Induction of Hepatocyte Growth Factor in Fibroblasts by Tumor-derived Factors Affects Invasive Growth of Tumor Cells: In Vitro Analysis of Tumor-Stromal Interactions

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ABSTRACT

Invasive and metastatic potentials of several types of carcinoma cells are regulated through interactions with host stromal cells, e.g., tumor-stromal interactions. Because hepatocyte growth factor (HGF), a ligand for the c-Met proto-oncogene product, is a mesenchymal-stromal-derived factor that induces mitogenic, motogenic, and morphogenic responses, we examined the mechanisms involved in tumor-stromal interactions in vitro. The c-Met/HGF receptor was expressed in A431 human epidermoid carcinoma cells, A549 human non-small cell lung cancer cells, HuCC-T1 human cholangiocarcinoma cells, and SBC-3 human small cell lung carcinoma cells. HGF stimulated cell growth, scattering, and invasion of these cells. Although these cells did not produce biologically significant levels of HGF, these cells did secrete soluble factors that potentially stimulated HGF production in human skin fibroblasts. These carcinoma cell-derived HGF inducers proved to be interleukin-1 (IL-1) in A431 cells, IL-1 plus basic fibroblast growth factor (bFGF) in A549 and HuCC-T1 cells, and bFGF plus platelet-derived growth factor in SBC-3 cells. When these carcinoma cells were cocultured with fibroblasts, HGF levels in the coculture system were much higher than the levels in fibroblasts alone, without cocultured carcinoma cells. Together with the increase in HGF levels, the number of invasive cells increased, but in vitro invasion of carcinoma cells in the coculture system was strongly inhibited by anti-HGF antibodies. Thus, there are mutual interactions between carcinoma cells and fibroblasts: IL-1, bFGF, and platelet-derived growth factor derived from tumor cells play a role in inducing HGF expression in stromal fibroblasts, whereas fibroblast-derived HGF, in turn, leads to invasive growth in carcinoma cells. The mutual interactions, as mediated by HGF and HGF inducers, may play a significant role in the occurrence of invasion and metastasis of carcinoma cells.

INTRODUCTION

An essential characteristic of malignant cancer cells is their potential to proliferate and infiltrate surrounding normal tissue. In most cancers, lethality is the result of local invasion and the metastasis of neoplastic cells from the primary tumor to other tissues. Most human malignant tumors are carcinomas, and several lines of studies done in vivo (1) and in vitro (2, 3) indicated that the growth and invasive potentials of carcinoma cells are influenced through interactions with host stromal cells. In vivo growth of certain carcinoma cells was markedly accelerated by a broad spectrum of fibroblasts, and in vitro invasion of oral squamous carcinoma cells was induced by cocultivation with stromal fibroblasts (4). A soluble factor that stimulates the invasive growth of tumor cells was partially purified from the conditioned media of fibroblasts (3). Thus, host stromal-derived factor is likely to be one of the key molecules that regulates tumor cell invasion and metastasis. In addition to this stromal involvement in tumor malignancy, several studies have shown that stromal alterations precede the malignant progression of tumor cells (3, 5), including the activated appearance of stromal fibroblasts surrounding the tumor cells. Therefore, local and mutual interactions between carcinoma cells and stromal fibroblasts are of particular importance in regulating the extracellular matrix degradation, migration, and invasion of tumor cells. The molecular mechanisms leading to tumor progression are unclear; hence, molecular mechanisms for tumor-stromal interactions that confer the malignant characteristics of tumor cells are of interest. HGF, initially identified and cloned as a potent mitogen for mature hepatocytes (6–8), is a mesenchymal-stromal-derived multipotent growth factor that elicits mitogenic, motogenic, and glandular morphogenic activities on various types of cells, mostly in a paracrine fashion (9–13). HGF is also known to promote angiogenesis in vivo and in vitro (14, 15). The HGF receptor is a membrane-spanning heterodimeric tyrosine kinase, c-Met, and it is predominantly expressed in various types of normal and malignant cells (16, 17). Functional coupling between HGF and c-Met mediates epithelial-mesenchymal interactions for the development of several organs. Mice embryos deficient in HGF are lethal due to impaired development of the placenta and liver (18, 19). In vitro studies indicate that HGF supports morphogenesis of the kidney, mammary gland, and tooth (10–12, 20). Although interactions between the epithelium and mesenchyme mediate crucial aspects of normal development, these interactions may also be important in neoplasia. Molecular mechanisms underlying epithelial-mesenchymal interactions are likely to be functioning, at least in part, in interactions between epithelial tumors, i.e., carcinoma cells and surrounding stromal cells. HGF stimulates the cell migration and motility of several types of carcinoma cells, eventually promoting progression to a more malignant phenotype (21).

We now report that HGF is a predominant fibroblast-derived factor that stimulates mitogenesis, motogenesis, and the invasion of human carcinoma cells. Moreover, the mutual interactions between carcinoma cells and fibroblasts are of significant importance; carcinoma cells secrete various types of HGF inducers for fibroblasts. These mutual interactions, as mediated by HGF and the inducers, may play a significant role in the occurrence of invasion and metastasis of carcinoma cells.

MATERIALS AND METHODS

Materials. Human recombinant HGF was purified from the culture media of Chinese hamster ovary cells transfected with an expression plasmid containing human HGF cDNA, and the purity of HGF exceeded 98% (8, 22). Human recombinant bFGF was a kind gift from Dr. K. Nishikawa (Kanazawa Medical College, Kanazawa, Japan). Bovine aFGF and human recombinant FGF-4 (HST-1/FGF-4) were obtained from Toyobo Co. (Osaka, Japan) and

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The abbreviations used are: HGF, hepatocyte growth factor; IL, interleukin; IL-1R, IL-1 receptor; FGF, fibroblast growth factor; bFGF, basic FGF; PDGF, platelet-derived growth factor; aFGF, acidic FGF; KGF, keratinocyte growth factor; EGF-R, epidermal growth factor receptor; RT-PCR, reverse transcription-PCR.
Fig. 1. Effects of HGF on cell growth and DNA synthesis of various carcinoma cell lines. Effects on cell growth (○) and DNA synthesis (□) were presented as the fold increase relative to that of unstimulated control cells. Data, mean ± SD of triplicate measurements.

R&D Systems (Minneapolis, MN), respectively. Human recombinant KGF was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). Human recombinant IL-1β and human recombinant PDGF were obtained from Genzyme Corp. (Boston, MA). Polyclonal antibody against human HGF was prepared from the serum of a rabbit immunized with human recombinant HGF, and antihuman HGF IgG (1 μg/ml) completely neutralized the biological activities of 1 ng/ml human HGF. Polyclonal antibody against the human c-Met/HGF receptor (C-12) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal mouse antihuman EGF-R antibody (α-EGF-R) was obtained from Genzyme Corp. Monoclonal mouse antihuman bFGF antibody (α-bFGF) was a kind gift from Dr. K. Nishikawa. Recombinant human IL-1R antagonist and polyclonal goat antihuman PDGF antibody (α-PDGF) were obtained from R&D Systems and Promega (Madison, WI), respectively.

**Assay for Cell Growth, DNA Synthesis, and Cell Motility.** A431 human epidermoid carcinoma cells, A549 human non-small cell lung cancer cells, HuCC-T1 human cholangiocellular carcinoma cells, and SBC-3 human small cell lung carcinoma cells were obtained from the Japanese Cancer Research Resources Bank. Normal human skin fibroblasts were initially proliferated outward from dermal tissue obtained during plastic surgery. These cells were cultured in DMEM supplemented with 10% FCS.

To measure cell growth, 10⁴ cells were seeded on 6-well plates (Costar Corp., Cambridge, MA) and cultured for 24 h. After washing with DMEM, the cells were cultured for 7 days in DMEM supplemented with 1% FCS (A549 and SBC-3 cells) or 2% FCS (A431 and HuCC-T1 cells) in the absence or presence of various concentrations of HGF. The medium was changed every 3 days. The number of cells was counted using a hemocytometer after dissociation by trypsin. To measure DNA synthesis, the cells were seeded at a density of 10⁴ cells/cm² on 24-well plastic plates (Costar Corp.) and cultured for an additional 24 h. The medium was changed to serum-free medium, and the cells were cultured for 24 h. The cells were stimulated with various concentrations of HGF for 15 h and then pulse-labeled with 0.3 μCi/ml [³H]labeled deoxyuridine (2200 Ci/mmol; DuPont New England Nuclear, Boston, MA) for 12 h. The radioactivity incorporated into nuclei was counted using a gamma counter. The stimulatory effect on cell motility (cell scattering) was measured as described elsewhere (23).

**In Vitro Invasion Assay.** In vitro invasion of carcinoma cells was measured using Biocoat Matrigel invasion chambers (Collaborative Biomedical Products, Bedford, MA). The cells were seeded on Matrigel invasion chamber plates at a density of 5 × 10⁴ cells/cm² and cultured in DMEM containing 10% FCS. HGF was added to the lower medium, and cells were cultured for 3 days. Invasive cells that penetrated through pores and migrated to the underside of the membrane were stained with H&E and counted with microscopic vision.

For cocultivation of carcinoma cells and fibroblasts, human dermal fibroblasts were initially seeded on 24-well plates at a density of 5 × 10⁴ cells/cm² and cultured in DMEM containing 10% FCS. HGF was added to the lower medium, and cells were cultured for 3 days. Invasive cells that penetrated through pores and migrated to the underside of the membrane were stained with H&E and counted with microscopic vision.

**Measurement of HGF in Culture Media.** To prepare conditioned media from carcinoma cells, confluent carcinoma cells were washed three times and incubated in DMEM supplemented with 1% FCS for 3 days. Human fibroblasts were seeded on 48-well plates at a density of 5 × 10⁴ cells/cm² and cultured for 24 h. After replacing the media with fresh DMEM supplemented
with 1% FCS and 2 μg/ml heparin, cytokines or conditioned media from carcinoma cells were added, and the cells were cultured for 24 h. The concentration of HGF in the media was determined by ELISA, as described elsewhere (24).

**Western Immunoblotting of HGF.** HGF in the conditioned medium from fibroblasts was partially purified using Hi-Trap heparin (Pharmacia/Biotech) and subjected to SDS-PAGE using a 4–20% gradient gel under nonreducing conditions, as described elsewhere (25). After SDS-PAGE, the proteins were electrophoretically transferred onto a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). Immunoblotting was done as described elsewhere (25).

**Western Immunoblotting of the c-Met/HGF Receptor.** After reaching confluence, carcinoma cells were scraped, and cells were collected by centrifugation and then solubilized in 500 μl of ice-cold lysis buffer composed of 150 mM NaCl, 20 mM Tris-HCl (pH 7.5), 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 0.5% (v/v) Triton X-100. After centrifugation, supernatants were incubated with antibodies against the c-Met/HGF receptor for 1 h at 4°C, and immune complexes were recovered with protein A-Sepharose 4FF (Pharmacia/Biotech). After SDS-PAGE under reducing conditions, the proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad), and the membrane was incubated with antibodies against the c-Met/HGF receptor. The membrane was incubated with biotinylated goat antirabbit IgG (Vector Laboratories, Inc., Burlingame, CA) and subsequently incubated with peroxidase-conjugated avidin-biotin complex and then visualized using a chemiluminescent kit (Amersham, Bucks., United Kingdom).

**Analysis of ¹²⁵I-labeled HGF Binding to the Receptor.** Recombinant human HGF was radiiodinated by the chloramine-T method, and concentra-

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**Fig. 2.** Cell scattering induced by HGF. Cells were plated at a density of 1 × 10⁶ cells/well (A431 and HuCC-T1 cells), 1 × 10⁵ cells/well (A549 cells), or 4 × 10⁵ cells/well (SBC-3 cells) on 6-well plates and cultured for 24 h in the absence or presence of 5 ng/ml HGF. Bar, 200 μm.

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**Fig. 3.** Enhancement by HGF of cell invasion through Matrigel membrane. The cells were seeded on 24-well Matrigel invasion chamber plates at a density of 5 × 10⁴ cells/cm² and cultured in DMEM containing 10% FCS. HGF was added to the lower media, and cells were cultured for 3 days. Values, mean ± SD of triplicate measurements.
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Expression of c-Met/HGF receptor. A, concentration-dependent $^{125}$I-labeled HGF binding to its receptor on carcinoma cells. Insert, a Scatchard plot. $K_d$ and $B_{max}$ values were calculated from the Scatchard analysis. B, expression of c-Met/HGF receptor immunoprecipitated from the cell lysate of carcinoma cells. Cell lysate from cultured carcinoma cells was immunoprecipitated with antibodies against the c-Met/HGF receptor and subjected to SDS-PAGE under reducing conditions. The proteins were transferred and probed with antibodies against the c-Met/HGF receptor. C, expression of c-Met mRNA in carcinoma cells detected by RT-PCR. RT-PCR was carried out using specific primers for the c-Met/HGF receptor or $\beta$-actin gene (see "Materials and Methods"). PCR products were separated on 2% agarose and stained with ethidium bromide. Arrowhead, the product of the expected length of 516 bp.

Mitogenic and Motogenic Activities and Enhancement of in Vitro Invasion. We first examined the effects of HGF on proliferation and DNA synthesis of A431, A549, HuCC-T1, and SBC-3 cells (Fig. 1). HGF dose-dependently stimulated cell growth and DNA synthesis of the four cell lines, and the maximal stimulatory effects seen with 10–30 ng/ml HGF were 1.3-, 1.6-, 1.5-, and 2.2-fold in A431, A549, HuCC-T1, and SBC-3 cells, respectively. This means that HGF has weak mitogenic activity for A431, A549, HuCC-T1, and SBC-3 carcinoma cells.

Fig. 5. HGF-inducing activity in conditioned media of various carcinoma cells. Human fibroblasts were seeded on 48-well plates and cultured for 24 h. After replacing the medium with fresh DMEM supplemented with 1% FCS and 2 μg/ml heparin, appropriate amounts of conditioned medium from carcinoma cells were added, and the cells were cultured for 24 h. The concentration of HGF in the medium was measured by ELISA. Values, mean ± SD of triplicate measurements.

RESULTS

Mitogenic and Motogenic Activities and Enhancement of in Vitro Invasion. We first examined the effects of HGF on proliferation and DNA synthesis of A431, A549, HuCC-T1, and SBC-3 cells (Fig. 1). HGF dose-dependently stimulated cell growth and DNA synthesis of the four cell lines, and the maximal stimulatory effects seen with 10–30 ng/ml HGF were 1.3-, 1.6-, 1.5-, and 2.2-fold in A431, A549, HuCC-T1, and SBC-3 cells, respectively. This means that HGF has weak mitogenic activity for A431, A549, HuCC-T1, and SBC-3 carcinoma cells.
To determine whether HGF would stimulate cell motility, these carcinoma cells were cultured in monolayer, and HGF was added (Fig. 2). HGF caused a dissociation of the colonies and stimulated motility, resulting in scatterings of A431, A549, and SBC-3 cells. Although HGF did not cause a remarkable dissociation of colonies in HuCC-T1 cells, the cells had a flattened and spindle-like appearance when HGF was added, and several cells were scattered, thereby suggesting that HGF stimulated motility. Thus, HGF has motogenic activity for A431, A549, HuCC-T1, and SBC-3 cells, whereas HuCC-T1 seems to form more adhesive cell-cell interactions than do A431, A549, and SBC-3 cells.

We next measured the invasion of carcinoma cells in vitro using a Matrigel invasion chamber. In the absence of HGF, there was no evidence of invasive cells. However, HGF potently stimulated the invasion of A431, A549, and SBC-3 cells but less potently stimulated the invasion of HuCC-T1 cells (Fig. 3). Again, in this case, the lesser invasive potential in HuCC-T1 cells might be due to the more adhesive characteristics of these cells. Nevertheless, these results do indicate that HGF is potent in stimulating the motility and invasion of these carcinoma cells.

Expression of the c-Met/HGF Receptor. Because the above results indicate that A431, A549, HuCC-T1, and SBC-3 carcinoma cells are targets of HGF, we analyzed the expression of c-Met in cultured carcinoma cells. Fig. 4A shows the concentration-dependent binding of 125I-labeled HGF to carcinoma cells in culture. 125I-labeled HGF specifically bound to these cells with \( K_d \) values between 31—36 nM, and the number of high-affinity binding sites/cell was calculated as 348 sites/cell in A431, 324 sites/cell in A549, 1685 sites/cell in HuCC-T1, and 114 sites/cell in SBC-3 cells, respectively (Fig. 4A).

We also detected c-Met/HGF receptor expression by Western immunoblotting (Fig. 4B). The \( \beta \) subunit (Mr 145,000; p 145 \( \beta \)) was identified in four carcinoma cell lines, and the precursor form of c-Met/HGF receptor (Mr 170,000; Pr 170) was identified in A549 and HuCC-T1 cells (Fig. 4B). The amount of c-Met/HGF receptor detected by immunoblotting was relatively correlated with the number of high-affinity binding sites detected by Scatchard analysis. Consist-

Fig. 6. Induction of HGF by FGFs, PDGF, and IL-1. A, induction of HGF mRNA expression. Human fibroblasts were cultured in the absence or presence of FGFs (10 ng/ml), IL-1\( \beta \) (0.25 ng/ml), or PDGF (10 ng/ml) for 12 h, and RNA was purified from the cells. Total RNA (30 \( \mu \)g) was electrophoresed, and RNA blots were hybridized with 32P-labeled human HGF cDNA. RNAs stained with ethidium bromide are shown in the lower photograph to indicate the amount of RNAs loaded onto the gel. B, stimulation of HGF production in human fibroblasts by FGFs, PDGF, and IL-1\( \beta \). Human fibroblasts were seeded on 48-well plates and cultured for 24 h. After replacing the medium with fresh DMEM supplemented with 1% FCS and 2 \( \mu \)g/ml heparin, cells were cultured in the absence or presence of FGFs (10 ng/ml), IL-1\( \beta \) (0.25 ng/ml), or PDGF (10 ng/ml) for 24 h. Values, mean ± SD of triplicate measurements. C, Western immunoblotting of HGF produced by human fibroblasts. Human fibroblasts were cultured in the absence or presence of 10 ng/ml FGFs for 48 h. HGF in the conditioned medium was partially purified and subjected to SDS-PAGE. After electroblotting, HGF was probed with rabbit antihuman HGF IgG. The first lane shows recombinant human HGF as an authentic sample.
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Fig. 7. Neutralization of HGF-inducing activity in conditioned media of various carcinoma cells. Human fibroblasts were seeded on 48-well plates and cultured for 24 h. After replacing the medium with fresh DMEM supplemented with 1% FCS and 2 μg/ml heparin, conditioned medium from carcinoma cells was added in the absence or presence of antibodies, an antagonist, and their combinations. Conditioned media were added to cultures (in v/v) as follows: A431, 6.25%; A549, 50%; HuCC-T1, 25%; and SBC-3, 50%. Antibodies and an antagonist were added to cultures as follows: α-EGF-R, 3 μg/ml; IL-1R antagonist, 300 ng/ml; α-bFGF, 6 μg/ml; and α-PDGF, 30 μg/ml. Values, mean ± SD of triplicate measurements.

consistent with these results, the expression of c-Met/HGF receptor mRNA was confirmed by RT-PCR (Fig. 4C). The PCR products with the expected size of 516 bp were detected in these cells, and their amounts correlated relatively with the number of high-affinity HGF receptors on these cells.

Enhancement of HGF Production by Carcinoma-derived Soluble Factor. During malignant progression through tumor-stromal interactions, carcinoma cells have significant effects on stromal fibroblasts, because the existence of activated fibroblasts was noted in stromal tissues associated with tumor tissues (27, 28). We then asked whether HGF production in stromal fibroblasts would be affected by factors derived from carcinoma cells. Normal human fibroblasts were cultured in the presence of conditioned media derived from carcinoma cells, and HGF concentration in the culture medium was measured (Fig. 5). The addition of conditioned media from A431 cells potently stimulated HGF production, and a 9-fold stimulation occurred. Likewise, HGF production in fibroblasts was stimulated by conditioned media from A549, HuCC-T1, and SBC-3 cells. Maximal stimulatory effects seen within the tested concentration range were 7-, 5-, and 11-fold in A549, HuCC-T1, and SBC-3 cells, respectively. Therefore, these carcinoma cell lines secrete soluble factors that stimulate HGF production in stromal fibroblasts.

Induction of HGF Expression in Fibroblasts by FGF, PDGF, and IL-1. Before determining which molecule is responsible for the induction of HGF in fibroblasts as a carcinoma-derived factor, we examined the effects of various growth factors and cytokines on the production of HGF in fibroblasts. As reported previously, aFGF, bFGF, HST-1/FGF-4, IL-1, PDGF, epidermal growth factor, transforming growth factor α, and prostaglandins stimulated HGF production in human fibroblasts (24, 25, 29, 30). Fig. 6A shows HGF mRNA expression in fibroblasts upon exposure to aFGF, bFGF, HST-1/FGF-4, KGF, IL-1β, or PDGF. HGF mRNA expression was not detectable or only slightly detectable in the control culture, whereas aFGF, bFGF, HST-1/FGF-4, IL-1β, or PDGF did induce HGF mRNA expression, but KGF did not do so. Consistent with the induction of HGF mRNA, HGF production in fibroblasts was stimulated by aFGF, bFGF, HST-1/FGF-4, IL-1β, and PDGF but not by KGF (Fig. 6B).

Western immunoblotting analysis indicated that the addition of aFGF or bFGF stimulated the production of the native type of HGF (Mr, 85,000), but not the two-kringle variant form of HGF (Fig. 6C).
IL-1 and PDGF also stimulated the native type of HGF production (data not shown). Native-type HGF is an 85-kDa heterodimeric molecule composed of the four kringle-containing α-chain and a serine protease-like β-chain, whereas the 28-kDa smaller form of HGF containing two kringle domains is a naturally occurring variant.

Identification of Carcinoma-derived HGF Inducers. We next studied whether carcinoma-derived HGF-inducing soluble factors are known growth factors or cytokines tested above. We used specific antibodies or antagonist for the ligand or receptor (Fig. 7). Among the antibodies and antagonist tested, stimulatory activity for HGF production in the conditioned medium of A431 cells was almost completely abrogated by an IL-1R antagonist; however, it was hardly inhibited by antibodies against EGF-R, bFGF, and PDGF. For HGF-inducing activity in conditioned medium of A549 cells, α-bFGF and an IL-1R antagonist showed partial inhibition, and a marginal inhibitory effect was seen with α-EGF-R and α-PDGF. Simultaneous addition of α-bFGF and an IL-1R antagonist mostly inhibited HGF-inducing activity in the A549 cells. In the case of HuCC-T1 cells, an IL-1R antagonist strongly inhibited HGF-inducing activity in the conditioned medium, whereas α-bFGF partially inhibited it. Simultaneous addition of an IL-1R antagonist and α-bFGF almost completely inhibited HGF-inducing activity in HuCC-T1 cells. For SBC-3 cells, α-bFGF and α-PDGF inhibited HGF-inducing activity in a conditioned medium of SBC-3 cells, and their simultaneous addition almost completely inhibited it. Thus, HGF-inducing factors in conditioned media proved to be as follows: IL-1 in A431 cells; IL-1 and bFGF in A549 and HuCC-T1 cells; and bFGF and PDGF in SBC-3 cells.

Induction of in Vitro Invasion of Carcinoma Cells by Fibroblast-derived HGF. Based on our findings that carcinoma cells secrete HGF-inducers for fibroblasts, whereas HGF potently stimulated migration and invasiveness of carcinoma cells, we set up a coculture method in which fibroblasts were cultured in the lower well, whereas carcinoma cells were cultured in the Matrigel invasion chamber (Fig. 8A). We then measured invasion of carcinoma cells and HGF production (Fig. 8, B and C). A431, HuCC-T1, and SBC-3 cells did not produce detectable levels of HGF, whereas A549 cells produced 0.31 ng/ml HGF during 3 days of culture, and bFGF, IL-1, and PDGF had no effect on HGF levels in these cancer cells (data not shown). On the other hand, without cocultured carcinoma cells, basal HGF production in fibroblasts was 0.31 ng/ml during the culture period of 3 days. However, the HGF level was stimulated 26–28-fold by cocultivation with A431 cells (Fig. 8, B and C), indicating that the A431-derived HGF inducer (IL-1) stimulated HGF production in cocultured fibroblasts. In accordance with the increase in HGF concentration, the number of invading A431 cells increased 3.5-fold by cocultivation with fibroblasts than it did without fibroblasts (Fig. 8B). Importantly, in vitro invasion of A431 cells was suppressed to the basal level seen without cocultured fibroblasts in the presence of anti-HGF antibody (Fig. 8, B and C). Likewise, IL-1R antagonist also inhibited the invasion of the cells, concomitant with suppression of the HGF level in this system (Fig. 8C).

Similar results were obtained with the other carcinoma cell lines.
tested. Cocultivation with A549, HuCC-T1, and SBC-3 cells increased HGF levels, and the number of invading carcinoma cells was increased by cocultivation with fibroblasts (Fig. 8B). The addition of anti-HGF antibody into the coculture system potently inhibited invasion of A549, HuCC-T1, and SBC-3 cells (Fig. 8B). In the case of A549 cells, the number of invading cells in the presence of anti-HGF antibody was significantly lower than that without fibroblasts. This suggests that a low amount of HGF produced by A549 cells may affect the invasiveness of the cells in an autocrine manner. However, the neutralizing antibodies and IL-1R antagonist had no effect on the invasion of carcinoma cells in the absence of fibroblasts (data not shown). Moreover, in coculture system with HuCC-T1 and fibroblasts, α-bFGF and IL-1R antagonist inhibited the invasion of the cells, concomitant with the suppression of HGF levels (Fig. 8C). These findings in the coculture system suggest that the fibroblast-derived invasion factor for these cell lines is HGF and that HGF production in normal human dermal fibroblasts is strongly stimulated by a carcinoma-derived soluble factor.

DISCUSSION

Studies done during the past several years suggest that HGF is at least one stromal-derived factor that may affect the motility of epithelial cells, including various types of carcinoma cells. HGF stimulates the migration of various types of carcinoma cells, and Matsumoto et al. (31) recently identified the fibroblast-derived invasion factor for oral squamous carcinoma cells as HGF. In the present study, we obtained evidence that HGF is the predominant fibroblast-derived factor that promotes the mitogenesis, motogenesis, and invasion of human carcinoma cell lines A431, A549, HuCC-T1, and SBC-3. Of importance in this study, we demonstrated that four carcinoma cell lines secrete inducing factors for HGF production in stromal fibroblasts, and we identified the carcinoma-derived HGF inducers as bFGF, IL-1, and PDGF. Thus, there is a mutual interaction between carcinoma cells and stromal fibroblasts mediated by carcinoma-derived HGF inducers and stromal-derived HGF, which eventually stimulates the migration and invasion of carcinoma cells. Other workers also noted the presence and characterization of tumor-derived inducing molecules for HGF (32, 33). We previously showed that human gallbladder carcinoma cells secrete IL-1 as an inducer of HGF production in fibroblasts and that fibroblast-derived HGF induces invasion of the cells into collagen gels (34).

We identified the carcinoma cell-derived HGF inducers to be IL-1, bFGF, and PDGF. Expression of IL-1, bFGF, and PDGF was noted in several types of carcinoma cells, and these factors may possibly regulate the growth of cells in an autocrine manner. The physiological significance of these factors for their own growth and their involvement in invasive growth are not clear (35–37). Our present data indicate that IL-1, bFGF, and PDGF secreted from these carcinoma cells are likely to play a role in inducing HGF production in certain types of stromal fibroblasts, which in turn results in the acquisition of invasive growth in these carcinoma cells through the influence of stromal-derived HGF. In this context, it is noteworthy that the expression of stromelysin 3 in stromal fibroblasts is also potently induced by growth factors, including bFGF, PDGF, and epidermal growth factor (38), similar to that of HGF. Taken together, it is probable that these factors derived from tumor cells activate neighboring fibroblasts, which in turn secrete proteins responsible for extracellular matrix degradation and concomitant cell movements.

Proteolytic degradation of the extracellular matrix, disruption of cell adhesion, and cell motility are key components for tumor cell invasion. HGF induces tyrosine phosphorylation of β-catenin on carcinoma cells, which contributes to the promotion of cell motility (39). HGF induces the expression of the urokinase plasminogen activator and the urokinase plasminogen activator receptor (40), which then activate the proteinase cascade that promotes focal degradation of the extracellular matrix. Ligand-dependent activation of c-Met in a leiomyosarcoma cell line induced a urokinase-related proteolytic network and concomitantly enhanced tumorigenicity, invasion, and metastasis (41). HGF, which regulates cell spreading and focal adhesion formation by the induction of tyrosine phosphorylation of focal adhesion kinase (p125FAK), eventually stimulates cell motility and invasion (31). Likewise, HGF activates small GTP-binding proteins, by which modulation of cytoskeleton reorganization and membrane ruffling eventually stimulate cell motility (42). Therefore, because enhanced cell motility by HGF is coupled with the dissociation of cell-cell interactions and extracellular matrix degradation, HGF seems to be particularly potent in enhancing the invasiveness of tumor cells.

Cell motility factors have been implicated to play a role in stimulating the neoplastic progression of cancer cells. Distinct types of autocrine motility factors that enhance cell motility in an autocrine manner have been noted in several tumor cell lines (43), and some growth factors seem to stimulate tumor cell motility in an autocrine or a paracrine manner, including transforming growth factor β (44). On the other hand, the potent activity of HGF on epithelial cell motility has been well demonstrated; it predominantly acts as a paracrine factor. Nearly 90% of human malignant tumors arise from epithelial tissue, and most carcinoma cells express the c-Met/HGF receptor; however, only a few carcinoma cells among more than 50 distinct cell lines produce any detectable level of HGF.4 Therefore, most carcinoma cells are likely to use HGF derived from stromal tissue, and importantly, carcinoma cells capable of inducing HGF production in stromal cells may be particularly susceptible to a more malignant progression through tumor-stromal interactions. Nevertheless, a few types of carcinoma cells do use HGF as an autocrine motility factor, including lung and bladder carcinoma (45, 46).

HGF is a mesenchymal- or stromal-derived paracrine mediator that regulates cell growth, cell motility, and morphogenesis during organogenesis and organ regeneration. Our present study suggests the mutual interaction between carcinoma cells and stromal fibroblasts, mediated by carcinoma (epithelial)-derived HGF inducers and stromal (mesenchymal)-derived HGF. We hypothesize that such mutual interaction may exist in several types of tumor tissues and may explain how tumor cells acquire more malignant phenotypes, depending on stromal tissues. The mutual interaction of tumor (carcinoma) cells and stromal fibroblasts is reminiscent of epithelial-mesenchymal (or-stromal) interactions during dynamic tissue organization and reconstruction. It may well be that several types of carcinoma cells use the overlapping mechanisms of epithelial-mesenchymal interactions during embryogenesis or tissue regeneration, but in the case of carcinoma, it results in tumor progression or extracellular matrix remodeling. Our ongoing study is directed toward the prevention of tumor invasion and metastasis by interfering with mutual interactions between tumor and stromal cells as mediated by HGF inducers and HGF.

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4 Unpublished results.
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REFERENCES


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