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Regulation of Gelatinase Production in Metastatic Renal Cell Carcinoma by Organ-specific Fibroblasts

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We have recently established a human renal cell carcinoma KG-2 line that is tumorigenic in the subcutis (ectopic) and kidney (orthotopic) of nude mice but spontaneously metastasizes to the lung only after orthotopic implantation. KG-2 cells growing in the kidney (orthotopic) and lung metastases secreted higher levels of gelatinase than did cells growing in the subcutis (ectopic). We examined whether organ-specific fibroblasts play a role in the regulation of gelatinase production and invasion by renal carcinoma cells. The gelatinase level in the culture supernatants of KG-2 cells was increased by their cultivation with mouse kidney or lung fibroblasts. In contrast, cocultivation of KG-2 cells with mouse skin fibroblasts resulted in a significant reduction of gelatinase activity. Similar results were obtained by culturing KG-2 cells in the media conditioned by the different mouse fibroblasts. We, therefore, investigated effects on KG-2 cells of cytokines and growth factors known to be produced by fibroblasts of various origins. Of ten cytokines and growth factors tested, basic fibroblast growth factor, hepatocyte growth factor, and transforming growth factor-β_1 (TGF-β_1) stimulated gelatinase expression by the cultured KG-2 cells. Parallel immunohistochemical analyses revealed that mouse kidney and lung fibroblasts produced higher levels of TGF-β_1 than did skin fibroblasts. These results indicate that gelatinase production by KG-2 renal cell carcinoma cells is influenced by the organ microenvironment. Specifically, organ-specific fibroblasts regulate the production of degradative enzymes by KG-2 cells and, hence, profoundly influence their invasive and metastatic capacity.

Key words: Renal cell carcinoma — Metastasis — Gelatinase — Fibroblast

The outcome of cancer metastasis depends on multiple interactions of metastatic tumor cells with various host factors.[1)] Since the process begins with the invasion of host stroma by tumor cells and their entry into the circulation,[2)] the ability of tumor cells to degrade connective tissues and basement membranes is a prerequisite for invasion and metastasis.[3–16)] Metastatic tumor cells produce matrix metalloproteinases with degradative activity for interstitial collagen, collagen type IV and V, gelatin and proteoglycans.[5, 6, 11, 12, 13)] In several independent rodent and human tumor systems, production of gelatinases (type IV collagenases) is directly correlated with invasive-metastatic potential.[4, 11, 17)]

To study the metastatic behavior of human tumor cells isolated from surgical specimens, it is necessary to implant them into an anatomically relevant (orthotopic) organ of nude mice.[1, 18–20)] For example, human colon cancer cells implanted into the subcutis of nude mice can produce local benign tumors, whereas the same cells implanted into the cecal wall produce distant metastases.[21–24)] Similarly, the orthotopic implantation of human KG-2 renal carcinoma cells (into the kidney) produced local tumors and distant metastases, whereas subcutaneous implantation resulted in only benign tumors.[25)] These divergent results suggested the possibility that an organ environment may differentially influence the metastatic behavior of KG-2 renal carcinoma cells. We, therefore, examined the production of gelatinases by KG-2 tumors growing in the kidney and subcutaneous tissues. We also examined whether the production of gelatinases by KG-2 cells was influenced in culture with organ-specific fibroblasts or their conditioned media. Finally, we report the effects of cytokines and growth factors, which are differentially expressed by fibroblasts from different organs, on gelatinase activity of the tumor cells.

MATERIALS AND METHODS

Animals Male athymic BALB/c nude mice were obtained from the animal production area of the NCI-Frederick Cancer Research Facility (Frederick, MD). The mice were maintained under specific pathogen-free

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conditions in facilities approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current regulations and standards of the United States Department of Agriculture, Department of Health and Human Services, and National Institutes of Health. The mice were used for experiments when they were 8–10 weeks old.

Tumor cells The human renal cell carcinoma KG-2 line was established in culture with cells dissociated enzymatically from a surgical specimen classified histologically as a clear cell carcinoma.[25] KG-2 cells were maintained on plastic as monolayer cultures in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS), sodium pyruvate, nonessential amino acids, L-glutamine, and a 2-fold vitamin solution (GIBCO, Grand Island, NY). The cells were free of *Mycoplasma*, reovirus type 3, pneumonia virus of mice, mouse adenovirus, murine hepatitis virus, lymphocytic choriomeningitis virus, ectromelia virus, and lactate dehydrogenase virus (Microbiological Associates Bethesda, MD).

Mouse fibroblast cultures Primary cultures of nude mouse skin, lung, and kidney fibroblasts were derived from 18- to 19-day-old fetuses. Cultures of skin fibroblasts were established from explants of minced dermis. Cultures of lung or kidney fibroblasts were derived from cells isolated from the organs by enzymatic dissociation with collagenase type I (200 U/ml) and DNases (270 U/ml) (Sigma Chemicals, St. Louis, MO) as described previously.[26] The fibroblast cultures were maintained in supplemented EMEM (see above) with 15% FBS. Confluent cultures were harvested with 0.25% trypsin-0.02% EDTA. Cells from passages 4 to 6 were used in all *in vitro* assays.

Growth and production of metastasis Nude mice were injected with 1×10^6 viable tumor cells into the subcutis or the subrenal capsule exactly as described previously.[26] When subcutaneous tumors reached 2 cm in diameter (60–80 days), the mice were killed and autopsied. Local tumors were fixed in 10% buffered formalin solution and the lungs were placed in Bouin fixative. The number of peripheral lung nodules (larger than 0.1 mm in diameter) was determined with the aid of a dissecting microscope. Histological examination verified the presence or absence of tumors.

Tumor cell growth in media conditioned by mouse fibroblasts Fibroblast cultures (70% confluency) in 75-cm^2 flasks were washed three times with serum-free MEM and refed with 10 ml of serum-free DMEM/F12 (GIBCO). After 24-h incubation, the culture supernatants were collected, sequentially centrifuged at 800g and 18,000g, and filtered through a 0.45-μm membrane. KG-2 cells (5×10^4/60 mm dish) were incubated for 24 h in supplemented EMEM. The adherent cells were rinsed three times with serum-free DMEM/F12 and refed with or without conditioned medium from organ-specific fibroblasts (1:1 and 1:3, v/v). After different incubation periods, triplicate cultures/group were harvested by using 0.25% trypsin-0.02% EDTA solution and the number of viable cells (trypan blue exclusion) was determined.

Gelatin zymography Gelatinolytic enzymes secreted by cultured cells were identified and quantified by electrophoresis of serum-free conditioned medium in a gelatin-embedded polyacrylamide gel. Briefly, cells were incubated in supplemented EMEM for 24 h. The adherent cells were washed three times with serum-free MEM and refed with serum-free DMEM/F12 or conditioned medium. After 24-h incubation, the medium was collected and sequentially centrifuged at 800g and 18,000g. The supernatants were concentrated with Centricon 30 miocroconcentrators (Amicon Corporation, Beverly, MA) and immediately mixed with SDS sample buffer without 2-β-mercaptoethanol. Electrophoresis was carried out as previously described[4] using acrylamide gels containing 1 mg/ml gelatin (heat-denatured porcine skin collagen type I, Sigma Chemicals). The gels were then rinsed twice with 2.5% Triton X-100 in 50 mM Tris-HCl buffer (pH 7.5) and incubated at 37°C for 16 h in 0.15 M NaCl, 10 mM $CaCl_2$, and 50 mM Tris-HCl buffer (pH 7.5) containing 0.05% NaN_3. The gels were stained with 0.05% Coomassie blue, 10% isopropanol and 10% acetic acid in deionized water and then destained with 10% isopropanol and 10% acetic acid in deionized water. Gelatinolytic enzymes were detected as transparent bands on the background of Coomassie blue-stained slab gels. Quantitative analysis of the gelatinolytic enzyme was performed by a Pharmacia LKB-UltroScan XL laser densitometer. The area under each peak representing gelatinolytic activity was measured. The average integrated intensity of three individual experiments was calculated as a relative gelatinase activity.

Gelatinolytic activity in media conditioned by KG-2 tumors growing in nude mice KG-2 tumors growing in the subcutis, kidney and lungs were harvested by aseptic procedures and their wet weight was measured. The tumors were dissected, freed of surrounding tissues and any zones of necrosis, minced, and then cultured in serum-free DMEM/F12 (10 ml of medium per 100 mg of tumor tissue). After 24 h incubation, the conditioned media were collected and sequentially centrifuged at 800g and 18,000g. The supernatants were collected and 1-ml aliquots were concentrated as described above for gelatin zymography.

Gelatinase production by tumor cells cocultured with organ-specific mouse fibroblasts Fibroblasts from different organs were incubated to confluency in 30-mm culture dishes. KG-2 cells (2.5×10^5/dish) were plated on

top of the fibroblast monolayers. After 24-h incubation in supplemented EMEM, the cultures were rinsed three times with serum-free DMEM/F12, and 2.5 ml of serum-free DMEM/F12 was added. The media were collected 24-h later, concentrated and analyzed for gelatinase activity by zymography.

Gelatinase production by tumor cells cultured in media conditioned by mouse fibroblasts To remove gelatinases produced by mouse fibroblasts, their conditioned media were passed through a gelatin-Sepharose column (Pharmacia LKB Biotechnology, Uppsala, Sweden) and then filtered through a 0.45-μm Millipore filter. KG-2 cells (1×10^6) were plated into a 75-mm^2 flask containing 10 ml of supplemented EMEM. After 24 h, the adherent cells were rinsed with serum-free DMEM/F12 and incubated for an additional 24 h in DMEM/F12 with or without conditioned media from organ-specific fibroblasts (1:1, v/v). The culture supernatants were then collected and centrifuged, and 2-ml aliquots (associated with 2.5×10^5 viable cells) were concentrated and analyzed for gelatinase activity by zymography.

Gelatinase production by tumor cells treated with various growth factors or cytokines KG-2 cells were plated at a density of 2×10^5 cells/30 mm dish and incubated for 24 h in supplemented EMEM. The cultures were rinsed and refed with serum-free DMEM/F12. After a 24-h incubation, the medium was replaced with serum-free DMEM/F12 containing different concentrations of the following human growth factors or cytokines: recombinant basic fibroblast growth factor (FGF), platelet derived growth factor (PDGF), recombinant epidermal growth factor (EGF) (Collaborative Research, Bedford, MA), transforming growth factor-β_1 (TGF-β_1) (R&D Systems, Minneapolis, MN), recombinant interleukin-1-β (IL-1β), tumor necrosis factor-α (TNF-α) (Boehringer-Mannheim, Indianapolis, IN), recombinant hepatocyte growth factor/scatter factor (HGF/SF) (Toyobo, Osaka) and recombinant granulocyte colony stimulating factor (G-CSF), recombinant monocyte colony stimulating factor (M-CSF), recombinant granulocyte-monocyte colony stimulating factor (GM-CSF) (Kirin Brewery Company, Ltd., Tokyo). All growth factors and cytokines were diluted with serum-free DMEM/F12. The KG-2 cells were incubated for 24 h and then the culture supernatants were harvested and gelatinase activity was determined by zymography. To ascertain that the tumor cells were viable and proliferating, cell number/culture was determined as described before.

Immunohistochemical analysis for growth factors produced by mouse fibroblasts Organ-derived fibroblasts (1×10^4) were plated into wells of a Lab Tek chamber slide (Nunc, Naperville, IL) and incubated for 72 h in supplemented EMEM. The cultures were then rinsed three times with phosphate-buffered saline (PBS), fixed for 5 min with a 1:1 mixture of acetone and chloroform, and incubated for 20 min in PBS containing 1% normal goat serum and 1% normal horse serum. The fibroblasts were then incubated overnight at 4°C in a humidified chamber with either anti-bovine basic FGF type I monoclonal IgG$_1$ (Upstate Biotechnology, Inc., Lake Placid, NY), rabbit polyclonal anti-TGF-β_1 antibody (R&D Systems), or mouse monoclonal anti-HGF (a gift from Dr. Naomi Kitamura, Kansai Medical University, Osaka). The slides were then rinsed with PBS and incubated for 1 h at room temperature with gold-labeled secondary antibodies (Amersham, Arlington Heights, IL). Following several rinses with PBS, the slides were fixed for 10 min with 2% glutaraldehyde and rinsed with several changes of distilled water. The cells were then treated with Silver intense according to the manufacturer's directions (Amersham), counterstained with Mayer's hematoxylin, mounted using Crystal mount (Fisher Scientific, Pittsburgh, PA) and examined by both bright field and epipolarization microscopy.

RESULTS

Tumorigenicity and production of spontaneous metastasis by KG-2 cells KG-2 cells produced tumors in all mice injected into the subcutis or the subrenal capsule (n=10).

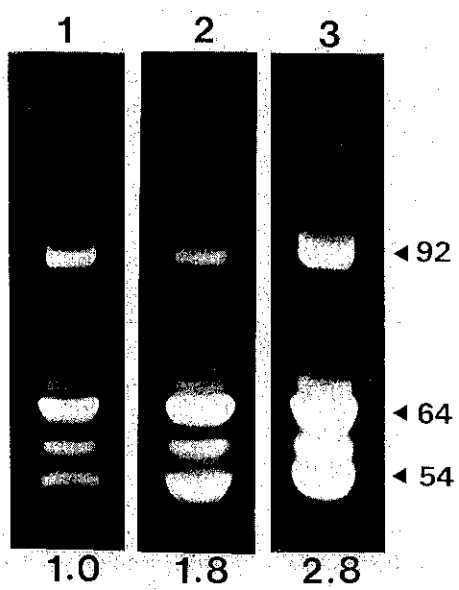

Fig. 1. Identification of gelatinolytic activity in media conditioned by KG-2 tumors growing in nude mice. Lane 1, subcutaneous tumor; lane 2, kidney tumor; lane 3, lung metastases. Numbers shown on the right side of the zymogram are molecular weights (in thousands) of standard proteins. Numbers on the bottom of the zymogram are the relative gelatinolytic activity.

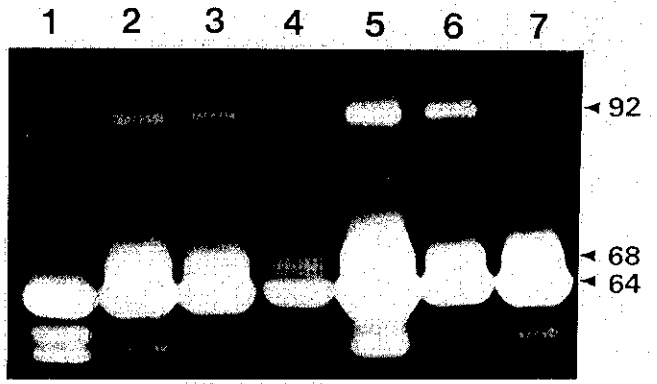

Fig. 2. Gelatinolytic activity of mouse fibroblasts cultured alone or with KG-2 cells. Lane 1, KG-2 cells; lane 2, mouse kidney fibroblasts; lane 3, mouse skin fibroblasts; lane 4, mouse lung fibroblasts; lane 5, KG-2 cells and kidney fibroblasts; lane 6, KG-2 cells and skin fibroblasts; lane 7, KG-2 cells and lung fibroblasts. Numbers shown on the right side indicate molecular weight in thousands.

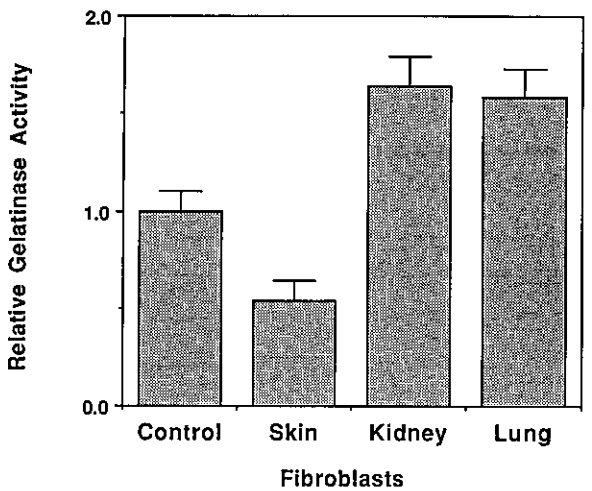

Fig. 3. Relative gelatinolytic activity in cocultures of KG-2 and mouse fibroblasts. Gelatinolytic activity in the cocultures of KG-2 cells and fibroblasts was compared with the total activity in the conditioned medium of KG-2 cells cultured alone or fibroblasts cultured alone. Relative gelatinase activity was determined by means of the following formula: Relative gelatinase activity = gelatinase activity in the conditioned medium of KG-2 cell-fibroblast coculture/(gelatinase activity in the KG-2 cell culture medium + gelatinase activity in the fibroblast culture medium).

In agreement with our earlier data,[25] KG-2 tumors growing in the kidney produced spontaneous lung metastases (median of 10) whereas those in the subcutis did not. Histologic examination revealed that the subcutaneous tumors were not well vascularized and were surrounded by a fibrous pseudocapsule.

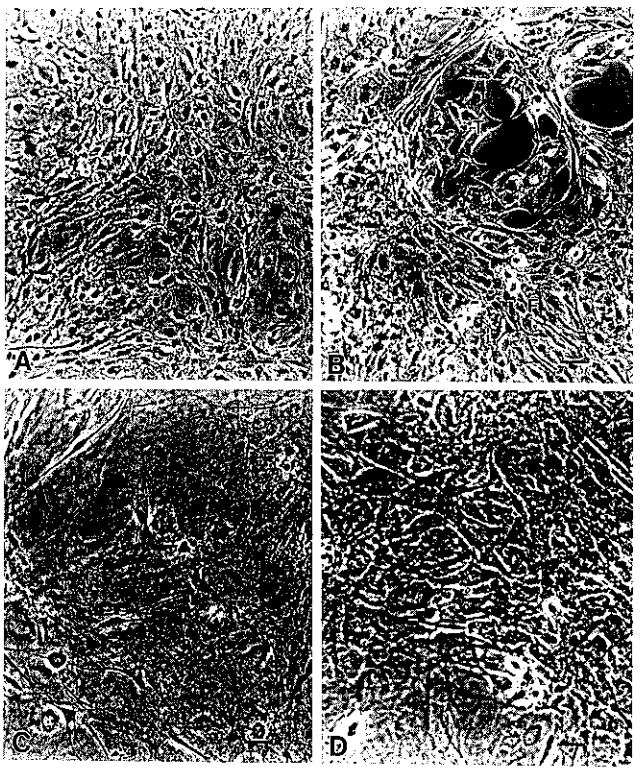

Fig. 4. Phase-contrast micrographs of KG-2 cells cultured with mouse fibroblasts. A, Monolayer of mouse kidney fibroblasts. B, KG-2 cells and mouse kidney fibroblasts, Note degradation of mouse kidney fibroblasts (F) by KG-2 tumors cells (T). C, Monolayer of mouse skin fibroblasts. D, KG-2 cells growing on top of mouse skin fibroblasts. No degradation is seen. Bars indicate the length of 50 μm.

Gelatinolytic activity in conditioned media from KG-2 tumors growing in nude mice The gelatinolytic activity in the media conditioned by KG-2 cells growing in the subcutis, kidney and lung of nude mice is shown in Fig. 1. The highest activity was found in the medium conditioned by KG-2 cells growing as metastases in the lung followed by kidney and subcutaneous tumors. The relative enzyme activity was 1.0 for the subcutaneous tumors, 1.8 for kidney tumors, and 2.8 for lung metastases.

Gelatinase production by KG-2 cells cocultured with organ-specific mouse fibroblasts The media conditioned by cultures of KG-2 cells contained 72-kDa gelatinase activity (68-kDa and 64-kDa clear bands shown in Fig. 2) but not significant 92-kDa gelatinase activity. Mouse kidney, skin and lung fibroblasts produced both 72-kDa and 92-kDa gelatinases. The gelatinase levels in the media conditioned by cocultures of KG-2 cells and

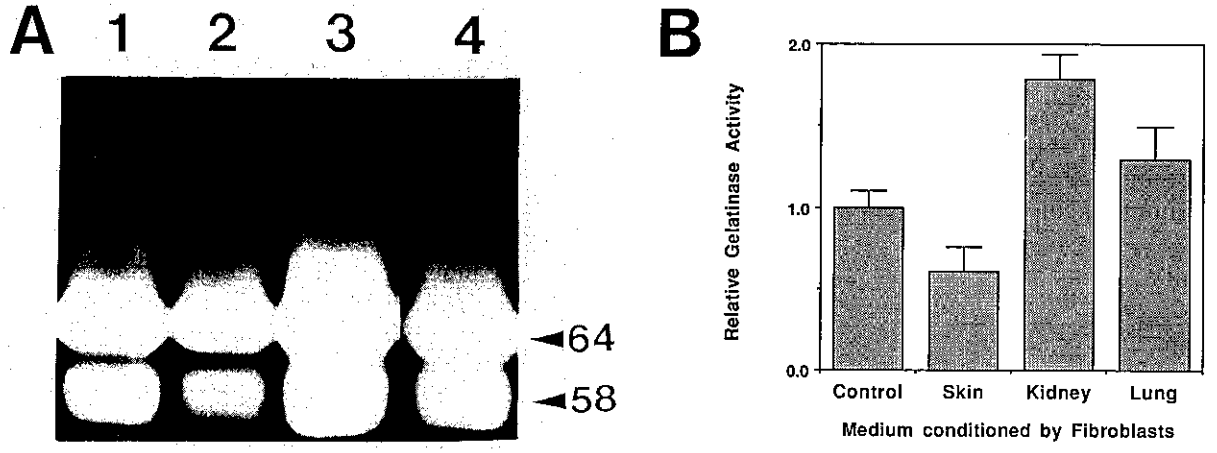

Fig. 5. A) Identification of gelatinolytic activity secreted by KG-2 cells cultured in control media and media conditioned by mouse fibroblasts. Lane 1, KG-2 cells cultured in control media; lane 2, KG-2 cells cultured with skin fibroblast-conditioned media; lane 3, KG-2 cells cultured with kidney fibroblast-conditioned media; lane 4, KG-2 cells cultured with lung fibroblast-conditioned media. Numbers shown on the right side of the zymogram indicate molecular weights in thousands. B) Relative gelatinolytic activity of KG-2 cells cultured in media conditioned by mouse fibroblasts. KG-2 cells were cultured in the presence or absence of medium conditioned by fibroblasts derived from different mouse organs. The zymogram shown in Fig. 5A was scanned by a Pharmacia LKB-UltroScan XL laser densitometer. The average integrated intensity of three individual experiments is shown as the relative gelatinase level.

mouse skin fibroblasts were lower than those found for either cells cultured alone. In contrast, the gelatinase levels in the media conditioned by cocultures of KG-2 cells with mouse kidney or lung fibroblasts were higher than those found for either KG-2 cells or fibroblasts cultured alone (Fig. 2). The relative gelatinolytic activities in conditioned media from KG-2 cells cocultured with mouse kidney, skin, or lung fibroblasts were 1.4, 0.4, and 1.3, respectively, relative to a value of 1.0 assigned to gelatinase activity found in the conditioned medium of KG-2 cells growing alone (Fig. 3).

We next examined the interaction of KG-2 cells with mouse fibroblasts by microscopy. KG-2 cells proliferated on all monolayers of fibroblasts. After 24 h, the KG-2 cells invaded through confluent monolayers of kidney and lung fibroblasts but not skin fibroblasts (Fig. 4).

Influence of conditioned media from organ-specific fibroblasts on KG-2 cell growth and gelatinase production The growth of KG-2 cells was unaffected by medium conditioned by mouse fibroblasts. However, the production of gelatinases by KG-2 cells was stimulated in medium containing 1:1 conditioned medium of mouse kidney and lung fibroblasts and was decreased by the conditioned medium of mouse skin fibroblasts (Fig. 5A). The relative gelatinolytic activities of KG-2 cells in the presence of conditioned media from kidney, skin, and lung fibroblasts were 1.8, 0.6, and 1.2, respectively (Fig. 5B).

Influence of growth factors and cytokines on the *in vitro* production of gelatinase by KG-2 cells The gelatinolytic activity of KG-2 cells was enhanced by their incubation with human basic FGF. Treatment with basic FGF at 0.1, 1.0, and 10 ng/ml increased the KG-2 cell gelatinolytic activity 2.0, 4.8, and 4.5 times, respectively (Table I). Treatment for 24 h of KG-2 cells with 10 and 100 pg/ml TGF-β_1 increased the gelatinolytic activity 1.7- and 2.8-fold, respectively. In contrast, human EGF, IL-1β, and PDGF did not have significant effects on the gelatinolytic activity of KG-2 cells (Table I).

Immunohistochemical analysis of cultured mouse fibroblasts The cytoplasm of mouse kidney and lung fibroblasts reacted intensely with anti-TGF-β_1 antibodies, whereas skin fibroblasts did not (Fig. 6). No significant difference was observed among the three fibroblast cultures in reactivity to antibodies against basic FGF or HGF (data not shown).

DISCUSSION

We studied the influence that different mouse organs (and their fibroblasts) exert on the production of gelatinase by metastatic human renal carcinoma KG-2 cells. The KG-2 cells produce slow-growing tumors in the subcutis and kidney of nude mice.[26] The kidney tumors are highly vascularized, nonencapsulated and produce spontaneous lung metastases. In contrast, the subcutane-

Table I. Gelatinolytic Activities Secreted by KG-2 Cells Treated with Various Growth Factors and Cytokines

Factor	Concentration	Relative gelatinase activity
Human TGF-β_1	1.0 pg/ml	1.07
	10	1.74
	100	2.83
Human EGF	1.0 ng/ml	1.10
	10	1.24
	100	1.13
Human basic FGF	0.1 ng/ml	2.00
	1.0	4.84
	10	4.53
Human HGF	0.1 ng/ml	1.76
	1.0	3.04
	10	3.54
Human PDGF	0.1 ng/ml	1.10
	1.0	0.90
	10	1.00
Human G-CSF	0.1 ng/ml	1.04
	10	1.02
Human M-CSF	0.1 ng/ml	1.00
	1.0	1.02
	10	0.94
Human GM-CSF	0.1 ng/ml	1.02
	1.0	1.03
	10	1.08
Human TNF-α	1.0 U/ml	1.10
	10	1.00
	100	1.20
Human IL-1 β	1.0 U/ml	1.10
	10	1.00
	100	1.20

Relative levels of gelatinases in conditioned media of KG-2 cells were determined by zymography. Zymograms were scanned by a Pharmacia LKB-UltroScan XL laser densitometer. The average integrated intensity of three individual experiments is shown as a relative gelatinase level (SD$<$17%). The gelatinase activity of KG-2 cells growing in control media$=$1.0.

ous tumors are poorly vascularized and encapsulated with central necrosis, and do not produce spontaneous metastasis. The different biological behaviors of the KG-2 cells growing at different organ sites in nude mice suggest that the microenvironment influences the metastatic potential of the KG-2 cells.

The ability to degrade connective tissues and basement membranes is prerequisite for the production of metastasis, and the production of type IV collagenases (gelatinases) by tumor cells correlates with their invasive and metastatic potential.[3, 5, 8, 11, 17, 22, 27] We have previously shown that the organ environment can influence the production of gelatinases and hence invasion by human colon cancer cells.[4] The present data extend these observations. We measured the gelatinolytic activity of human renal cell carcinoma growing in the subcutis, kidney, and lung (metastases) of nude mice and observed a direct correlation between the *in vivo* production of gelatinase and metastasis. KG-2 kidney tumors (metastatic) and lung metastases produced higher gelatinolytic activity than KG-2 subcutaneous tumors (not metastatic).

There is now increasing evidence that fibroblasts derived from different anatomical sites in the adult display functional phenotypic heterogeneity in their morphology, interactions with steroid hormones, growth capacity, and production of cytokines.[28] Unique interactions of orthotopic fibroblasts with tumor cells have been demonstrated in *in vitro* systems utilizing both rodent and human samples.[29–32] Diffusible factors produced by fibroblasts subsequent to their interactions with tumor cells have been shown to stimulate the growth of mouse mammary tumor cells, human breast cancer cells, and human colon carcinoma cells.[33–37] We therefore examined the relationship between the organ microenvironment and the behavior of renal carcinoma cells.

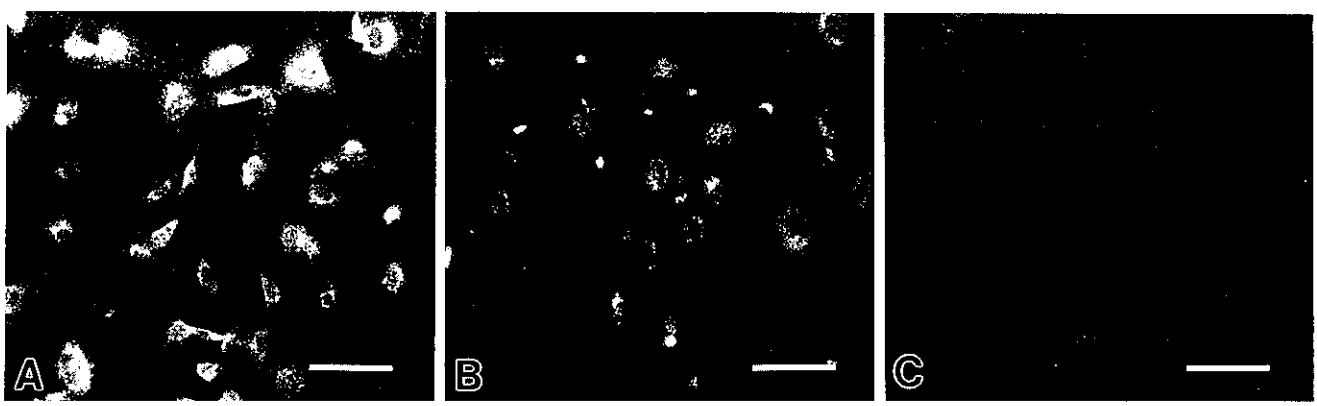

Fig. 6. Immunogold silver staining of cultured fibroblasts isolated from different organs of nude mice. Cytoplasm of fibroblasts from the kidney and the lung reacted more intensely with the anti-TGF-β_1 antibody as compared to skin fibroblasts. The cells were examined in the epipolarized mode. A, Kidney fibroblasts. B, Lung fibroblasts. C, Skin fibroblasts. Bars indicate the length of 100 μm. ($\times$155)

We isolated fibroblasts from orthotopic (kidney, lung) and ectopic (skin) tissues and cocultured the KG-2 cells on the fibroblast monolayers. The growth of KG-2 cells was unaffected by cocultivation with different mouse fibroblasts or their conditioned media. However, the gelatinolytic activity of the KG-2 cells was increased by coculture with mouse kidney or lung fibroblasts and decreased by coculture with mouse skin fibroblasts. Moreover, the KG-2 cells degraded monolayers of mouse kidney and lung fibroblasts but not mouse skin fibroblasts. These results are consistent with the *in vivo* behavior of the KG-2 cells. The finding that mouse lung fibroblasts increased gelatinase production in the KG-2 cells is consistent with the observation that the lung is the most common site for their spontaneous metastases in nude mice. The KG-2 cells from the lung metastases produced the highest activity of gelatinase as compared with the cells growing in other sites, suggesting that other tissue factors in addition to lung fibroblasts may play a role in the enhancement of gelatinase production by the KG-2 cells in the lung.

There are several mechanisms by which stromal cells and tumor cells interact. Epithelial cells can produce a variety of growth factors that can influence fibroblast function, whereas fibroblasts produce tissue-specific extracellular matrix.[38-40] While growth factors can induce and alter extracellular matrix gene expression,[36,38] the matrix can influence the type and level of growth factors and their receptors.[41] The expression of gelatinase by normal and tumor cells is known to be modulated by various growth factors, cytokines, and extracellular matrix.[42-46] Since gelatinase production by fibroblasts can be stimulated by interleukin-1,[42] PDGF,[43] EGF and TGF-β,[44] we examined whether these and other growth factors secreted from fibroblasts also influenced gelatinase production by KG-2 cells. The incubation of KG-2 cells with TGF-β_1, basic FGF, or HGF/SF increased gelatinolytic activity. TGF-β has been shown to regulate production of 72-kDa gelatinase and interstitial collagenase by fibroblasts.[44] Similarly, treatment of rat mammary adenocarcinoma cells with TGF-β enhanced the production of 72-kDa and 92-kDa gelatinases, and pretreatment of the tumor cells with TGF-β enhanced their metastatic potential.[17,47] In the present study, we found that a 24-h treatment of KG-2 cells with 10 pg/ml TGF-β increased the production of 72-kDa gelatinase. Since fibroblasts from mouse kidney and lung stained intensely with anti-TGF-β_1 antibodies wherease skin fibroblasts did not, the data are consistent with the finding that coculture of KG-2 cells with kidney and lung fibroblasts enhanced the production of gelatinase.

Basic FGF stimulates the proliferation and migration of vascular endothelial cells[48,49] and enhances gelatinase and urokinase production by vascular endothelial cells.[50,51] HGF is a fibroblast-derived scatter factor which stimulates migration of normal and transformed epithelial cells.[52-56] We found that both basic FGF and HGF increased gelatinase production by KG-2 cells. However, since we did not find discernible differences in production of these growth factors by fibroblasts from different mouse tissues, the relevance of these molecules to the present findings is unclear.

We observed that skin fibroblasts and their conditioned media decreased gelatinase production and invasion by KG-2 cells. Therefore, the skin fibroblasts probably secrete a factor(s) which depresses the expression of gelatinase in KG-2 cells. We are currently investigating a negative regulatory factor(s) produced by skin fibroblasts.

In summary, our results demonstrate that fibroblasts from orthotopic and ectopic organs differentially affect the production and secretion of degradative enzymes by tumor cells and, hence, their invasive and metastatic properties.

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