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Human Lung Cancer Cell Line Producing Hepatocyte Growth Factor/Scatter Factor

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Hepatocyte growth factor (HGF)/scatter factor (SF) is a cytokine which is produced by mesenchymal cells and stimulates the motility of some epithelial cells, including cancer cells and vascular endothelial cells. Two human lung cancer cell lines, PC-1 and PC-13, were found to produce a protein which was indistinguishable from HGF/SF with regard to biological activities and immunological characteristics, although they were derived from epithelial cells. In general, highly aggressive cancer cells often show some mesenchymal characteristics, and production of HGF/SF by cancer cells is also considered as a phenomenon of acquisition of mesenchymal phenotype, which may be involved in cancer invasion and progression. These cell lines showed no apparent response to exogenous HGF/SF. In addition, no c-*met* proto-oncogene product was detectable in these cells by Western blot analysis. Although the function of HGF/SF produced by cancer cells, either autocrine or paracrine stimulation, remains to be studied, this is the first report to describe cancer cells producing HGF/SF.

Key words: Hepatocyte growth factor — Scatter factor — Tumor invasion — Angiogenesis

Tumor invasion and metastasis are the major reasons for the poor prognosis of many cancer patients. Despite extensive research efforts, the mechanisms responsible for these phenomena are still unknown, but it appears that at least active tumor cell motility is required.[1,2] Since a report by Yoshida *et al.*,[3] several factors mediating tumor cell motility have been found by different research teams. The most notable and interesting of these are: 1) tumor autocrine motility factor (AMF), a tumor-secreted cytokine which stimulates both random and directed cell migration of self-producing cells, and which has been detected in urine samples from patients with bladder cancer[4,5]; 2) migration stimulating factor (MSH), a protein secreted by fibroblasts of human fetus and breast cancer patients, which stimulates the migration of non-producing normal adult fibroblasts[6]; 3) hepatocyte growth factor (HGF)/scatter factor (SF), a protein produced by mesenchymal cells, which stimulates the migration of epithelial cells including some cancer cells and vascular endothelial cells.[7–9] HGF/SF is a multifunctional factor which increases the proliferation of primary cultured hepatocytes, renal tubular epithelial cells, keratinocytes and melanocytes.[10–13] The physiological role of HGF/SF *in vivo* remains largely unknown, but it is thought to be related to tissue regeneration, wound healing, embryogenesis, angiogenesis and cancer invasion.[14–16]

We have found that HGF/SF is accumulated in human cancerous lesions, but have been unable to localize the cells producing HGF/SF immunohistochemically (Yoshinaga *et al.*, unpublished). Noji *et al.* have shown that the expression of HGF/SF in rat liver is confined to non-parenchymal cells (endothelial cells and Kupffer cells) by *in situ* hybridization.[17]

Recently, Adams *et al.* demonstrated a human keratinocyte strain secreting HGF/SF, and proposed that autocrine stimulation of cell motility might occur.[18] Apart from this single paper, there are no reports of epithelial cells producing HGF/SF. It would be interesting and important to determine whether the action of HGF/SF is mediated only in a paracrine manner or by both paracrine and autocrine mechanisms, particularly in the development of certain cancers. In the present study, we describe for the first time human lung cancer cell lines producing HGF/SF, detected by bioassay and immunoblot analysis.

MRC5 human embryonic lung fibroblast cells and Madin Darby canine kidney (MDCK) cells were obtained from the Japanese Cancer Research Resources Bank (Tokyo). Human cancer cell lines used in this study were poorly differentiated squamous cell carcinoma PC-1, large cell carcinoma PC-13 derived from lung cancer[19] and bladder cancer T24. A polyclonal antibody

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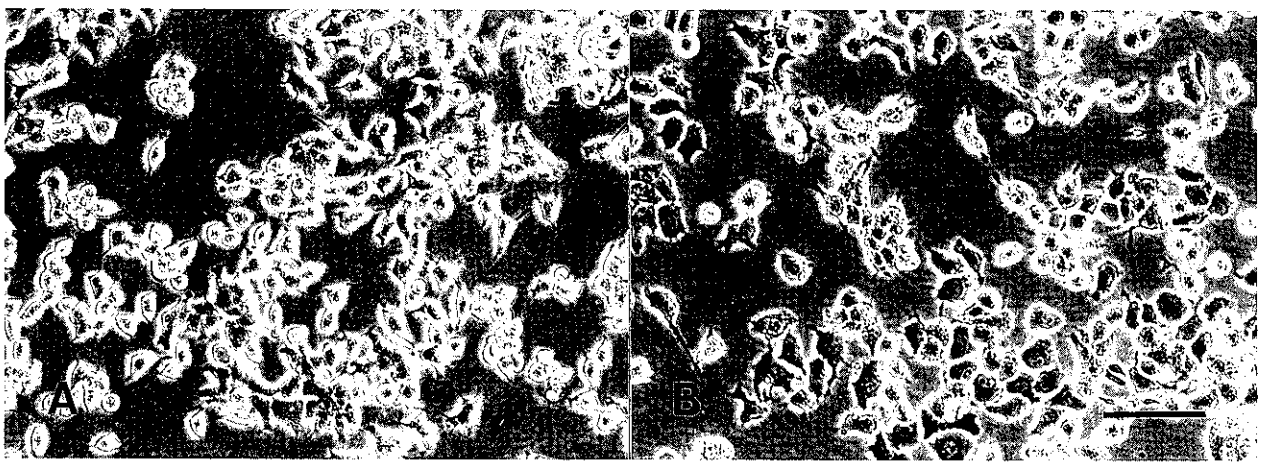

Fig. 1. Morphology of PC-1 (A) and PC-13 (B) in culture. Both PC-1 and PC-13 cells appear dissociated or in small clusters. Bar, 100 μm.

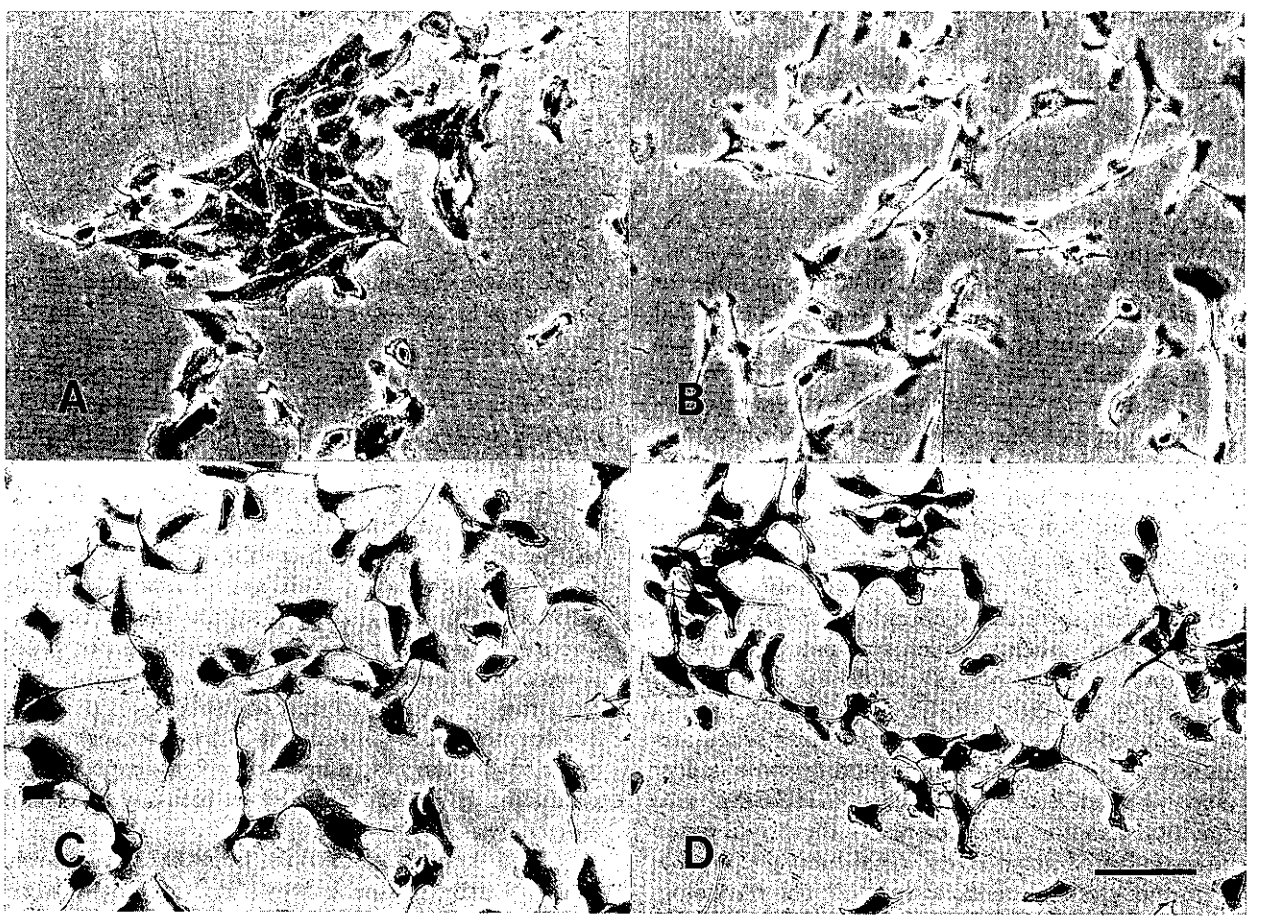

Fig. 2. Scatter factor activity in CMs of PC-1 and PC-13. Scattering of MDCK cells was assayed in 96-well plates by using serial 2-fold dilutions of these CMs. MDCK cells (3×10^3 cells/well) were incubated with or without serum-free CM in an equal volume of DMEM with 10% FCS overnight at 37°C. Cultures were fixed and stained with crystal violet. A: control without addition, B: with 4-fold-diluted CM of MRC5, C: with 2-fold-diluted CM of PC-1, D: with 2-fold-diluted CM of PC-13. Bar, 100 μm.

was raised in rabbit against recombinant human HGF.[20] Mouse monoclonal antibody against human c-*met* product was provided by Dr. George F. Vande Woude.[21] Biological scattering assay was carried out as described previously.[22] Briefly MRC5, PC-1 and PC-13 were cultured in serum-free Dulbecco's modified Eagle's medium (DMEM) or RPMI 1640 for 72 h. Scattering of MDCK cells was assayed in 96-well plates using serial 2-fold dilutions of the conditioned medium (CM) of MRC5, PC-1 and PC-13. MDCK cells (3×10^3 per well) in an equal volume of DMEM with 10% fetal calf serum (FCS) were added, and the plates were incubated overnight at 37°C. Cell scattering was assessed after fixation and staining. The titer was taken as the highest dilution of the sample that caused clear cell scattering. Western blot analysis was carried out as follows. Recombinant human HGF[23] at 0.5 μg/ml, and 40-fold-concentrated CMs of MRC5, PC-1, PC-13 and T24 were subjected to SDS-PAGE on 12.5% polyacrylamide gel under non-reducing conditions, and transferred to Immobilon membrane (Millipore, Bedford, MA). HGF/SF was detected using polyclonal antibody against recombinant human HGF diluted 1:500 and peroxidase-conjugated anti-rabbit IgG (DAKOPATTS, Denmark), and visualized using an ECL kit (Amersham, UK). PC-1, PC-13 and T24 were lysed in Laemmli's sample buffer[24] with brief sonication. The lysates were fractionated on a 7.5% SDS-acrylamide gel under reducing conditions and transferred to Immobilon membrane, and the c-*met* products were detected by using anti-c-*met* protein diluted 1:5 and peroxidase-conjugated anti-mouse IgG (IBL, Fujioka).

PC-1, a poorly differentiated squamous cell carcinoma, and PC-13, a large cell carcinoma, are human lung cancer cell lines established from different patients. In culture, these cells became attached loosely to the dish, and many cells did not form tight cohesive colonies. Many of the cells grew in a dissociated manner or in small clusters with loose mutual adhesion (Fig. 1). As HGF/SF had been purified previously from MRC5 CM,[8] MDCK cells were dissociated by incubation with MRC5 CM (Fig. 2B). We also examined the effect of PC-1 and PC-13 CMs by means of the scattering assay using MDCK cells. Upon addition of serum-free CM of PC-1 (Fig. 2C) or PC-13 (Fig. 2D), MDCK cells became dissociated (scattering) and showed morphological changes (spindle shape, pseudopodia protrusion) very similar to those induced by MRC5 CM. The titer of scattering activity of CMs of PC-1 and PC-13 per dish was about 1:16–1:32, and less than that of MRC5 CM, 1:64–1:128. We also examined the effect of MRC5 CM as HGF/SF on PC-1 and PC-13 by scattering assay. There were no particular changes in scattering or cell morphology for either PC-1 or PC-13. These two cell lines apparently did not respond to exogenous HGF/SF, and moreover exogenous HGF/SF did not affect the growth of these cell lines (data not shown). HGF/SF in concentrated MRC5 CM was detected at 82 kDa by Western blot analysis under non-reducing conditions. Figure 3 shows that there was also a protein reactive with anti-recombinant human HGF antibody along with other species of the same molecular weight in concentrated PC-1 and PC-13 CMs. These results indicate that human lung cancer cell lines PC-1 and PC-13 release into their CMs a protein which is indistinguishable from HGF/SF in terms of biological activity and immunological characteristics. T24 CM also showed a weak scattering activity on MDCK scattering assay, but this CM did not react with anti-HGF antibody in Western blot analysis. We are trying to identify other unknown scatter factor(s) in T24 CM.

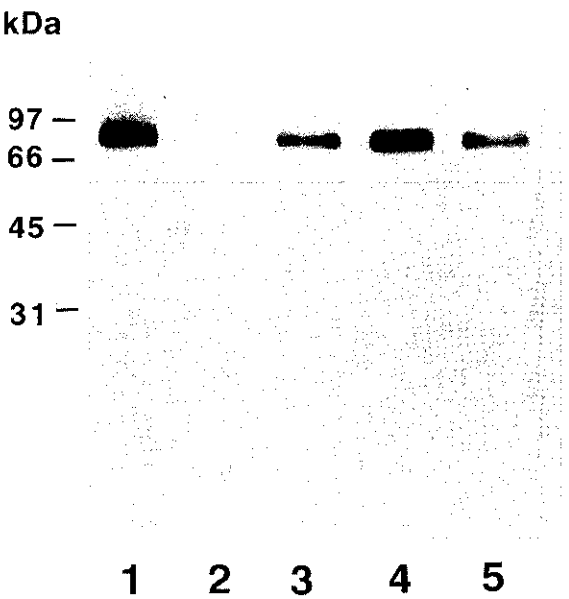

Fig. 3. Western blot analysis of HGF/SF in concentrated CMs of PC-1 and PC-13 using anti-recombinant human HGF. Recombinant human HGF (0.5 μg/ml) (lane 1), 40-fold-concentrated serum-free CM (2.5 μg, protein/each lane) of T24 (lane 2), MRC5 (lane 3), PC-1 (lane 4), and PC-13 (lane 5) were loaded on 12.5% polyacrylamide gel under non-reducing conditions, and transferred to Immobilon membranes. HGF/SF was detected using anti-recombinant human HGF polyclonal antibody diluted 1:500, and visualized using an ECL kit. Bars show positions of molecular weight markers in $Mr\times10^3$.

We also analyzed the presence of c-*met* proto-oncogene product in PC-1 and PC-13 cells by Western blot analysis using anti-c-*met* product antibody. Figure 4 shows that c-*met* proto-oncogene product was detected at 145 kDa and 190 kDa in the lysate of T24 (positive control) but not in PC-1 and PC-13.

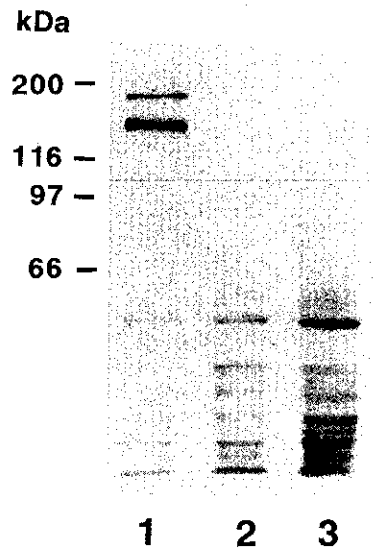

Fig. 4. Western blot analysis of c-*met* protein in lysate of PC-1 and PC-13 using anti-human c-*met* product. Cell lysate in Laemmli's buffer of T24 (positive control) (lane 1), PC-1 (lane 2), and PC-13 (lane 3) were loaded on 7.5% polyacrylamide gel at 10 μg protein/lane under reducing conditions, and transferred to Immobilon membranes. c-*met* products were detected using anti-human c-*met* product monoclonal antibody diluted 1:5, and visualized using an ECL kit. Bars show positions of molecular weight markers in $Mr \times 10^3$.

In this study we demonstrated that two human cancer cell lines, PC-1 and PC-13, among 16 cell lines (including lung cancer, stomach cancer, colon cancer, bladder cancer, melanoma and leukemia) examined, were able to produce HGF/SF. A protein secreted from PC-1 and PC-13 was identified as HGF/SF on the basis of MDCK scattering assay and immunoreactivity with anti-HGF antibody in Western blot analysis. We also confirmed that these two cell lines produced HGF/SF mRNA by Northern blot analysis (data not shown).

Previous studies in several laboratories have shown that HGF/SF is produced in mesenchymal cells, and that epithelial cells and vascular endothelial cells are the targets of its paracrine stimulation.[7-9, 14, 15] Adams *et al.*[18] were the first to report an epithelial cell line producing HGF/SF, and in the present study we demonstrated for the first time that cancer cells are able to produce this substance. In general, highly aggressive cancer has mesenchymal characteristics,[25] and this might be the case for PC-1 and PC-13. Our present results are of interest because HGF/SF is thought to be involved in cancer invasion, and some investigators speculate that HGF/SF may act in an autocrine manner.[16] However, we were unable to obtain direct evidence that this is the case, since PC-1 and PC-13 did not respond to exogenous HGF/SF. Moreover, we failed to detect any c-*met* product, which has been identified as the receptor of HGF/SF,[21, 26] in the lysate of PC-1 and PC-13. Although c-*met* protein was clearly detected in lysates of T24 human bladder cancer, Lu-90 human lung cancer and A-431 human vulvar cancer by Western blot analysis using this anti-c-*met* monoclonal antibody, only the T24 cells showed clear scattering upon incubation with MRC5 CM (data not shown). Therefore the detection of c-*met* protein by this antibody is not always correlated with cell motility activated by HGF/SF, at least in the standard scattering assay.

From these data the following possibilities arise. i) These PC-1 and PC-13 do not possess the receptor coded by c-*met*, and HGF/SF secreted from the cells may act on vascular endothelial cells as an angiogenic factor in a paracrine manner. ii) The receptor coded by c-*met* may already be occupied and internalized after binding to HGF/SF produced by these cells.[14] iii) The autocrine signal transduction may be exerted by intracellular autocrine loops.[27] iv) The expression of c-*met* may be regulated by interaction with surrounding mesenchymal cells, for example fibroblasts or inflammatory cells. At present, however, we can not identify which is the real function of HGF/SF produced by cancer cells.

The role of HGF/SF in cancer invasion may not be simple. It seems necessary to identify the subcellular changes occurring in cells upon stimulation with HGF/SF.

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