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Hepatocyte growth factor inhibits growth of hepatocellular carcinoma cells

(autocrine growth factor/albumin expression vector)

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ABSTRACT **Hepatocyte growth factor (HGF) is a potent mitogen for primary hepatocytes. Therefore, we examined HGF as a possible autocrine growth factor in hepatocellular carcinoma (HCC). We introduced an albumin–HGF expression vector into Fao HCC cells and transgenic mice. Expression of the albumin–HGF vector in Fao HCC cells inhibited their growth *in vitro*. *In vivo*, FaoHGF cells produced tumors that averaged 10% of the sizes of G418-resistant controls when transplanted into nude mice. In contrast, hepatocytes from transgenic mice expressing HGF grew more rapidly than did those from normal siblings. Further, growth of eight additional HCC cell lines was inhibited by the addition of recombinant HGF. Finally, of 35 tumor cell lines surveyed, only 6 cell lines expressed HGF mRNA, and no HCC cell line expressed HGF. Although HGF stimulates normal hepatocytes, it is a negative growth regulator for HCC cells.**

Hepatocyte growth factor (HGF) is an important regulator of liver regeneration in response to injury (1). In addition, HGF is a potent mitogen for mature hepatocytes *in vitro* (2, 3). Despite its initial characterization as a liver-specific factor, HGF is found in numerous additional tissues and displays several alternative activities (4, 5). HGF stimulates growth of melanocytes, endothelial cells, renal tubular cells, and keratinocytes (6–8). HGF inhibits the growth of certain sarcoma cells (9). Finally, HGF is identical to scatter factor, a nonmitogenic factor that promotes cell migration (10, 11). Thus HGF, like other growth factors, has different effects in different cells.

Transformed cell growth that results from endogenous production of growth factors has been demonstrated in many tumors. We initially considered that HGF was a likely candidate for autocrine stimulation of hepatocellular carcinoma (HCC) cells because serum HGF is elevated in patients with chronic hepatitis and liver cirrhosis (12). These conditions cause persistent hepatocellular damage and regeneration; consequently, they are associated with the subsequent development of HCC. To test whether HGF can function as an autocrine growth factor, we developed an expression system that targets high levels of HGF expression to hepatocytes. Contrary to our expectations, endogenous HGF expression in Fao HCC cells and treatment of HCC cells with recombinant HGF produced a marked inhibition of cell growth.

MATERIALS AND METHODS

Recombinant DNA Constructs. Standard methods were used to construct all plasmids (13). Our fusion gene, in plasmid pAlbHGF (Fig. 1*A*), contains the albumin enhancer and promoter from pAN/T2-NB′, inserted between the indicated *Apa* I and *Xba* I sites (15); the HGF cDNA, included between the indicated *Xba* I sites (16); and the SV40 intron and polyadenylylation signals from pSV2gpt, included between the indicated *Xba* I and *Mlu* I sites (17); the construct is cloned in pGEM-7 (Promega). For microinjection, pAlbHGF was cut at the *Apa* I and *Mlu* I sites to remove vector sequences. This microinjection fragment was purified by CsCl gradient purification and dialyzed extensively against injection buffer (5 mM NaCl/0.1 mM EDTA/5 mM Tris·HCl, pH 7.4) (18).

Cell Lines and Culture Conditions. Human and rat HCC cell lines (obtained from Jack Wands and Douglas Jefferson) included HepG2, Hep3B, SK-HEP-1, TON6, HA22T, HuH7, FOCUS, and Fao (references available on request). Cells were grown in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal bovine serum, penicillin (50 units/ml), and streptomycin sulfate (50 μg/ml) unless otherwise specified. Additional cell lines including IMR-90, U-138MG, THP-1, and NGP were obtained from the ATCC. HGL4 glioblastoma cells were the gift of Frank Pardo.

Fao cells were cotransfected by calcium phosphate coprecipitation with 30 μg of pAlbHGF and 5 μg of pAN/T2-NB′ per 100-mm dish (19). Colonies able to grow in the presence of G418 (400 μg/ml) were isolated and analyzed for expression of HGF by RNA blots. Cell growth was assessed by plating 5×10^5 cells per 60-mm plate (Nunclon). At each indicated time point, individual plates were harvested by trypsinization, cells in an aliquot were counted on a hemocytometer, and the total cells in that well were plotted versus day of harvest.

Transgenic Mice. The *Apa* I–*Mlu* I fragment of pAlbHGF was microinjected (18) into fertilized oocytes (from FVB inbred mice; Taconic Farms, NY) at the single-cell stage. Transgenic mice were identified by DNA blot techniques using DNA isolated from individual mouse tails. F_1 mice and their siblings were used to obtain isolated hepatocytes for *in vitro* growth studies.

Assay of HGF Activity. HGF was assayed according to ref. 14. Briefly, inocula of 2.5×10^5 parenchymal liver cells, isolated by collagenase perfusion from CD-1 rats (180–200 g; Charles River Breeding Laboratories), were cultured in 1 ml of Williams medium E (Sigma) supplemented with 5% fetal bovine serum, 1 nM insulin, and 1 nM dexamethasone in a 5% CO_2 incubator at 37°C. After 4 hr, culture medium was replaced with serum- and hormone-free medium for 24 hr. At that point, 0.1 ml of conditioned media from cell lines or 50 μl of serum from candidate mice, 100 nM insulin, and epidermal growth factor (EGF; 10 ng/ml) were added in a

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Abbreviations: EGF, epidermal growth factor; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; TGF, transforming growth factor.

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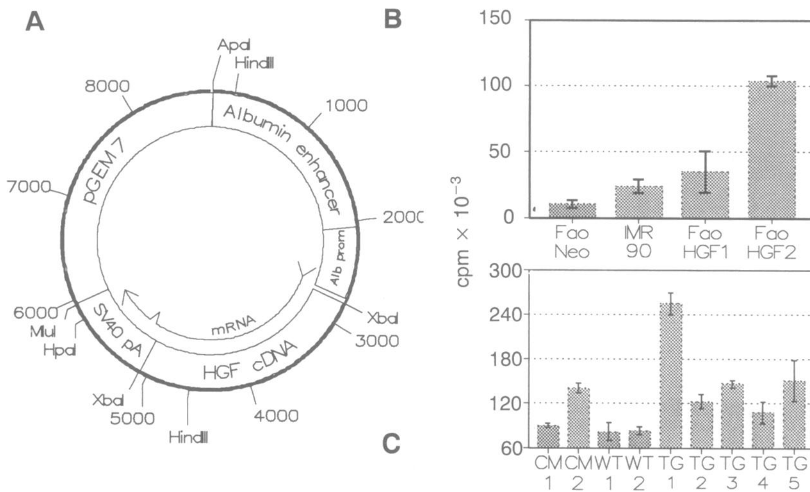

FIG. 1. (*A*) pAlbHGF expression vector. Position numbers indicate length in base pairs; Alb prom, albumin promoter; SV40 pA, simian virus 40 intron and polyadenylylation signals. (*B*) HGF expression in transfected Fao cells. Two clones of Fao HCC cells transfected with pAlbHGF were analyzed by a biologic assay for HGF (14). Conditioned medium (100 μl) from FaoHGF cells was added to HGF-assay culture medium. HGF activity is revealed by stimulation of [^{3}H]thymidine incorporation into primary rat hepatocytes. Results were plotted as mean ± SD for two replicas at each point. (*C*) HGF expression in sera from transgenic mice. Serum (50 μl) from F_1 mice in each of the indicated transgenic strains (TG 1–5) was added to HGF-assay culture medium. Control sera were included from wild-type siblings in the first two strains of transgenic mice (WT 1 and WT 2 compared with TG 1 and TG 2). Conditioned media from FaoNeo (CM 1) and FaoHGF2 (CM 2) cells were added as negative and positive controls, respectively.

final volume of 1 ml to the cells. Fifteen hours later hepatocytes were labeled with 5 μCi (185 GBq) of [^{3}H]thymidine (New England Nuclear) in the presence or absence of 10 mM hydroxyurea for 24 hr. Thymidine incorporation into DNA was measured by cold 10% (wt/vol) trichloroacetic acid precipitation followed by extraction into hot 10% trichloroacetic acid (20). HGF activity was defined as the mean of hydroxyurea-sensitive [^{3}H]thymidine incorporated for each sample.

In vitro growth studies of hepatocytes from transgenic mice and their normal siblings were performed in the same conditions except that no conditioned media were added as exogenous sources of HGF. Instead, 5 μCi of [^{3}H]thymidine was added to culture media with EGF and insulin after 24 hr of serum deprivation. Cells were harvested 24 hr later and DNA synthesis was measured as above.

Stimulation of DNA synthesis by purified recombinant HGF was assessed by similar procedures except that 6.25 × 10^4 isolated rat hepatocytes in 0.5 ml were labeled in 24-well plates with [^{125}I]iododeoxyuridine (0.3 μCi/ml; 2 Ci/mmol) in the presence of various concentrations of human recombinant HGF or EGF (20).

Assay for Tumorigenicity in Nude Mice. Fao HCC cells transfected with pAN/T2-NB′ with and without pAlbHGF were injected into two subcutaneous sites in 6-week-old nude mice (Swiss *nu/nu*, Taconic Farms). Samples (1 × 10^6, 5 × 10^6, and 1 × 10^7 cells) of each cell line were injected into two sites in two mice (four injections per sample). Mice were sacrificed 28 days later for analysis of tumor sizes.

Antibody Reversal of HGF Effects in FaoHGF Cells. The growth of Fao cells transfected with AlbHGF or neomycin-resistant controls was examined in the presence of a polyclonal anti-HGF rabbit antibody. IgG from this antiserum was purified by protein A-Sepharose. For each aliquot, 5 × 10^4 cells were labeled for 15 hr with 1 μCi of [^{3}H]thymidine (New England Nuclear) in 100 μl of Dulbecco's modified Eagle's medium in individual wells of a 96-well plate. Purified rabbit immunoglobulin (20 ng) was added to the indicated cultures of FaoHGF and FaoNeo cells to assess the reversibility of the HGF effect.

Antiproliferative Assay. Human recombinant HGF was purified from culture medium of CHO or C-127 cells transfected with plasmid containing the HGF cDNA (20). Briefly, HGF was purified sequentially on S-Sepharose FF (Pharmacia), heparin-Sepharose CL-6B (Pharmacia) and phenyl 5PW (TOSO). Recombinant HGF purity was assessed by SDS/PAGE followed by silver staining.

Antiproliferative activity was assayed as described (21, 22). Wells of 96-well microtiter plates were seeded with 10^4 cells in 0.2 ml of medium containing various amounts of recombinant HGF. Cells were incubated in 5% CO_2 at 37°C for 72 hr, washed with phosphate-buffered saline, and stained with 0.5% crystal violet in 20% methanol. After washes with 20% methanol, the dye was extracted (30.5 mM disodium citrate/19.5 mM HCl/47.5% ethanol) and its A_{570} was used to determine the cell number. The antiproliferative assay was also performed after the addition of EGF (10 ng/ml; Pepro Tech; Rocky Hill, NJ), 100 nM insulin (Sigma), or transforming growth factor β (TGF-β; R and D Systems) in the presence and absence of the indicated concentrations of recombinant HGF.

RNA Analysis. RNA was isolated (23) and HGF expression in individual tumor cell lines was analyzed by RNase protection (24). An antisense RNA probe, labeled with [^{32}P]UTP (New England Nuclear), was transcribed with T3 RNA polymerase from pBKS-HGF (16) linearized with *Bgl* II. Total RNA (20 μg) from the indicated cell lines and tissues was used in each assay. RNA from IMR-90 cells, which express high levels of HGF protein (9), was used as a positive control.

RESULTS AND DISCUSSION

Albumin Enhancer/Promoter-Driven HGF Fusion Gene and Its Expression in HCC Cells. We developed an expression construct (pAlbHGF) that contains an HGF cDNA regulated by the albumin gene enhancer and promoter (Fig. 1*A*). Fao HCC cells were cotransfected with pAlbHGF and pAN/T2-NB′ and selected for growth in G418. After 4 weeks, 15 clones were isolated and screened for HGF expression by RNA blot analysis. Positive clones were confirmed by the HGF activity assay. Two clones, FaoHGF1 and FaoHGF2, were identified (Fig. 1*B*). Fao cells transfected with pAN/T2-NB′ alone contained no HGF transcripts in a sensitive RNase protection assay and were used as negative controls in subsequent experiments (FaoNeo). Indeed, FaoNeo cells expressed no other activity that affected hepatocyte growth in primary culture. (The background stimulation from untransfected cells or conditioning medium is due to the presence of serum in the medium that is necessary for survival of the FaoNeo cells.) Culture supernatants from FaoHGF cells had 4 times as much activity as control IMR-90 cells (Fig. 1*B*).

Expression of HGF in Transgenic Mice Carrying a Fragment of pAlbHGF. Purified AlbHGF DNA was injected into the pronucleus of 128 fertilized oocytes from FVB mice over 5 days of microinjection. Thirty potential founder mice were born; five of these were identified as founder animals by DNA blot analysis of tail DNA. These founders were further bred to FVB mice and F_1 mice were similarly identified. Sera

from F_1 mice from each of the five lines of transgenic mice and two of their normal siblings were collected at sacrifice and analyzed for HGF expression (Fig. 1*C*). Circulating HGF was elevated in all five lines of transgenic mice (TG 1–5) when compared with two cage/sex/sibling-matched controls (WT 1 and WT 2) from the first two lines of transgenic mice (TG line 1 and TG line 2). RNA blots of various mouse organs demonstrated that expression of the human HGF transgene was confined to the liver.

***In Vitro* Growth of HGF-Expressing HCC Cells.** Using FaoHGF cells, we examined the role of HGF as a potential autocrine growth factor in HCC cells (Fig. 2). We first examined FaoHGF cell growth in medium with reduced serum. In medium containing 7% serum, FaoHGF1 and FaoHGF2 cells grew more slowly than the FaoNeo cells (Fig. 2*A*). In 3% serum, both lines of FaoHGF cells grew significantly more slowly than the FaoNeo controls. In serum-free medium (0%), FaoNeo cells continued to grow and divide while both lines of FaoHGF cells died. Note that the magnitude of this inhibition is in proportion to the levels of HGF expression in the FaoHGF1 cells compared with FaoHGF2 cells. These results suggested that HGF expression reduced tumorigenicity of the Fao HCC cell line. Indeed, we were successful in cotransfecting only 2 of 15 G418-resistant clones of Fao cells tested; therefore, HGF expression provided a negative selection in transfection experiments. Attempts to transfect HepG2 and SK-HEP-1 cells with AlbHGF were unsuccessful.

Morphologic changes in HGF-expressing cells were immediately apparent. Fao cells normally look like fibroblasts. After transfection with the control plasmid, pAN/T2-NB′, this morphology was not altered (Fig. 2*B*). In contrast, cell shapes were altered in FaoHGF cells; after HGF transfection these cells were rounded and formed tight, self-adherent clumps (Fig. 2*C*).

Antibodies to HGF Normalize the Growth of FaoHGF Cells. A polyclonal antibody for HGF reversed the inhibitory effect of autocrine HGF production (Table 1). Anti-HGF antibodies had no effect on growth of FaoNeo cells. In contrast, the addition of antibodies prevented high levels of autocrine production of HGF in FaoHGF1 and FaoHGF2 cultures and their normal growth rate was restored.

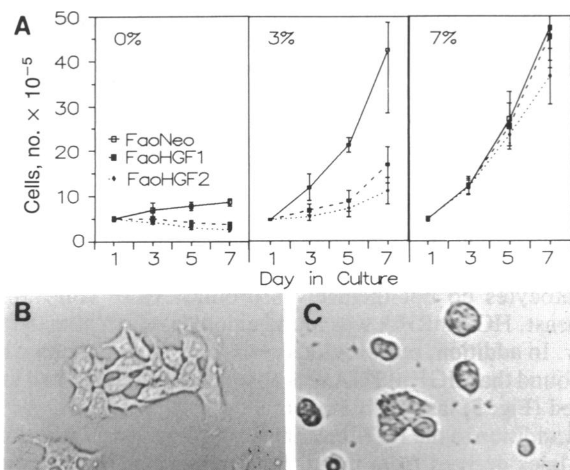

FIG. 2. (*A*) Endogenous expression of HGF slows the growth of Fao cells. On day 1, dishes (60 mm) were seeded with 5×10^5 cells of either FaoNeo or the FaoHGF cell lines. Cells were grown in 0%, 3%, or 7% fetal bovine serum. Data points represent the mean ± SD from four individual dishes for each condition on the indicated days. The experiment was terminated when the FaoHGF cells in 0% serum were no longer found in the cultures. (*B*) Fao cells normally demonstrate a fibroblastic morphology. (×85.) (*C*) In contrast, FaoHGF cells are rounded, refractile, and tend to grow in clumps. (×85.)

Table 1. HGF antibodies normalize FaoHGF cell growth

Cell type	[³H]Thymidine incorporation, cpm (mean ± SD): Without antibody	With antibody
FaoNeo	95,410 ± 17,954	79,986 ± 10,469
FaoHGF1	42,843 ± 10,195	57,110 ± 9,643
FaoHGF2	3,753 ± 896	46,163 ± 8,135

Decreased Tumorigenicity in HGF-Expressing HCC Cells. To test tumorigenicity of HGF-expressing Fao cells directly, we inoculated both FaoNeo and FaoHGF2 cells into nude mice (Table 2). With FaoHGF2 cells, tumors were formed in three of four sites inoculated with either 1×10^6 cells or 5×10^6 cells. Tumors were formed in all four sites inoculated with 1×10^7 FaoHGF2 cells. In contrast, tumors formed in every inoculation (four of four) of FaoNeo cells at each concentration. In addition, a clear reduction of tumorigenicity was observed in the tumor sizes of FaoHGF2 cells. Tumor weights of FaoHGF2 transplants were, on average, 10% and 25% of the control weights at the lowest and highest inocula, respectively. These data suggest that HGF produced by FaoHGF cells inhibited their growth in an autocrine fashion *in vivo*.

Stimulation of Normal Hepatocytes by HGF. We compared the HGF effect on Fao HCC cells with its effect on normal hepatocytes (Fig. 3). Our recombinant HGF, purified from transfected CHO cells, was >98% pure as assessed by standard SDS/PAGE (Fig. 3*A*).

Hepatocytes were isolated from the livers of transgenic mice expressing the AlbHGF fusion gene. These cells produced high levels of HGF in an autocrine fashion similar to the production in FaoHGF cells (Fig. 1). In contrast, however, the autocrine production of large quantities of HGF in transgenic hepatocytes stimulated their growth 4-fold in comparison to their wild-type siblings (Fig. 3*B*). In addition, we assessed the effect of purified recombinant HGF on isolated normal rat hepatocytes (Fig. 3*C*). A dose-dependent stimulatory effect (maximal at 15 ng/ml) was seen at all concentrations of added HGF and no dose was inhibitory to normal rat hepatocytes.

Antiproliferative Activity of HGF on Eight HCC Cell Lines. Because endogenous HGF expression inhibited the growth of Fao cells, we tested the ability of exogenous recombinant HGF to inhibit growth in Fao and seven other HCC cell lines. An antiproliferative assay for tumor necrosis factor was adapted for this purpose (21). In all cases, HGF led to decreased cell numbers after short-term exposure of the HCC cells (Fig. 4 *A* and *B*). This inhibitory effect was seen in the same concentration range that stimulated the growth of primary rat hepatocytes (2.5–100 ng/ml). Fao cells were actually the least sensitive cell line tested. Other cell lines revealed greater inhibitory effects at lower doses with a maximum inhibitory effect of 60% in FOCUS cells with HGF at 50 ng/ml.

Table 2. Tumorigenicity in nude mice

Inoculum, cell no.	Cell type	Tumor incidence	Tumor mass, g (mean ± SD)
1×10⁶	FaoNeo	4/4	1.78 ± 0.78
	FaoHGF2	3/4	0.17 ± 0.21*
5×10⁶	FaoNeo	4/4	2.94 ± 1.13
	FaoHGF2	3/4	0.49 ± 0.54**
1×10⁷	FaoNeo	4/4	2.38 ± 1.08
	FaoHGF2	4/4	0.61 ± 0.58***

Comparison with FaoNeo by Student's *t* test: *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.01$.

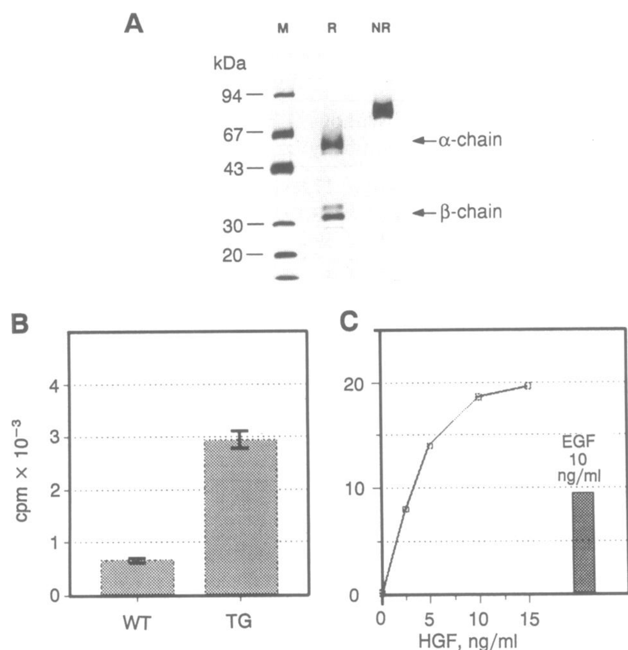

FIG. 3. HGF stimulates growth of normal hepatocytes. (*A*) Purification of HGF from transfected CHO cells yields a single protein in nonreducing conditions (lane NR) on SDS/PAGE. This protein dissociates into two subunits under reducing conditions (lane R). Lane M, size markers. (*B*) To analyze the effect of autocrine production of HGF on normal cells, hepatocytes were isolated from two transgenic mice (TG) and two of their normal siblings (wild type, WT). Cells were isolated by collagenase perfusion and were allowed to adhere to culture plates in medium containing fetal bovine serum, insulin, and dexamethasone as in the HGF assay. After culture for 24 hr in serum-free medium, insulin and epidermal growth factor were added overnight and DNA synthesis was assayed by [^{3}H]thymidine incorporation the following day. For each mouse, the assay was repeated in quadruplicate. Data are plotted as the mean of the eight data points with error bars showing the SDs. DNA synthesis was increased 4-fold in hepatocytes from the transgenic mice. (*C*) Purified recombinant HGF was added to isolated rat hepatocytes. DNA synthesis was stimulated at all concentrations of HGF; maximal stimulation was seen at 15 ng/ml. Bar shows stimulation with EGF at 10 ng/ml.

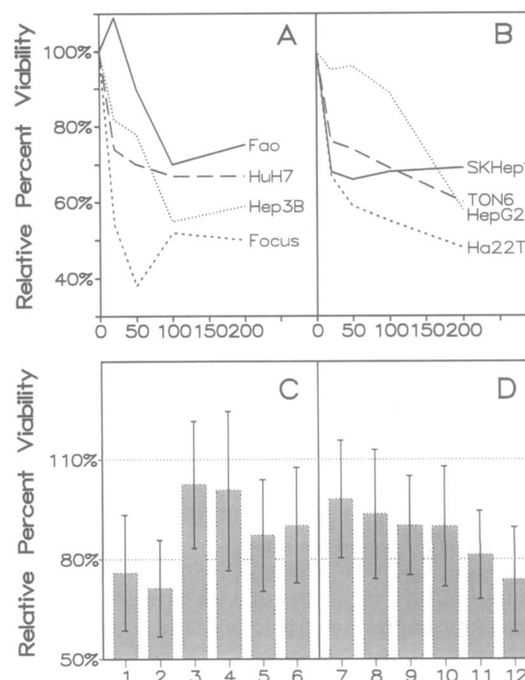

FIG. 4. Growth inhibition assays. (*A*) Decreased viability of HCC cells in medium with recombinant HGF. The indicated HCC cell lines were cultured with various concentrations of HGF for 72 hr; the abscissa displays HGF concentrations in ng/ml. The surviving cell number (21) in each well was plotted as a fraction of the cell number in the control well containing no added HGF (22). [Relative percent viability = (mean OD, drug-treated/mean OD, non-drug-treated) × 100%.] Each data point is plotted as the mean of four determinations for that concentration of HGF in the culture medium. (*B*) Inhibition of four additional HCC cell lines by recombinant HGF. (*C*) HGF was compared with other growth factors. Each bar represents the results for six cell lines (Hep3B, SK-HEP-1, TON6, HA22T, HuH7, and FOCUS); each cell line was tested in quadruplicate, so that for each point, $n = 24$. When HGF was used alone (50 and 100 ng/ml; bars 1 and 2, respectively), it inhibited all cell lines, as described in *A* and *B*. In contrast, EGF (10 ng/ml) stimulated proliferation of HCC cells (bar 3) but did not reverse the inhibitory effect of HGF (5 and 20 ng/ml; bars 4 and 5). The addition of both EGF (10 ng/ml) and insulin (100 nM) still did not reverse the inhibitory effect of HGF (20 ng/ml; bar 6). Thus, inhibition of HCC cells by HGF was independent of other stimulatory factors. (*D*) HGF was compared with TGF-β to examine possible inhibitory interactions. TGF-β (1, 5 and 10 pM) gave a dose-dependent reduction in HCC cell viability (bars 7–9, respectively), as expected. The combination of HGF (20 ng/ml) and TGF-β (1, 5 and 10 pM) was inhibitory in all combinations (bars 10–12) and their effect was apparently synergistic.

We compared HGF with factors known to be important in liver cell growth (Fig. 4 *C* and *D*). For these experiments, we focused on the six human HCC cell lines (Hep3B, SK-HEP-1, TON6, HA22T, HuH7, and FOCUS) and pooled data from all of them. EGF stimulated the growth of these HCC cells to a small extent, as expected (Fig. 4*C*, bar 3). However, the addition of EGF to HGF did not completely reverse the inhibition of HCC (compare bars 4 and 5 with bars 1 and 2). Further addition of both insulin and EGF to HGF did not cause HGF to become a stimulatory factor for any HCC cells (compare bar 6 with bar 2).

TGF-β was inhibitory at 5 and 10 pM (Fig. 4*D*, bars 7–9). Although the concentration of TGF-β employed was lower than the amounts of HGF used, the magnitude of the inhibition was higher for HGF (Fig. 4*D*, bars 8 and 9, versus Fig. 4*C*, bars 1 and 2). When TGF-β was added to HGF, the net result was a synergistic inhibition of HCC cell growth at each concentration of TGF-β (Fig. 4*D*, bars 10–12). Thus, HGF acts synergistically with TGF-β in inhibiting HCC cell growth while acting synergistically with EGF and insulin in stimulating normal hepatocytes.

HGF Expression in Cell Lines. Sera of patients with acute hepatitis, chronic hepatitis, and liver cirrhosis contain elevated levels of HGF (12). Because persistent liver damage is closely related to the occurrence of HCC, we initially speculated that endogenous expression of HGF in developing HCC might explain elevated HGF levels. Consequently, we examined eight HCC cell lines for HGF expression by RNase protection analysis. First, we confirmed that normal human hepatocytes do not themselves produce HGF (Fig. 5). In contrast, HGF mRNA was found at low levels in human fetal liver. In addition, our previous results were confirmed when we found that HGF mRNA was absent from all HCC cell lines tested (Fig. 5, last eight lanes).

In addition to the HCC cell lines, we surveyed 27 additional cell lines derived from brain, lung, pancreas, ileum, colon, bone, and leukocytes by RNA blot analysis and RNase protection. Only 6 cell lines exhibited positive signals; this suggests that HGF is rarely expressed in tumors. Positive cell lines included two glioblastomas (HGL4 and U-138MG), one neuroblastoma (NGP), and one monocytic leukemia (THP-1).

Is HGF a Transforming Protein? We began this study to determine whether HGF, a mitogen for hepatocytes, could act as a transforming protein. In fact, endogenous expression of transforming proteins and growth factors can cause a

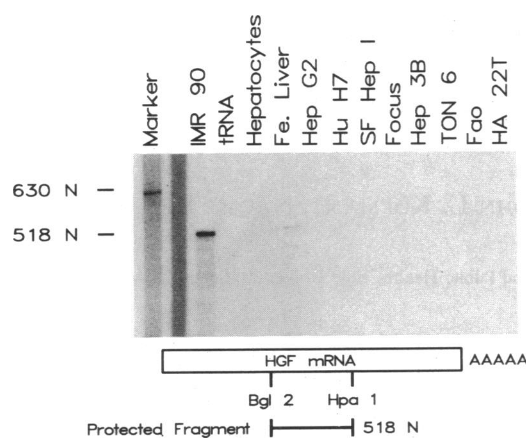

FIG. 5. HGF mRNA is absent from all HCC cell lines tested by RNase protection. The antisense RNA probe used in this experiment detects a protected fragment of 518 nucleotides (N) between the *Bgl* II site where the template plasmid is linearized and the *Hpa* I site located at the 3′ end of the inserted DNA fragment in pBKS-HGF. We used 20 μg of total RNA from each of the indicated cell lines and tissues for this experiment. The positive control line, IMR-90, has been shown to express HGF (9). Negative controls included the same amount of yeast tRNA and normal human liver RNA (Hepatocytes). In addition, 20 μg of human fetal liver RNA was tested in the indicated lane (Fe. Liver). The 630-nucleotide fragment of *Hae* III-digested ϕX174 DNA was included as a size marker.

variety of effects in cells. They can induce transformation, as in the case of TGF-α (25). In contrast, other factors can work in opposing directions in different circumstances. For example, endogenous expression of protein kinase C in fibroblasts increases their tumorigenic potential, but protein kinase C expression inhibits colon carcinoma cell lines (26). Similarly, we found that HGF is not a simple mitogenic factor for normal hepatocytes; it also inhibits HCC cell growth.

What, then, is the role of the elevated serum HGF levels seen in patients with diseases associated with the development of HCC? HGF is normally a paracrine regulator of hepatocyte growth. It is produced in lung, spleen, and nonparenchymal liver cells in response to hepatic injury (27). Because HGF is a signal for liver repair, it seems likely that elevated HGF expression may actually protect against HCC development.

The difference between the effect of HGF on normal hepatocytes and its effect on transformed cells of the same cell lineage is interesting. This may be related to the stem-cell origin of HCC cells in contrast to the mature phenotype of cultured hepatocytes. Alternatively, the inhibitory effect of HGF may be related to some transformation event, perhaps to alterations in the HGF receptor, the c-*met* gene product. Thus, our findings suggest that HGF should be studied not only as an inhibitor of the development of HCC but also as a tool to assess changes in regulatory signals that are part of the neoplastic process.

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