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Expression of the Human Hepatocyte Growth Factor cDNA in Primary Cultures of Rat Hepatocytes

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Hepatocyte growth factor (HGF) and epidermal growth factor (EGF) are primary mitogens for hepatocytes in culture. hepatocytes express the HGF-receptor MET but not HGF itself. To investigate the influence of autocrine HGF expression on the proliferative potential of hepatocytes, primary cultures were submitted to retrovirus-mediated transduction of the human hgf (huHGF) cDNA. Expression of the transduced cDNA revealed a minimum 2-fold increase in HGF-mRNA, whereas expression of the *Escherichia coli* β-galactosidase gene remained even. Estimation of huHGF copy numbers showed there was a minimum 4-fold increase, suggesting an increase in the population of transduced cells. Immunoprecipitation of excreted huHGF and growth bioassays proofed that HGF was present and functional. HGF is excreted into the medium and therefore, by diffusion, available to transduced and non-transduced cells. The increase in huHGF-transduced cells suggests that the autocrine pathway as opposed to the paracrine pathway, which are both present at the same time, confers a growth advantage to these cells.

HGF and its receptor MET (1), ordinarily constitute a paracrine signaling system in which cells of mesenchymal origin produce the ligand (HGF) which binds to its receptor (MET) that is predominantly expressed in cells of epithelial origin (2). In primary cultures of hepatocytes, exogenously added HGF can stimulate growth, motility and morphogenic events (3). *In vivo,* HGF is an important regulator of normal liver regeneration in response to injury (4) via a paracrine mechanism.

Co-expression of HGF and its receptor, resulting in an autocrine loop, is thought to contribute to abnormal growth and altered differentiation (5, 6, 7); however, there is also evidence suggesting that autocrine expression of HGF/MET can occur under normal circumstances (8, 9, 10). Furthermore, while the effects of exogenous HGF on promoting normal hepatocyte growth have been well characterized, there appears to be a contradictory effect on neoplastic hepatocytes. When HepG2 cells were transfected with an HGF cDNA, they grew slower than control cells and were less tumorigenic in nude mice (11). infusion of HGF in rats bearing tumors induced by diethylnitrosamine resulted in suppression of DNA synthesis in the majority of the tumors (12). Finally, interbreeding of transgenic mice expressing HGF in hepatocytes under the albumin promoter with transgenic mice expressing c-*myc* in their hepatocytes resulted in a decrease in the number of spontaneous tumors (13). when TGFα transgenic mice were used instead of the HGF mice there was a marked increase in the number of tumors in the livers of the double transgenics (14). These combined studies suggest that HGF has inhibitory effects on neoplastic hepatocyte proliferation, despite the positive effect on normal hepatocytes (15, 16).

In this study we have examined the effects of forced, autocrine expression of HGF in serum-free long-term primary cultures of rat hepatocytes. huHGF was expressed by transducing the hepatocytes with a retroviral vector containing the huHGF cDNA. The intact protein was precipitated out of the media of transduced cultures and was active in a proliferation bioassay. Therefore, it can be regarded as being available to transduced and non-transduced cells in culture. We present evidence that the forced expression of HGF in primary cultures of rat hepatocytes leads to an increase in the population of transduced cells compared to non-transduced cells in the same cultures. HGF mRNA increased in transduced cultures, while in control cultures lacZ-mRNA expression stayed stable. Also, an increase in the copy number of the integrated huHGF-cDNA was observed. The results suggest that autocrine expression presents an advantage for the proliferation and survival of normal, transduced cells compared to paracrine action taking place in the same cultures. Since autocrine expression of HGF in HepG2 cells is deleterious, our results imply that there are fundamental differences in the way normal and neoplastic hepatocytes respond to HGF.

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MATERIALS AND METHODS

Materials. Human recombinant HGF was a gift from Snow Brand Milk Products (Tochigi, Japan) and corresponds to the naturally occurring five amino acid deleted form. EGF was purchased from Collaborative Research (Waltham, MA). Monoclonal and polyclonal antibodies against HGF were obtained from R&D Systems (Minneapolis, MN).

Isolation and culture of hepatocytes. These were performed as described before (17).

Construction of retroviral vectors. Construction of the MFG-lacZ retroviral vector has previously been described (18). For the MFG-huHGF retroviral vector the human HGF cDNA was isolated from pSPORT1 as 2.6 kb *Bam*HI-fragment. The 5′-end of the fragment was cut at its *Bst*EII site, 5 nucleotides downstream of the ATG. The ATG was reinitiated by ligating an oligonucleotide fragment containing a *Nco*I site to the *Bst*EII site. The 3′-end of the fragment was generated by digestion with *Dra*I at position +2252 followed by ligation to a *Bam*HI linker. The resulting cDNA fragment was then inserted into the *Nco*I- and *Bam*HI-sites of the MFG vector.

Producer cell line. BOSC 23, an ecotropic packaging cell line derived from the human embryonic kidney cell line 293, was cultured and transfected as described using calcium phosphate (19). To enhance the titer and for a broader host range, cotransfections with vesicular stomatitis virus G glycoprotein (20) and the pol-gag helper plasmid (21) were performed. The viral supernatants were harvested 72 hours post transfection. The retroviral vectors produced are helper-free. Titers in excess of 10^6-10^7 per milliliter of supernatant were obtained, determined as previously described (19).

Transduction of rat hepatocytes. Hepatocytes were cultured completely serum-free for 72 hours in HGM (22) with HGF (40 ng/ml) and EGF (20 ng/ml) prior to transduction. The media was removed and supernatants containing retroviral vectors were diluted 2:1 with HGM and growth factors and a final concentration of 8 μg/ml polybrene (Aldrich) were added. Cultures were incubated for 12-16 hours, the retrovirus-containing medium was removed, the cells were washed twice with HGM without growth factors, and cultures were re-fed with HGM. MFG-huHGF-transduced cells were cultured only with EGF, whereas MFG-lacZ-transduced cells were maintained in HGM with HGF and EGF.

X-gal staining of hepatocyte cultures. Cultures were washed three times with PBS after the medium was removed. Cells were fixed in 0.25% glutaraldehyde, 1.75% formaldehyde in PBS for 5 min at 4°C, washed three times for at least 10 min with agitation in PBS with 1 mM $MgCl_2$, and stained in a solution containing 250 mM $K_4Fe(CN)_6$, 2 mM $MgCl_2$, 1% x-Gal in TBS buffer (25 mM Tris pH 8.0, 140 mM NaCl, 5 mM KCl at 37°C for 2-4 hours. Staining solution was removed and the number of blue cells determined by counting at least three viewing fields per culture dish under a light microscope.

In situ hybridisation. This was performed as previously described (23). Hepatocytes were transduced with MFG-huHGF, trypsinized after 48 hours and centrifuged onto slides (roughly 40.000 cells/cm^2). Slides were used for *in situ* hybridization. Results were evaluated by counting at least 3 viewing fields per slide at a magnification x 200.

RNA isolation and Northern blot analyses. These were performed as previously described (17). Membranes were hybridized using a pSPORT-huHGF 600 bp *Eco*RI fragment or for normalization a 28S RNA 700 bp *Eco*RI fragment (HHCH 75, ATCC).

Protein isolation, immunoprecipitation, and Western blot analyses. Conditioned medium was precipitated using ice cold acetone at a final concentration of 80% and incubated for 1 hour on ice. After centrifugation (30 min, 13000 x g), pellets were washed three times with 70% ethanol and air dried. Pellets were re-dissolved in 10 mM Tris, pH 7.4 with 1% SDS in the presence of a phosphatase inhibitor (100 mM sodium vanadate) and multiple protease inhibitors [1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml pepstatin A, 10 μg/ml trans-Epoxysuccinyl-L-leucylamido-(4-guanidino)butane (E-64), 10 μg/ml antipain]. Samples were sonicated and the protein concentration was determined using the bicinchoninic acid assay (Sigma, St. Louis, MO). for immunoprecipitation, 5 μg of polyclonal anti-HGF antibody was mixed with 500 μg protein in immunoprecipitation buffer (1% Triton-X-100, 150 mM NaCl, 10 mM Tris-HCl pH 7.4, 1 mM EDTA, 1 mM EGTA, 0.2 mM sodium vanadate, 0.2 mM phenylmethylsulfonyl fluoride, 0.5% Nonidet P40). The components were vortexed and incubated on ice for one hour. 50 μl of a solution containing 10% protein A-sepharose was added to the samples and the incubation was continued with agitation for 30 min at 4°C. samples were washed three times with immunoprecipitation buffer and the sepharose pellet was resuspended in 25 μl of gel loading buffer. Proteins were subjected to SDS-PAGE and electrotransferred to Immobilon-P membranes (Millipore, Bedford, MA) using a filtered solution containing 50 mM Tris base, 95 mM glycine, and 0.005% SDS. Membranes were blocked and antibodies were applied as described previously (17, 24).

Determination of gene copy number. Genomic DNA was isolated from transduced cells as described previously (25). 20 μg DNA was digested with *Eco*RI and separated on a 0.5%/0.5x TBE (45 mM Tris-borate, 1 mM EDTA, pH 8.0) agarose gel. As a standard for determination of copy numbers of the integrated foreign cDNA, pSPORT-huHGF (26) was linearized with *Nco*I. Calculated amounts of DNA (27) corresponding to 0.3, 0.5 and 1 copy number were added to 20 μg control DNA, also digested with *Eco*RI. The DNA was denatured, neutralized and transferred onto nylon membrane (GeneScreen Plus) as recommended by the manufacturer. Membranes were probed using a 680 bp *Eco*RI fragment derived from pSPORT-huHGF. The resulting autoradiographs were analyzed by scanning densitometry. Conditions were utilized in which the endogenous gene was not detectable.

Determination of total DNA. Cells were transduced with MFG-huHGF, grown in HGM with EGF and harvested at times indicated. The medium was removed and the cells washed four times with 0.9% NaCl. Cells were lysed in 0.2% SDS, 1x SSC (150 mM NaCl, 15 mM Na-citrate pH 7.0), 5 mM EDTA. Aliquots were used to determine the total DNA in the cultures using Hoechst dye H 33258 and a fluorometer (Hoefer, DyNA Quant 200).

Bioassay. Medium of transduced hepatocyte cultures was collected on day 10 after transduction. Freshly isolated hepatocytes were grown in HGM with the addition of HGF and EGF for 48 hours. Medium was changed to HGM without growth factors and cells incubated for 6-8 hours. HGM was replaced by fresh media as indicated, containing ^{3}[H]thymidine at 2.5 μCi/ml. HGF action of medium collected from cultures transduced with MFG-HGF was blocked by pre-incubation with polyclonal anti-human HGF antibody for 1 hour on ice at the recommended concentration. As a control antibody we used anti-goat IgG. After addition of growth factors and/or 200 μl medium harvested from transduced cultures hepatocytes were grown for 48 hours. The bioassay was terminated by removing the media, washing three times with PBS (140 mM NaCl, 2.7 mM Kcl, 6.5 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) and cell lysis in 1x SSC, 5 mM EDTA, 0.2% SDS. incorporation of radioactivity into DNA was determined by scintillation counting.

RESULTS

Optimizing the transduction efficiency in rat hepatocyte cultures. Since the administration of growth factors can enhance the transduction efficiency (28), we used four different culture conditions to determine the most efficient conditions for retroviral integration into hepatocytes in our culture system. These experiments

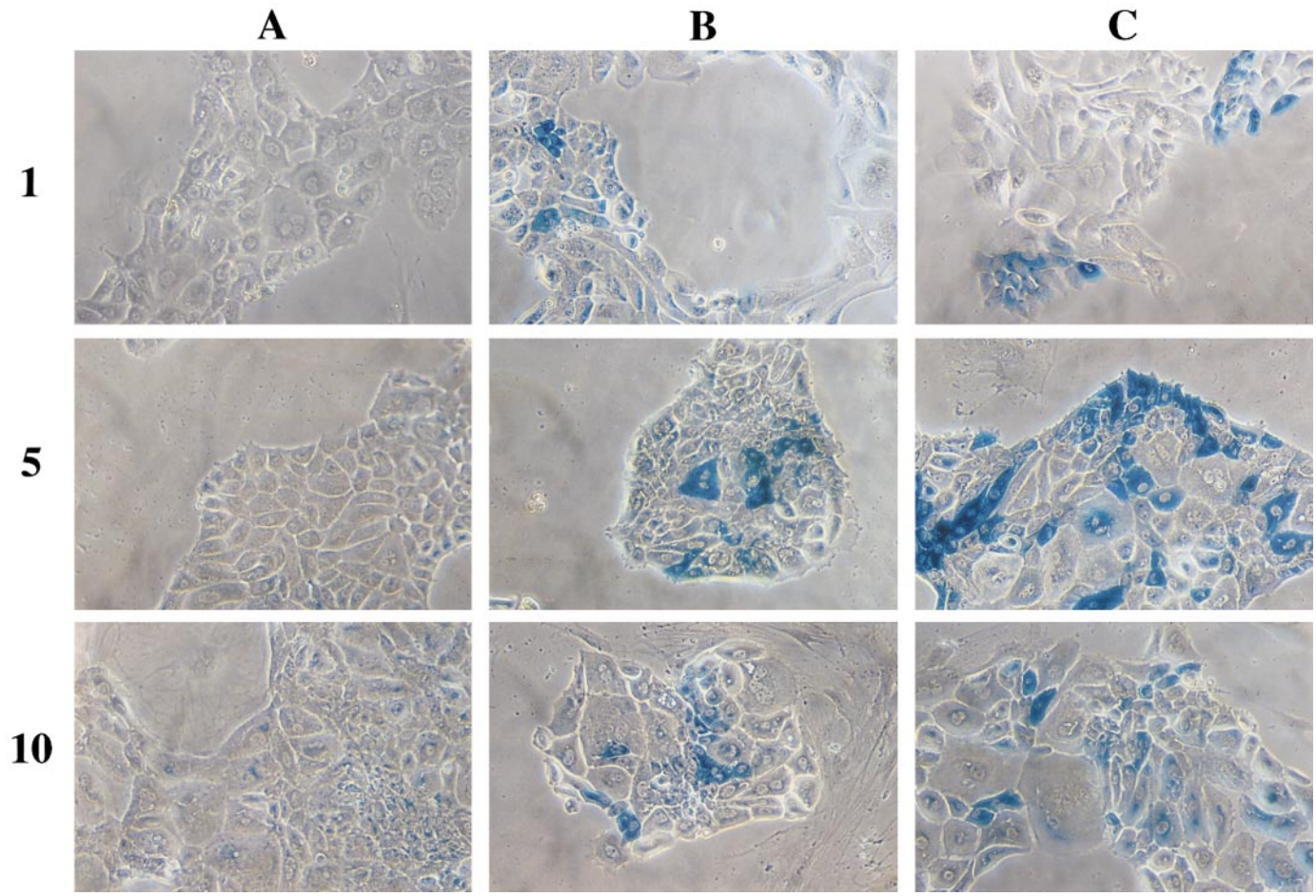

FIG. 1. HGF and EGF promote retroviral transduction of rat hepatocytes. primary cultures of rat hepatocytes were grown in HGM plus HGF (40 ng/ml) and EGF (20 ng/ml) for 72 hours before being subjected to a transduction procedure using either MFG-lacZ retroviral vector or, as a control, non-transfected producer cell supernatants. During transduction, control cultures received HGF and EGF (column A) and transfected cultures received either no growth factors (column B) or HGF and EGF (column C). after 12-16 hours the medium on all cultures was changed to HGM plus HGF and EGF. Staining for β-galactosidase activity was performed 1, 5 and 10 days after transduction as indicated.

were performed using MFG-lacZ constructs which did not contain the HGF cDNA. In HGM with HGF and EGF added, hepatocytes start to proliferate after 48 to 72 hours (22). Therefore, all transductions were performed 72 hours after the cells were plated. Retrovirus containing supernatants of BOSC23 cells were mixed with HGM in the presence of either EGF, HGF, EGF plus HGF, or no growth factors and added to the hepatocyte cultures. As a control, culture supernatants of non-transfected BOSC23 cells were added to HGM containing HGF plus EGF (Table 1). In all cases, after 12-16 hours the medium was changed to HGM with HGF plus EGF and the cultures were maintained with subsequent medium changes every 48 hours. Transduction efficiency was determined by staining for β-galactosidase activity and counting at least three different viewing areas per plate on days one, five and ten after transduction. A transduction efficiency of about 20% was observed when no growth factors or only EGF was included during transduction (Table 1, column 2 and 3). The addition of HGF alone or HGF plus EGF led to a transduction efficiency of about 30% (Table 1, last two columns). In Fig. 1, representative stains of cultures transduced in the absence of growth factors (column B) or presence of HGF and EGF (column C) are shown. Mock-transduced control cultures were always negative (Fig. 1, column A). Regardless of the initial efficiency, the increase in transduced cells over time was comparable under all culture conditions, suggesting that subsequent to transduction, cell division occurred at a consistent rate in the cultures.

Expression of huHGF increased in transduced cultures over time whereas expression of the β-galactosidase did not. Once the optimal conditions were determined for MFG-lacZ, all experiments were carried out using HGF plus EGF during transduction. In the post-transduction period, MFG-lacZ cultures received HGF plus EGF whereas MFG-huHGF cultures received EGF only. Expression of both the β-galactosi-

TABLE 1

Percentage of Blue Stained Hepatocytes in Cultures Transduced with MFG-lacZ

Time after transduction	No growth factors during transduction	+EGF during transduction	+HGF during transduction	+EGF+HGF during transduction
1 day	16 ± 2.0	18 ± 6.0	31 ± 3.5	31 ± 4.5
5 days	23 ± 1.8	23 ± 2.9	34 ± 4.1	37 ± 3.9
10 days	20 ± 6.9	24 ± 1.5	32 ± 3.7	38 ± 1.8

Note. Cultures were treated with the indicated growth factors only during the transduction period of 12-16 hours. All cultures were grown in HGM with HGF (40 ng/ml) and EGF (20 ng/ml) for 72 hours prior to transduction. Medium and growth factors were removed and cultures washed extensively. Fresh HGM with growth factors was added as indicated together with retrovirus containing supernatants. After the transduction period of 12-16 hours medium was again changed to HGM with HGF and EGF. All results represent the mean and standard error of at least three separate cultures.

dase mRNA and huHGF mRNA were monitored in transduced cultures over a period of 20 days using northern blot analyses of total RNA. While the expression of β-galactosidase started out higher than huHGF, mRNA expression stayed level or started to declined after ten days in culture (Fig. 2, white bars). In contrast, HGF mRNA levels more than doubled (Fig. 2, black bars) during the monitored period of 20 days.

Increase in huHGF-expression was due to an increase in the cell number of transduced cells. To determine, whether the increasing huHGF-mRNA amounts were due to an expansion of the cell population of transduced cells instead of an increase in transcription, copy number experiments were performed using high molecular weight DNA harvested two and 15 days after transduction. In the experiment shown in Fig. 3, two different exposures of the same filter are posted in order to verify the presence of a weak signal at two days after transduction (Fig. 3A, lane four). Southern blot analysis revealed that ≤0.1 copies of the huHGF-cDNA integrated into the host genome in cultures on day two after transduction, whereas on day 15 about 0.4 copies were detectable (Fig. 3B). This indicates a minimum 4-fold increase in transduced cells relative to the rest of the population. The cultures remained viable and growing over the monitored time period as indicated by an ongoing increase in total DNA (Fig. 3C), suggesting that the increase was due to an expansion of the transduced cells rather than an increase in the rate of transcription. In control cultures no signal was obtained (data not shown).

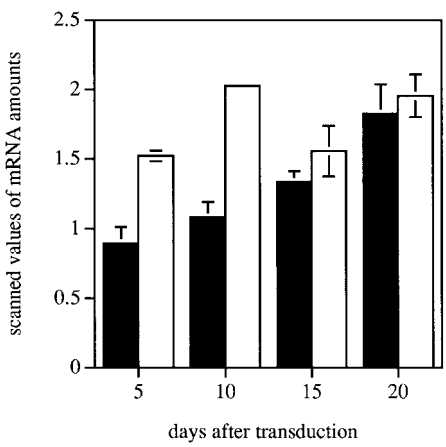

FIG. 2. Expression of the huHGF-cDNA in transduced hepatocyte cultures. Cultures of rat hepatocytes were subjected to retroviral transduction, total RNA was isolated on days 5, 10, 15 and 20 after transduction. 20 μg of total RNA was electrophoresed through 1.0% denaturing agarose gels, transferred onto nylon membranes and hybridized with a radiolabeled fragment of huHGF or lacZ, than stripped and reprobed for 28S RNA. Graph combines results obtained from scan and normalization of autoradiograms of three independent experiments.

Transduction efficiency of MFG-huHGF is lower than of MFG-lacZ. To address the question of whether the low copy number on day 2 (≤0.1 or 10% of the population) was due to a lower transduction efficiency of MFG-huHGF, we performed *in situ* hybridizations. Hepatocyte primary cultures were subjected to retroviral transduction using either MFG-huHGF or, as a control, MFG-lacZ. 48 hours after transduction the cells were released with trypsin and centrifuged onto slides. These preparations were than used for *in situ* hybridization targeting the HGF mRNA as previously described (23). as shown in Fig. 4, control cultures were always negative (Fig. 4A), while MFG-huHGF transduced cultures contain cells expressing HGF-mRNA on day 5 after transduction (Fig. 4B and C). The positive cells constituted 5-15% of the population, determined by counting at least three viewing fields per slide at a magnification x 200. This was considerably lower than what we observed with MFG-lacZ but in good correlation with our results from copy number experiments.

HGF is synthesized and excreted into the medium by transduced hepatocytes. To prove that the cultures produce HGF, media of transduced hepatocyte cultures was subjected to immunoprecipitation. At days 1, 2 and 4 after transduction, HGF production of two different cultures at each time point was examined. As a standard, 200 ng HGF was included. Normalization of the signals derived for the media samples revealed an HGF-concen-

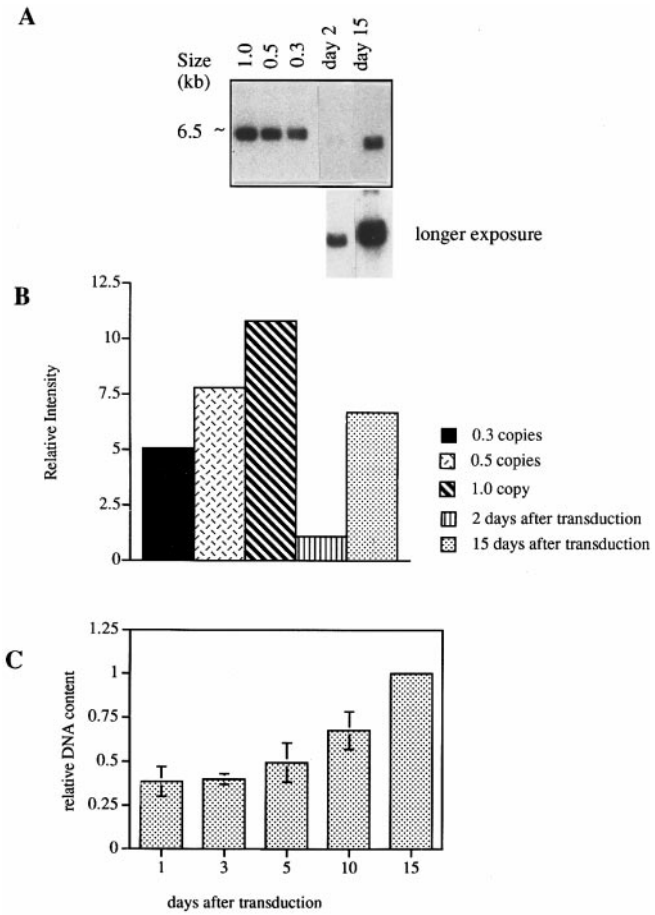

FIG. 3. Estimated gene copy number of the huHGF cDNA in transduced hepatocyte cultures. The first three lanes were loaded with linerized pSPORT-huHGF in amounts corresponding to 0.3, 0.5 and 1 gene copies of insert per haploid genome in 20 μg of total rat DNA. genomic DNAs (20 μg) isolated from transduced hepatocyte cultures two and 15 days after transduction were digested with *Eco*RI. Electrophoresis, Southern transfer, hybridization with pSPORT-huHGF DNA insert and autoradioramms were carried out as described in Materials and Methods. The resulting autoradiogramms were scanned using a densitometer. Panel A, representative autoradiogram, composed of two different exposures of the same nylon membrane, featuring the standards (0.3, 0.5 and 1 copy number) and total rat DNA day 2 and day 15 after transduction, below a longer exposure of day 2 and 15 to verify the faint band at day 2. Panel B, bar graph of relative intensities of the signals determined by scanning of the autoradiograph. Panel C, DNA content of transduced cultures followed up to day 15 indicating proliferating hepatocyte cultures were used in this experiment.

tration of approximately 5-10 ng/ml, cultures expressing varying amounts of HGF protein (Fig. 5) over time.

Transduced cultures synthesize functional HGF. The presence of biologically active HGF in the supernatant of transduced hepatocyte cultures was examined by performing a bioassay to determine the mitogenic activity. Medium of transduced rat hepatocyte cultures was collected five days after transduction with MFG-huHGF. Freshly isolated hepatocytes were grown in HGM without growth factors (Fig. 6, column 1), plus EGF (Fig. 6, column 2) or HGM plus HGF and EGF (Fig. 6, column 3) as controls, and compared to cultures with HGM plus media of MFG-lacZ (Fig. 6, column 4) or MFG-HGF (Fig. 6, column 5) transduced cultures. DNA synthesis as mirrored by [^{3}H]thymidine uptake of the hepatocytes was determined after 48 hours. HGF action was omitted by pre-incubation of the medium collected from MFG-HGF transduced cultures with neutralizing anti-HGF antibody (Fig. 6, column 6), unspecific antibody did not inhibit HGF (Fig. 6, column 7). There was no significant difference between control cultures that received HGF and EGF compared to cultures that received medium of MFG-HGF transduced cultures (compare Fig. 6, columns 3 and 5). Neutralizing antibody completely reduced HGF action ($p = 0.0006$) compared to unspecific antibody, resulting in a ^{3}[H]thymidine uptake equal to cells stimulated with EGF alone.

DISCUSSION

Several cases of HGF autocrine activity have been reported, either occurring naturally in non-differentiated human keratinocytes (8, 9, 10), or obtained following transfection experiments using hepatocarcinoma cells (11), MDCK cells (29), NIH 3T3 cells (7) or NBT-II cells (5). HGF is a mitogenic factor for hepatocytes in culture and *in vivo* following liver injury, partial hepatectomy (30, 31) or matrix breakdown after collagenase perfusion of the intact organ (32). We show here that forced HGF expression by primary cultures of rat hepatocytes has a mitogenic effect especially on the transduced cells providing them with a growth advantage.

Optimal conditions for transduction of rat hepatocytes in culture were established using HGF and EGF in our serum free HGM. Under these conditions, hepatocytes proliferate and integration of retrovirus does occur at a rate of more than 30% (Fig. 1). The increase in huHGf-mRNA (Fig. 2) and copy number of the integrated human HGF-cDNA over time (Fig. 3A and B) in a growing population of cells (Fig. 3C) indicates, that the transduced cells in the mixed population in the culture dish have a growth advantage. This is in contrast to recent studies which suggest that expression of HGF by hepatocytes may have anti-proliferative and anti-tumor effects (11, 12, 13, 14). We have previously shown that hepatocytes maintained in hepatocyte growth medium loose most of the differentiation specific gene expression patterns and behave as cells with hepatoblast properties. They retain levels of HNF1, HNF4 and HNF3 transcription factor family members, have decreased levels of C/EBPα, and increased levels of AP1 and NFκB (17, 22, 33). The increased proliferative effect of expression of HGF on transduced cells in our system may be due to the fact that the cells have "dedifferentiated" and thus behave differently than normal hepatocytes, on which HGF expression appears

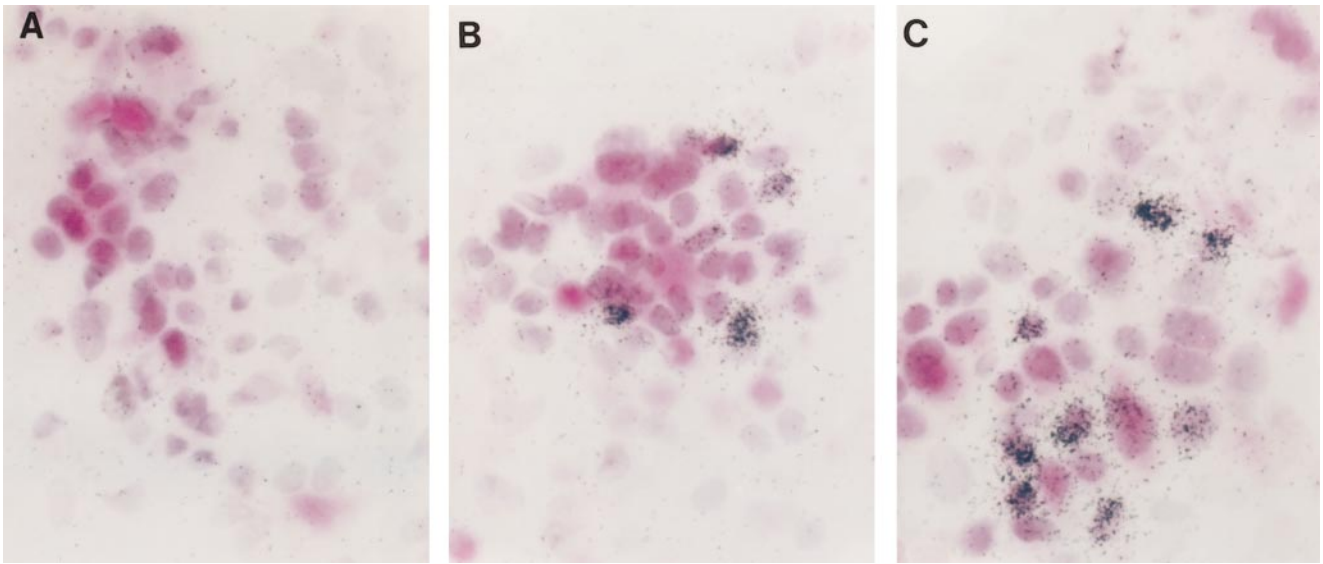

FIG. 4. *In situ* hybridization of hepatocytes transduced with MFG-huHGF. Hepatocytes were transduced as described and grown for two days. Hepatocytes were harvested using trypsin and centrifuged onto slides. *In situ* hybridization was performed on non-transduced hepatocytes (A) and MFG-huHGF (B and C representing two different cultures) transduced hepatocytes.

to have a mito-inhibitory effect whereas exogenously added HGF has a mitogenic effect.

In different scenarios a growth advantage for a small number of hepatocytes over the majority of cells in the liver has been observed *in vivo*. One example is the albumin-urokinase transgenic mouse model where the transgene is toxic to hepatocytes and somatic loss of the urokinase gene in liver cells confers a selective growth advantage. This results in a complete repopulating of the liver with cells that have spontaneously deleted the transgene (34). A comparable effect has been documented in a mouse model for hereditary tyrosinaemia type I, a recessive liver disease caused by a deficiency of fumarylacetoacetate hydrolase. As few as 1000 transplanted wild type hepatocytes were able to repopulate the mutant

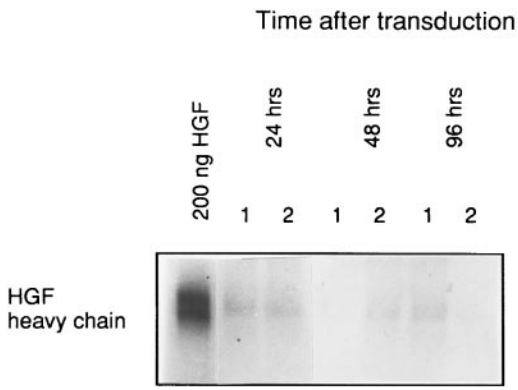

FIG. 5. Western blot analysis of excreted HGF isolated from media of transduced hepatocyte cultures. Media of hepatocyte cultures transduced with MFG-huHGF were collected from two different cultures at 24, 48 and 96 hours after transduction and the proteins acetone precipitated. As a control, 200 ng recombinant human HGF was used (lane 1). HGF contained in 1 ml medium was immunoprecipitated using a polyclonal antibody against human HGF. To the control HGF 500 ng BSA was added as carrier protein before immunoprecipitation. After separation on SDS-PAGE and electrotransfer, protein was detected using a monoclonal antibody against HGF.

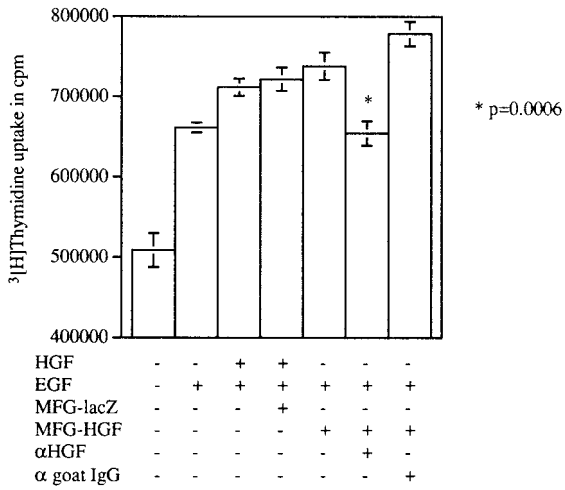

FIG. 6. Activity of HGF produced by hepatocyte cultures transduced with MFG-huHGF. The proliferation inducing effect of medium of MFG-HGF and MFG-lacZ transduced cultures on freshly plated hepatocytes was determined by ^{3}[H] thymidine incorporation. As controls HGM without growth factors (column 1), plus EGF alone (column 2) and plus HGF and EGF (column 3) were used. 200 μl of medium harvested from MFG-lacZ transduced cultures plus HGF and EGF (column 4) or MFG-HGF transduced cultures plus EGF (column 5) five days after transduction. Column 6 features medium of MFG-HGF transduced cultures pre-incubated with neutralizing antibody before being used in the growth assay. Unspecific antibody (column 7) did not have any effect.

liver after defective hepatocytes were corrected *in situ* by retroviral gene transfer (35). Similarly, clonal growth of hepatocytes expressing HGF in culture is an *in vitro* example of positive selection for growth.

The copy number experiment revealed a relatively low number of <10% transduced cells (Fig. 3), a discrepancy with the 30% transduction efficiency of the MFG-lacZ retroviral vector documented in Table 1. *In situ* hybridization for huHGF mRNA did reveal a low number of transduced cells, namely 5-15% (Fig. 4). Therefore, the discrepancy can be related to the different constructs, either to the different sizes or their abilities to integrate into the host genome. The expression analyses comparing lacZ- and HGF-mRNA amounts (Fig. 2) reflected a much higher expression of the β-galactosidase gene than of the huHGF cDNA, suggesting a lower infection rate in MFG-huHGF transduced cultures.

None the less, intact and active HGF protein is secreted by transduced cells (Fig. 5) in a range (5-10 ng/ml) shown to promote hepatocyte growth in culture (36). The Growth Assay (Fig. 6) confirmed the proliferative effect of HGF produced by transduced cells, that could be completely abolished using a specific antibody against HGF. The present study suggests that autocrine expression of HGF by hepatocytes with hepatoblast characteristics does confer a growth advantage for cells subjected to an autocrine pathway compared to paracrine signalling that does occur via intact HGF secreted into the medium or added to hepatocyte cultures.

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