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Influence of cytokines and growth factors on matrix metalloproteinase-2 production and invasion of human renal cancer

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Abstract This study evaluated the influence of cytokines and growth factors on the production of matrix metalloproteinase-2 (MMP-2, 72-kDa type IV collagenase, gelatinase A) and invasion of the human renal cell carcinoma (HRCC) cell line KG-2. The cells were treated with cytokines and growth factors, and the gelatiolytic activity and in vitro invasion were examined. Basic fibroblast growth factor (bFGF) stimulated MMP-2 production by KG-2 cells to 2.0-, 4.84- and 4.53-fold that of the untreated group at 0.1, 1.0 and 10 ng/ml, respectively. Transforming growth factor-β1 (TGF-β1) at very low concentrations of 10 pg/ml and 100 pg/ml stimulated enzyme production in KG-2 cells by 1.74- and 2.83-fold, respectively. In contrast, interferon-γ (IFN-γ) decreased MMP-2 production by KG-2 cells at 10 and 100 U/ml to 69% and 41% of the level in the untreated group, respectively. At those concentrations, IFN-γ did not cause cytostasis in KG-2 cells. Moreover, bFGF and TGF-β1 (low concentrations) stimulated in vitro invasion of KG-2 cells, but IFN-γ decreased the invasive activity, which was well correlated with the levels of MMP-2. However, the expression of MMP-2 mRNA of KG-2 cells treated with 10 ng/ml bFGF, 100 pg/ml TGF-β1 and 100 U/ml IFN-γ was shown to be 3.8-, 3.4- and 0.7-fold, respectively, those in untreated groups. Thus the production of MMP-2 in HRCC was influenced by cytokines and growth factors, and MMP-2 plays an important role in the invasion and metastasis of certain types of HRCC.

Key words Renal cancer · matrix metalloproteinase-2 · Invasion · Cytokine · Growth factor

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Introduction

In cancer metastasis, extracellular matrix (ECM) degradative enzymes such as matrix metalloproteinases (MMPs), serine proteinase and cystine proteinase are necessary to degrade the ECM and vascular basement membrane [6, 10, 13]. The production of these ECM degradative enzymes is regulated by an ECM substrate or cytokines and growth factors that are secreted from non-neoplastic cells [8]. The relationship of cancer cells and non-neoplastic cells originating from host tissues is thus important for cancer metastasis [8]. In the present study, we examined the influence of cytokines and growth factors that are secreted from host stromal cells on the production of matrix metalloproteinase 2 (MMP-2) in a human renal cell carcinoma (HRCC) cell line. We also determined the influence of these factors on the in vitro invasive activity of the cell line.

Materials and methods

HRCC cells

One HRCC cell line (KG-2) was used. This cell line was established from grade 2, clear cell HRCC; it produced tumors at both the subcutis (SC; ectopic) and subrenal capsular sites (SRC; orthotopic) [4]. The SRC tumors metastasized to the lung and regional lymph nodes; SC tumors did not metastasize [4]. KG-2 cells were cultured in Eagle's minimum essential medium (EMEM; Gibco, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (FBS), sodium pyruvate, non-essential amino acids and 2-fold vitamin. For subculturing, cells were treated with 0.25% trypsin/0.02% EDTA (Sigma, St. Louis, Mo.) for 2–3 min, tapped slightly and subcultured. The cells were free of *Mycoplasma* and pathogenic mouse viruses.

Cytokines and growth factors

All cytokines and growth factors used in this study and their concentrations are shown in Table 1. All cytokines and growth

factors were of human origin. Recombinant basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), recombinant epidermal growth factor (EGF; Collaborative Research, Bedford, Mass.), transforming growth factor-β1 (TGF-β1; R&D Systems, Minneapolis, Minn.), recombinant interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α; Boehringer-Mannheim, Indianapolis, Ind.), recombinant interferon-α 2A (IFN-α; Hoffman-La-Roche, Nutley, N.J.) and recombinant interferon-γ (IFN-γ; Genentech, San Francisco, Calif.) were used. All cytokines and growth factors were diluted with serum-free F-12/DMEM (Gibco) to several concentrations.

Microculture tetrazolium assay

An assay for the in vitro antiproliferative effect of KG-2 cells treated by cytokines and growth factors was carried out as described by Fan et al. [2]. Cells suspended in EMEM were seeded in 96-well tissue-culture plates (1×10^4 cells/well) and incubated for 16 h. The cells were then rinsed and suspended in fresh serum-free F-12/DMEM, or media containing various concentrations of cytokines and growth factors. After a 96-h incubation, the antiproliferative effect was assessed by the microculture tetrazolium (MTT) microassay. Briefly, the cells were incubated for 2 h with 400 μg/ml MTT and then lysed in 100% dimethylsulfoxide. The conversion of MTT to formazan by metabolically viable cells was monitored by a Dynatech MR 5000 microplate reader at 570 nm (Chantilly, Va.). Growth inhibition by cytokines and growth factors was calculated by the following formula:

$$\text{Cytostasis}\ (\%) = (1 - A/B) \times 100$$

where A is absorbance at 570 nm of the test sample treated with cytokines and growth factors, and B is absorbance at 570 nm of the control sample in medium alone. Each assay was performed in triplicate.

Gelatin zymography

KG-2 cells (5×10^6 viable cells) were cultured in DMEM media supplemented with 10% FBS. After 24 h, the dishes were rinsed with Hanks' balanced salt solution (HBSS) and the KG-2 cell line cultured in serum-free F-12/DMEM with cytokines or growth factors at various concentrations for 24 h. The conditioned medium was collected and centrifuged at 800 *g* for 5 min. Then the supernatants were centrifuged at 18 000 *g* for 20 min. Gelatinolytic enzymes secreted by cultured KG-2 cells were identified and quantified by electrophoresis of serum-free conditioned medium in a gelatin-embedded polyacrylamide gel [9]. The supernatants prepared from serum-free conditioned media (5×10^4 KG-2 cells) were immediately mixed with one-fourth volume of sample buffer containing 2% $NaDodSO_4$, 10% glycerol and 0.00125% bromophenol blue in 62.5 mM TRIS-HCl (pH 6.8), and electrophoresed at 60 mA at 16 °C in a 7.5% polyacrylamide slab gel containing 1 mg/ml gelatin (heat-denatured porcine skin collagen type I: Sigma). The gel was rinsed twice with 2.5% Triton X-100 in 50 mM TRIS-HCl buffer (pH 7.5) and incubated at 37 °C for a certain period 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 (v/v), then destained with 10% isopropanol and 10% acetic acid in deionized water (v/v). Gelatinolytic enzymes were detected as transparent bands against the background of Coomassie-blue-stained slab gels. The active and inactive forms of MMP-2 were shown as bands with different molecular weights.

Quantitative analysis of the gelatinolytic enzyme was performed with a Pharmacia LKB UltraScan XL laser densitometer (Uppsala, Sweden). The area under each peak representing gelatinolytic activity was measured and the average integrated intensity from three independent experiments was calculated. Relative gelatinase production was defined as the average gelatinase production in the culture media of KG-2 cells treated with cytokines or growth factors divided by that in the culture media of KG-2 cells without treatment.

mRNA extraction and Northern-blot hybridization analyses

KG-2 cells in 80% confluent cultures were rinsed with serum-free F-12/DMEM and re-fed with serum-free F-12/DMEM with or without cytokines and growth factors. After 24 h of incubation, the medium was replaced with fresh serum-free F-12/DMEM with or without cytokines and growth factors, and the cells incubated for another 24 h, then harvested for RNA extraction. Total cellular RNA was extracted from 10^7 to 10^8 cells using a guanidinium thiocyanate hot-phenol method. For Northern blot analysis, poly(A)$^+$RNA was prepared by oligo(dT)-cellulose chromatography and fractionated by electrophoresis on a 1% agarose gel under denaturing conditions with formaldehyde. RNA (2 μg) was electrotransferred at 0.6 mA to a GreenScreen nylon membrane (DuPont-NEN, Boston, Mass.) and UV-crosslinked with 120 000 μJ/cm^2 using a UV Stratalinker 1800 (Stratagene, La Jolla, Calif.). The membranes hybridized with ^{32}P-labeled DNA probes were washed three times at 65°C with 30 mM NaCl and 3 mM sodium citrate (pH 7.2) containing 0.1% $NaDodSO_4$ (w/v) and exposed to X-OMAT imaging film (Kodak, Rochester, N.Y.).

Probes for mRNA sequences

Two DNA probes were used: (1) a 1.1-kb *Eco*RI restriction endonuclease fragment from the plasmid pH3a representing the human MMP-2 gene (courtesy of W. G. Stetler-Stevenson, Laboratory of Pathology, National Cancer Institute, Bethesda, Md.); and (2) a 1.2-kb *Pst*I gene fragment corresponding to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). DNA probe fragments were purified by agarose gel electrophoresis, recovered by GeneClean (BIO 101, LaJolla, Calif.) and radiolabeled by the random primer technique using [α-^{32}P]deoxyribonucleotide triphosphate. Bands were quantified by an LKB Ultrascan XL laser densitometer as described above. Relative MMP-2 mRNA expression was defined as the bands of mMMP-2 in KG-2 cells treated with cytokines or growth factors divided by that in KG-2 cells without treatment.

In vitro invasion assay

The invasion assay was carried out as previously described by Nakajima et al. [9] using Coster 6.5-mm transwell culture plates equipped with 8.0-μm pore size polycarbonate membranes (no. 3422; Coster, Cambridge, Mass.) [7]. The upper surface of the membrane was coated with Matrigel (200 μg protein, Collaborative Research). The bottom chamber was filled with a mixture of 500 μl of the medium conditioned with normal kidney fibroblasts and 500 μl of EMEM containing 10% FBS. HRCC cells were pretreated with or without cytokines and growth factors at various concentrations in EMEM with 10% FBS for 48 h. The cells were harvested and cultured in 200 μl of fresh EMEM with 10% FBS and seeded on the reconstituted Matrigel (5×10^5 cells/plate) with and without cytokines or growth factors. After incubation for 60 h in the presence or absence of cytokines and growth factors, cells that penetrated the polycarbonate membrane were harvested from the bottom chamber and centrifuged at 1000 *g* for 10 min. The cells were resuspended in fresh EMEM and counted at least three times in each chamber. Relative invasion was defined as the average number of KG-2 cells that penetrated the membrane after treatment with cytokines and growth factors divided by the average number of KG-2 cells that penetrated the membrane without treatment. Each assay was performed in triplicate.

Results

Effects of cytokines and growth factors on in vitro antiproliferation of HRCC cells

None of the cytokines or growth factors used showed any significant in vitro antiproliferative effect on KG-2 cells (Table 1).

Effects of cytokines and growth factors on MMP-2 production by HRCC cells

KG-2 cells produced and secreted MMP-2 into the culture medium without secreting matrix metalloproteinase-9 (MMP-9, 92-kDa type IV collagenase, gelatinase B)(Fig. 1). bFGF increased the production of MMP-2 (72-kDa type IV collagenase, gelatinase A) by KG-2 cells to 2.0-, 4.84- and 4.53-fold that detected in medium alone at concentrations of 0.1, 1.0 and 10 ng/ml, respectively (Table 1, Fig. 1). Zymography of conditioned medium from KG-2 cells treated with 1.0 ng/ml and 10.0 ng/ml bFGF showed both an active band and an inactive band. The major band of MMP-2 on zymography was an active form of 64 kDa; an indistinct band was an inactive form of 72 kDa (Fig. 1). Moreover, TGF-β1 at the low concentrations of 10 pg/ml and 100 pg/ml, respectively, increased production of MMP-2 of KG-2 cells to 1.74- and 2.83-fold the level in the untreated group (Table 1, Fig. 2). IFN-γ decreased MMP-2 production by KG-2 cells at 10 U/ml and 100 U/ml to 69% and 41% of the level in the untreated group, respectively (Table 1, Fig. 3). At those concentrations, IFN-γ did not cause cytostasis in KG-2 cells. Other cytokines and growth factors used in this study did not show any effect on MMP-2 production by KG-2 cells.

MMP-2 mRNA level in KG-2 cells treated with cytokines and growth factors

Northern blot hybridization of KG-2 cells revealed the expression of a 3.1-kb MMP-2 mRNA transcript and a 2.5-kb transcript (Fig. 4, lanes 1, 3 and 5). This additional transcript was not observed in any other human cell type analyzed including colon carcinoma cells and other HRCC cells (data not shown). mRNA levels were measured by densitometric scanning and normalized by levels of GAPDH mRNA expression as an internal control for possible loading differences. Treatment of KG-2 cells for 48 h with either 10 ng/ml bFGF or 100 pg/ml TGF-β1 resulted in an increase in MMP-2 mRNA expression (Fig. 4, lanes 2 and 4). The cells treated with 10 ng/ml bFGF and 100 pg/ml TGF-β1 demonstrated respectively 3.8 and 3.4 times the steady-state expression of MMP-2 specific mRNA transcripts in the untreated control. Treatment of KG-2 cells with

Table 1 Growth inhibition, relative gelatinase production and relative in vitro invasive activity of KG-2 cells treated with cytokines and growth factors

Cytokines and growth factors	Concentrations	Growth inhibition (%) (mean ± SD)	Relative gelatinase production[b] (mean ± SD)	Relative invasive activity[c] (mean ± SD)
None	–	100	1.00	1.00
bFGF	0.1 ng/ml	98 ± 5	2.00 ± 0.12	1.83 ± 0.08
	1	108 ± 8	4.84 ± 0.32	4.00 ± 0.19
	10	112 ± 6	4.53 ± 0.28	4.25 ± 0.34
TGF-β1	1.0 pg/ml	115 ± 11	1.07 ± 0.11	0.97 ± 0.04
	10	98 ± 7	1.74 ± 0.18	2.05 ± 0.23
	100	112 ± 13	2.83 ± 0.23	3.15 ± 0.24
EGF	1.0 ng/ml	95 ± 5	1.10 ± 0.08	1.00 ± 0.06
	10	112 ± 11	1.24 ± 0.15	1.30 ± 0.05
	100	130 ± 3	1.13 ± 0.18	1.20 ± 0.17
PDGF	1.0 ng/ml	105 ± 6	1.10 ± 0.12	1.00 ± 0.06
	10	110 ± 7	0.90 ± 0.15	1.10 ± 0.07
	100	115 ± 4	1.00 ± 0.08	1.15 ± 0.10
TNF-α	1.0 U/ml	95 ± 3	1.10 ± 0.06	1.10 ± 0.06
	10	86 ± 10	1.00 ± 0.15	1.10 ± 0.05
	100	83 ± 13	1.20 ± 0.13	1.05 ± 0.15
IL-1β	1.0 U/ml	103 ± 8	1.10 ± 0.06	0.95 ± 0.13
	10	115 ± 5	1.00 ± 0.11	0.95 ± 0.10
	100	118 ± 6	1.20 ± 0.25	1.10 ± 0.11
IFN-α	1.0 U/ml	96 ± 8	1.10 ± 0.18	1.15 ± 0.23
	10	90 ± 3	0.90 ± 20	1.20 ± 0.28
	100	82 ± 11	1.21 ± 16	1.18 ± 0.21
IFN-γ	1.0 U/ml	97 ± 8	1.10 ± 0.08	1.20 ± 0.23
	10	91 ± 7	0.69 ± 0.11	0.58 ± 0.10
	100	87 ± 10	0.41 ± 0.09	0.30 ± 0.06

[a] All cytokines and growth factors used in this study are of human origin

[b] Relative gelatinase production = average gelatinase production in the conditioned media of KG-2 cells treated with the cytokines or growth factors/average gelatinase production in KG-2 cell culture medium without treatment

[c] Relative invasive activity = average number of KG-2 cells penetrated through polycarbonate membrane when treated with cytokines or growth factors/average number of KG-2 cells penetrated through the membrane without treatment. Each assay was performed in triplicate

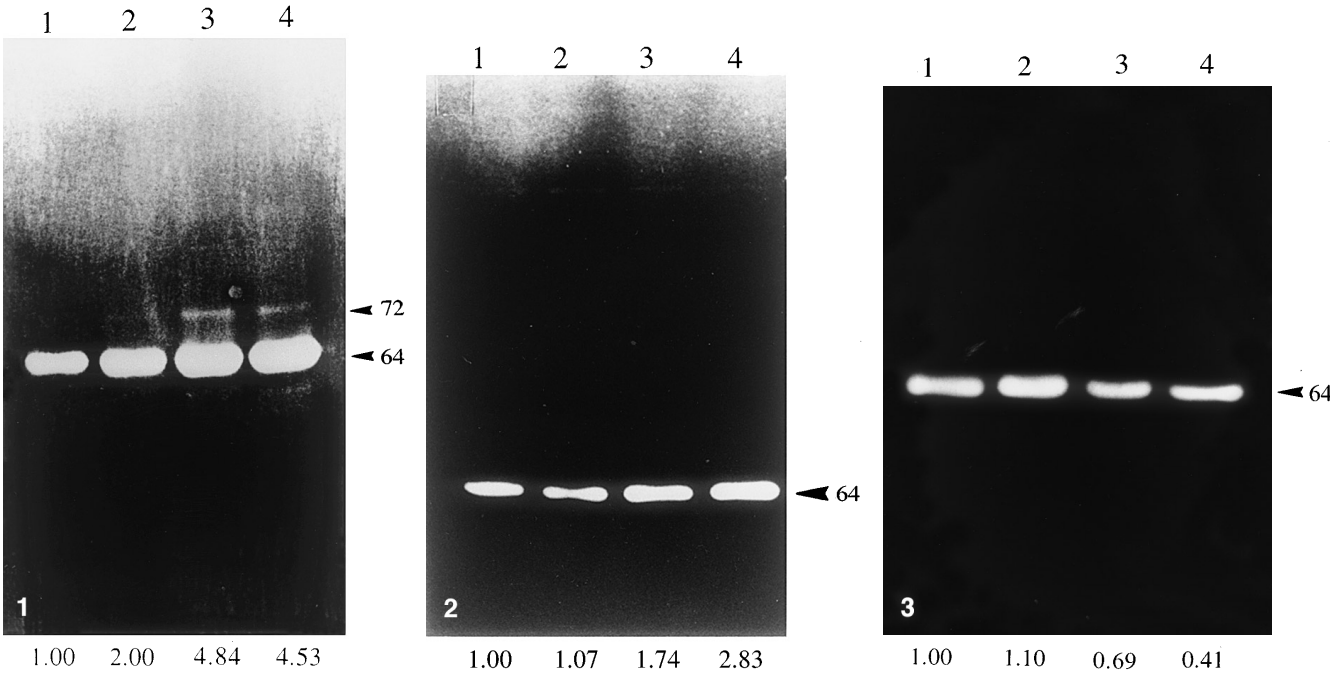

Fig. 1 Identification of matrix metalloproteinase-2 (MMP-2) secreted by control KG-2 human renal cell carcinoma (HRCC) cells or by cells treated with basic fibroblast growth factor (bFGF). *Lane 1* untreated (control), *lane 2* 0.1 ng/ml, *lane 3* 1.0 ng/ml, *lane 4* 10 ng/ml. The *numbers* on the *right* indicate the size of the molecule (kDa). The *numbers* at the *bottom* indicate the percentage relative gelatinase production as described in Materials and methods. The 64-kDa and 72-kDa bands indicate an active and an inactive form of MMP-2, respectively.

Fig. 2 Identification of MMP-2 secreted by control KG-2 cells or by cells treated with transforming growth factor-β1 (TGF-β1). *Lane 1* untreated (control), *lane 2* 1.0 pg/ml, *lane 3* 10 pg/ml, *lane 4* 100 pg/ml. The *number* on the *right* indicates the size of the molecule (kDa). The *numbers* at the *bottom* indicate the percentage relative gelatinase production. The 64-kDa band indicates an active form of MMP-2.

Fig. 3 Identification of MMP-2 secreted by control KG-2 cells or by cells treated with interferon-γ (IFN-γ). *Lane 1* untreated (control), *lane 2* 1.0 U/ml, *lane 3* 10 U/ml, *lane 4* 100 U/ml. The *number* on the *right* indicates the size of the molecule (kDa). The *numbers* at the *bottom* indicate the percentage relative gelatinase production. The 64-kDa band indicates an active form of MMP-2

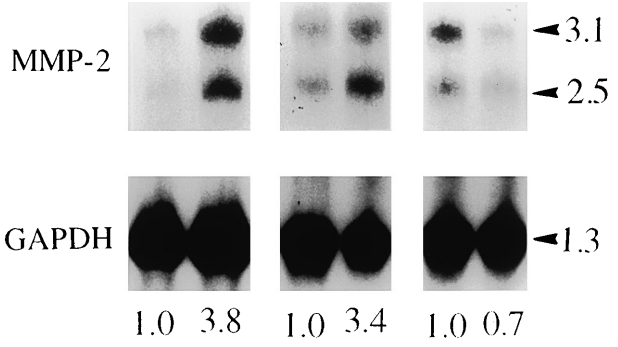

Fig. 4 Northern blot analyses of MMP-2 mRNA transcripts in KG-2 cells treated or untreated with bFGF, TGF-β1 and IFN-γ. *Lanes 1, 3 and 5* untreated, *lane 2* 10 ng/ml bFGF for 48 h, *lane 4* 100 pg/ml TGF-β1 for 48 h, *lane 6* 100 U/ml IFN-γ for 48 h. The *numbers* on the *right* indicate the size of the molecule (kb). The *numbers* at the *bottom* indicate the relative MMP-2 mRNA expression. *GAPDH* glyceraldehyde-3-phosphate dehydrogenase (internal control)

100 U/ml IFN-γ resulted in a decrease in MMP-2 mRNA expression (Fig. 4, lane 6) to 0.7-fold that of the untreated control. Other cytokines and growth factors used in the present study did not affect MMP-2 mRNA expression (data not shown).

Effects of cytokines and growth factors on in vitro invasive activity of HRCC cells

In vitro invasive activity induced by treatment with cytokines and growth factors is shown in Table 1. Treatment with bFGF at 0.1 ng/ml, 1.0 ng/ml and 10 ng/ml increased in vitro invasiveness of KG-2 cells to 1.83-, 4.00- and 4.25-fold that of untreated cells, respectively (Table 1). Treatment with TGF-β1 at 10 pg/ml and 100 pg/ml increased the in vitro invasive activity of KG-2 cells to 2.05- and 3.15-fold that of the untreated group, respectively (Table 1). The invasive activity of KG-2 cells following treatment with TGF-β1 was well correlated with enzyme production. Treatment with IFN-α did not show any effect on the in vitro invasive activity of KG-2 cells. However, treatment of KG-2 cells with IFN-γ at 10 U/ml and 100 U/ml decreased the in vitro invasive activity to 58% and 30% that of control KG-2 cells, respectively (Table 1).

Discussion

Metastasis is a complex process consisting of sequential and selective steps [3]. To metastasise, cancer cells have to degrade ECM or endothelial basement membranes

and intravasate into the vessels, then extravasate from the vessels at metastatic sites [6, 10, 13]. Liotta et al. [6] reported that the expression of MMPs in cancer cells was well correlated with their metastatic potential, and they demonstrated that this enzyme plays important roles in cancer invasion and metastasis. There are two types of MMP with different molecular weights; MMP-2, and MMP-9 [8]. In the present study, we demonstrated that KG-2 cells produced MMP-2. KG-2 cells, which were obtained from clear cell renal cell carcinoma, metastasized only from SRC sites. We previously demonstrated that the production of MMP-2 by KG-2 SRC tumours was much higher than that by KG-2 SC tumors, and that the difference in metastatic potential between SRC and SC tumors is due to differing production of MMP-2 [5]. Hence, MMP-2 could be an important enzyme in the invasion and metastasis of KG-2 cells.

MMP production is regulated by several cytokines and growth factors. Normal stromal cells, including fibroblasts, produced cytokines and growth factors such as bFGF, IL-1, EGF, TGF-β1, PDGF and IFNs. Fabra et al. [1] have demonstrated that collagenase production by human colon cancer cells is decreased by IFN-β and increased by IL-1, which are secreted by skin and colon fibroblasts, respectively, indicating that fibroblasts from different organs influence collagenase production by colon cancer cells and metastasis. Overall et al. [11] demonstrated that TGF-β1 increased the production of MMP-2 by human fibroblasts. A study by Welch et al. [14] revealed that a low concentration of TGF-β1 (50 pg/ml) increased production of MMP-2 and MMP-9 by rat 13762NF mammary carcinoma MTLn3 cells, and pretreatment of the tumor cells with TGF-β1 enhanced their metastatic potential. Our present results demonstrated that treatment of KG-2 cells with bFGF or TGF-β1 enhanced the production of MMP-2 and in vitro invasive activity; the degree of in vitro invasion of KG-2 cells was well correlated with production of the enzyme. Moreover, our results indicate that the upregulation of MMP-2 expression by bFGF or TGF-β1 is caused by gene transcriptional expression. Therefore, our results indicate that MMP-2 is important for invasion of KG-2 cells. In a previous study, the treatment of human alveolar macrophages with IFN-γ decreased the production of MMP-9, interstitial collagenase and stromelysin [12]. Similarly, in the present study, IFN-γ decreased the production of MMP-2 by KG-2 cells. Moreover, the downregulation of MMP-2 gene expression by IFN-γ is caused by transcriptional inhibition. The in vitro invasive activity of cells also decreased in parallel with decreased levels of MMP-2 following treatment with IFN-γ. However, IFN-α did not affect production of the enzyme or the invasiveness. Our results suggest that IFN-γ would be useful in preventing HRCC metastasis. Our results indicate that the invasiveness and metastatic potential of certain HRCC cell lines are influenced by cytokines and growth factors secreted from host stromal cells.

In conclusion, in certain types of HRCC cells, MMP-2 production and invasive activity were influenced by TGF-β1, bFGF and IFN-γ: TGF-β1 and bFGF stimulated the production of MMP-2 and invasive activity, while IFN-γ decreased enzyme production and inhibited invasion. Therefore drugs which inhibit the production of TGF-β1 and bFGF, or IFN-γ, could be useful for preventing HRCC metastasis.

References

1. Fabra A, Nakajima M, Bucana CD, Fidler IJ (1992) Modulation of the invasive phenotype of human colon-carcinoma cells by fibroblasts from orthotopic or ectopic organs of nude mice. Differentiation 52:101
2. Fan D, Bucana CD, O'Brian CA, Zwelling LA, Seid C, Fidler IJ (1990) Enhancement of murine tumor cell sensitivity to adriamycin by presentation of the drug in phosphatidylcholine-phosphatidylserine liposomes. Cancer Res 50:3619
3. Fidler IJ (1990) Critical factors in the biology of human cancer metastasis. The 28th G.H.A. Clowes Memorial Award Lecture. Cancer Res 50:6130
4. Gohji K, Nakajima M, Dinney CPN, Fan D, Pathak S, Killion JJ, von Eschenbach AC, Fidler IJ (1993) The importance of orthotopic implantation to the isolation and biological characterization of a metastatic human clear cell renal carcinoma in nude mice. Int J Oncol 2:23
5. Gohji K, Nakajima M, Kamidono S (1993) Establishment and characterization of a new human renal cell carcinoma cell line (KG-2) and metastatic model in nude mice (abstract in English). Jpn J Urol 84:1019
6. Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S (1980) Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature 284:67
7. Nakajima M, Welch DR, Belloni PN, Nicolson GL (1987) Degradation of basement membrane type IV collagen and lung subendothelial matrix by rat mammary adenocarcinoma cell clones of differing metastatic potentials. Cancer Res 47:4869
8. Nakajima M, Chop AM (1991) Tumor invasion and extracellular matrix degradative enzymes: regulation of activity by organ factors. Semin Cancer Biol 2:115
9. Nakajima M, DeChavigny A, Johnson CE, Hamada J, Stein CA, Nicolson GL (1991) Suramin: a potent inhibitor of melanoma heparanase and invasion. J Biol Chem 266:9661
10. Nicolson GL (1989) Metastatic tumor cell interactions with endothelium, basement membrane, and tissue. Curr Opin Cell Biol 1:1009
11. Overall CM, Wrana JL, Sodek J (1989) Independent regulation of collagenase, 72-kDa progelatinase, and metalloendoproteinase inhibitor expression in human fibroblasts by transforming growth factor-ß. J Biol Chem 264:1860
12. Shapiro SD, Campbell EJ, Kobayashi DK, Welgus HG (1990) Immune modulation of metalloproteinase production in human macrophages: selective pretranslational suppression of interstitial collagenase and stromelysin biosynthesis by interferon-γ. J Clin Invest 86:1204
13. Stetler-Stevenson WG (1990) Type IV collagenases in tumor invasion and metastasis. Cancer Mestatasis Rev 9:289
14. Welch DR, Fabra A, Nakajima M (1990) Transforming growth factor ß stimulates mammary adenocarcinoma cell invasion and metastatic potential. Proc Natl Acad Sci USA 87:7678