. Natl. Acad. Sci. USA*, **82**, 2267–2271.
25) Seyedin, S.M., Thompson, A.Y., Bentz, H., Rosen, D.M., McPherson, J.M., Conti, A., Siegel, N.R., Galluppi, G.R. & Piez, K.A. (1986) Cartilage-inducing factor-A: Apparent identity to transforming growth factor-β. *J. Biol. Chem.*, **261**, 5693–5695.
26) Shimo-Oku, M., Okamoto, N., Ogita, Y. & Sashikata, T. (1980) A case of mesenchymal chondrosarcoma of the orbit. *Acta Ophthal.*, **58**, 831–840.
27) Spearman, M., Taylor, W.R., Greenberg, A.H. & Wright, J.A. (1994) Antisense oligodeoxyribonucleotide inhibition of TGF-β1 gene expression and alterations in the growth and malignant properties of mouse fibrosarcoma cells. *Gene*, **149**, 25–29.
28) Sporn, M.B. & Roberts, A.B. (1988) Peptide growth factors are multifunctional. *Nature*, **332**, 217–219.
29) Steiner, G.C., Mirra, J.M. & Bullough, P.G. (1973) Mesenchymal chondrosarcoma: A study of the ultrastructure. *Cancer*, **32**, 926–939.
30) Thompson, N.L., Flanders, K.C., Smith, J.M., Ellingsworth, L.R., Roberts, A.B. & Sporn, M.B. (1989) Expression of transforming growth factor-β1 in specific cells and tissues of adult and neonatal mice. *J. Cell Biol.*, **108**, 661–669.
31) Twardzik, D.R., Ranchalis, J.E., McPherson, J.M., Ogawa, Y., Gentry, L., Purchio, A.,

Plata, E. & Todaro, G.J. (1989) Inhibition and promotion of differentiated-like phenotype of a human lung carcinoma in athymic mice by natural and recombinant forms of transforming growth factor-β. *J. Natl. Cancer Inst.*, **81**, 1182–1185.

32) Twentyman, P.R. & Luscombe, M. (1987) A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. *Br. J. Cancer*, **56**, 279–285.

33) Walker, R.A., Dearing, S.J. & Gallacher, B. (1994) Relationship of transforming growth factor-β1 to extracellular matrix and stromal infiltrates in invasive breast carcinoma. *Br. J. Cancer*, **69**, 1160–1165.

34) Welch, D.R., Fabra, A. & Nakajima, M. (1990) Transforming growth factor β stimulates mammary adenocarcinoma cell invasion and metastatic potential. *Proc. Natl. Acad. Sci. USA*, **87**, 7678–7682.

35) Wright, J.A. & Huang, A. (1996) Growth factors in mechanisms of malignancy: Roles for TGF-β and FGF. *Histol. Histopathol.*, **11**, 521–536.