POSITIVE AND NEGATIVE REGULATION OF HGF GENE EXPRESSION

Eiichi Gohda and Itaru Yamamoto

Department of Immunochemistry, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-naka, Okayama 700, Japan

Abstract: Hepatocyte growth factor (HGF), which is also known as a scatter factor or fibroblast-derived tumor cytotoxic factor, is most likely a physiological hepatotrophic factor. Regulatory mechanisms for HGF gene expression are important in understanding the control of liver regeneration. Analysis of the 5'-flanking region of the HGF gene has revealed several putative regulatory elements found in inducible genes, but functional characterization of the elements largely remains to be determined. HGF is produced by fibroblasts, vascular smooth muscle cells, a variety of human leukemia cell lines and human mesangial cells in culture. Fibroblasts and leukemia cells have been used to examine the factors that regulate HGF gene expression. cAMP-elevating agents, such as cholera toxin, forskolin and prostaglandin E2, membrane-permeable cAMP analogues and ascorbic acid, as well as protein kinase C (PKC)-activating phorbol esters, interleukin 1 and tumor necrosis factor-α, markedly up-regulate HGF gene expression. A reporter plasmid containing the mouse HGF gene promoter is responsive to interleukin 6 stimulation in transfected NIH 3T3 cells. In addition, heparin increases production of HGF, but without any increase in HGF mRNA levels. HGF gene expression is down-regulated by transforming growth factor-β, dexamethasone and 1,25-dihydroxyvitamin D3. Thus, HGF gene expression is positively and negatively regulated by various cytokines and steroid hormones.

Key words: hepatocyte growth factor (HGF), gene expression, cyclic AMP, liver regeneration

Introduction

Hepatocyte growth factor (HGF), which was initially identified as a potent growth factor for rat hepatocytes in primary culture, is a multifunctional cytokine with mitogenic, motogenic, morphogenic and tumor-suppressing activities. Thus, HGF is also known as a scatter factor (SF) or fibroblast-derived tumor cytotoxic factor (F-TCF). HGF is mainly produced by mesenchymal cells such as fibroblasts and vascular smooth muscle cells and acts on epithelial cells. HGF might be a molecule mediating mesenchymal epithelial communication. Recently, the receptor of HGF has been identified as the c-met protooncogene product. Since these findings, the literature on HGF is expanding at a rapidly increasing rate.

Despite the broad spectrum of its biological activities, HGF is believed to be the most physiological hepatotrophic factor that triggers liver regeneration. HGF levels in plasma and liver and HGF gene expression in the liver markedly increase before the induction of hepatic DNA synthesis after liver injury. Regulatory mechanisms for HGF production are crucial for understanding the control of liver regeneration. We reviewed the regulation of HGF gene expression and production in the Tissue Culture Research Communications last year. This review will concentrate on subsequent information concern-
HGF-Producing Cells in Culture

In addition to fibroblasts and vascular smooth muscle cells, some cell lines produce HGF. Nishino et al.\(^4\) have reported that the promyelocytic leukemia cell line HL-60 produces HGF. Nakamura et al.\(^5\) analyzed HGF production from 138 human leukemia and virus-transformed hematopoietic cell lines. A significant amount of HGF is detected in the conditioned medium of a variety of cell lines, including one T, four B, five non-T non-B, eight myeloid, one erythroid and two Epstein-Barr virus-transformed B cell lines. The amount of HGF produced during 72 h of culture by three of the myeloid cell lines, KCL-22 (33.48 ng/ml), KG-1A (26.21 ng/ml), and KG-1 (18.81 ng/ml), is comparable to the amount secreted by human embryonic lung fibroblasts such as MRC-5 and IMR-90. In fact, increased levels of HGF in blood and bone marrow plasma are frequently detected in patients with various types of leukemia\(^6\). Cultured human mesangial cells also secrete a large amount of HGF (23.92 ng/ml during 72 h of culture)\(^7\).

Nucleotide Sequence of 5'-Flanking Region of the HGF Gene

5'-Flanking regions of the rat and mouse HGF genes have recently been analyzed\(^8,9,10\). Several putative regulatory elements are present within 1.3 and 2.8 kbp upstream of the major transcription initiation sites of the rat and mouse HGF genes, respectively. The 5'-flanking region of the rat HGF gene contains two interleukin 6 response elements (IL-6-RE), a nuclear factor-IL-6 (NF-IL6) binding site, two potential interleukin 1 response elements (IL-1-RE), a nuclear factor 1 (NF-1) binding site, a potential glucocorticoid response element (GRE), two potential 12-O-tetradecanoylphorbol 13-acetate (TPA) response elements (TRE), two potential Sp1 binding sites, two tumor growth factor-β (TGF-β)-inhibitory elements (TIE), a potential retinoblastoma protein (Rb) control element (RCE) and a potential binding site for p53. In the 5'-flanking region of the mouse HGF gene, there are four IL-6-REs, two potential binding sites for NF-IL6, a TIE, a potential cAMP response element (CRE), two estrogen response elements (ERE), a potential vitamin D response element (VDRE) which overlaps a chicken ovalbumin upstream promoter (COUP) transcription factor binding element, two liver specific transcription factor (C/EBP) binding sites, a B cell- and macrophage-specific transcriptional factor binding site (Pu.1/ETS), two potential TREs and a potential GRE. Although the actual function of these elements largely remains to be determined, the presence of multiple putative regulatory elements in the HGF gene suggests that HGF gene expression is regulated by various cytokines and steroid hormones.

Positive Regulation of Gene Expression and Production of HGF

cAMP\(^11,12\), IL-6\(^10\), heparin\(^13\), and ascorbate\(^14\) have recently been reported as stimulating factors of HGF production, in addition to protein kinase C (PKC)-activating phorbol esters, IL-1, and tumor necrosis factor-α (TNF-α). Inaba et al.\(^11\) have found that gene expression and secretion of HGF by HL-60 cells is up-regulated by dibutyryl cAMP. We have also reported a marked stimulatory effect of forskolin, choler toxin, prostaglandin E\(_2\) (PGE\(_2\)), 3-isobutyl-1-methoxyxanthine and 8-bromo-cAMP, as well as dibutyryl cAMP on HGF secretion by human skin fibroblasts (Fig. 1)\(^12\). The maximal secretion of HGF by the fibroblasts exposed to forskolin, choler toxin, PGE\(_2\) or 8-bromo-cAMP was about 6, 8, 4 or 9 times that by the untreated cells, respectively. The dose-response curves of induction of HGF secretion by choler toxin and forskolin are nearly parallel with those of the intracellular cAMP levels (Fig. 1). The cellular HGF levels in all of the treated cultures are also greatly elevated. HGF mRNA levels do not significantly increase at 9 h, but increase considerably 15 h or more after the addition of choler toxin, forskolin or 8-bromo-cAMP (Fig. 2)\(^12\). PGE\(_2\) also causes appreciable
Regulation of HGF gene expression

Fig. 1. Effect of cAMP elevation on HGF secretion by human skin fibroblasts. Confluent cells, cultured in 24-well plastic dishes (Nunc), were incubated in MEM supplemented with 10% fetal bovine serum in the absence or presence of the indicated concentrations of cholera toxin (●), forskolin (▲) (A), PGE₂ (▲), 8-bromo-cAMP (●) and dibutyryl cAMP (○) (B) for 72 h. HGF in the conditioned medium was determined by ELISA. The cAMP levels in the cells treated with cholera toxin (©) or forskolin (●) (A) for 24 h were also determined using Yamasa cAMP assay kits. Values are means ± S.D. for triplicate cultures.

Fig. 2. Effects of cholera toxin, forskolin and 8-bromo-cAMP on HGF mRNA levels in human skin fibroblasts. Confluent cells grown in 9-cm dishes (Nunc) were incubated for the indicated periods without (○) or with 1 pM cholera toxin (●), 30 μM forskolin (▲) or 1 mM 8-bromo-cAMP (■). Total RNA was isolated and Northern blotted using ³²P-labeled cDNA probes for human HGF and then for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The amount of the 6.4-kb HGF mRNA was determined by quantifying the intensities of autoradiographic bands using a BAS 2000 Bioimage Analyzer and calculated as fold-induction relative to the control with no incubation, after normalization to the amount of the GAPDH mRNA. The data are expressed as averages of three experiments.
Confluent human skin fibroblasts from four different individuals were incubated with or without cholera toxin (2 pM), forskolin (30 μM) and 8-bromo-cAMP (1 mM) for 120 h. HGF in the conditioned medium was determined by ELISA. Values are means ± S.D. for triplicate cultures.

The agents also enhance highly active HGF secretion by MRC-5 fibroblasts and the human myeloid cell line KG-1 (Gohda et al., unpublished results).

Heparin also stimulates production of the HGF protein by MRC-5 cells, but without any increase in HGF mRNA levels. The maximal stimulation is obtained at 1 μg/ml heparin and stimulation is 4-fold compared to control cultures. The stimulating effect of heparin on HGF production is evident in various types of cells, such as IMR-90 and WI-38 human embryonic lung fibroblasts, human skin fibroblasts, HL-60 cells and human umbilical vein endothelial cells.

Hepatic DNA synthesis has been induced in the normal rats by injection of a mixture of dibutyryl cAMP, theophylline, triiodothyronine, amino acids and heparin. It is of interest that dibutyryl cAMP and heparin have a stimulatory effect on HGF production. However, triiodothyronine does not stimulate HGF production by human skin fibroblasts (Gohda et al., unpublished results).

Ascorbic acid and its stable derivative, ascorbic acid 2-O-α-glucoside induce gene expression and secretion of HGF by human skin fibroblasts. Maximal stimulation is about 50 percent. Both vitamins also enhance HGF secretion induced by IL-1β or phorbol 12-myristate 13-acetate (PMA).

As described above, the IL-6-RE exists in the 5′-flanking region of the rat, mouse and human HGF genes. A reporter plasmid containing the mouse HGF gene promoter is responsive to IL-6 stimulation in stably transfected NIH 3T3 cells, whereas IL-6 does not show any effect on HGF production by MRC-5 cells and HGF gene expression in rat Shay granulocyte sarcoma-derived cells.

Gene expression and production of HGF by MRC-5 and IMR-90 cells increase about 3-fold from 40 to 70 population doubling levels in culture. Both high glucose and hyperosmolality cause a 100-200 % increase in HGF production by the mesangial cells.

### Negative Regulation of Gene Expression and Production of HGF

HGF gene expression and HGF production are down-regulated by 25-hydroxyvitamin D₃ as well as TGF-β and dexamethasone. 1, 25-Dihydroxyvitamin D₃ dose-dependently abates either PMA- or dibutyryl cAMP-stimulated synthesis and release of HGF by HL-60 cells. It is effective at concentrations as low as 0.1 nM. 25-Hydroxyvitamin D₃ and 24, 25-dihydroxyvitamin D₃ also inhibit HGF secretion, but are approximately 100-fold less potent than 1,25-dihydroxyvitamin D₃. The relative potencies of the three vitamin D₃ analogues are in good agreement with their binding affinity for nuclear 1,25-dihydroxyvitamin D₃ receptor reported. It is not known whether 1,25-
dihydroxyvitamin D₃ inhibits HGF production by other leukemic cells and fibroblasts.

Hepatocyte Growth-Stimulating Effect of HGF in vivo

Elevated HGF in plasma and liver after hepatic injury may function in triggering proliferation of the remaining hepatocytes. In fact, injection of HGF to dogs and mice effectively stimulates liver growth. Francavilla et al.¹⁹ have demonstrated that infusion of HGF into the portal vein of dogs after the Eck's fistula increases the labeling index of hepatocytes. When HGF is injected intraperitoneally into mice subjected to 30 % hepatectomy and into mice administered carbon tetrachloride or α-naphthylisothiocyanate, the labeling index of hepatocytes is also markedly increased²⁰. The weight of the remnant liver in mice given HGF after 30 % hepatectomy was significantly greater than that in the untreated control mice²⁰. Repeated injection of HGF to normal and 70 % hepatomized rats also augments liver growth as evaluated by labeling index, mitotic index, liver weight and hepatic DNA content²¹. These findings indicate that HGF stimulates proliferation of hepatocytes in both normal and partially hepatectomized animals.

Conclusions

Mechanism of induction of HGF production is important for understanding the control of liver regeneration. Regulation of gene expression and production of HGF have mainly been examined using human embryonic lung fibroblasts, human skin fibroblasts and human leukemia cells. Several positive and negative regulators are now found. Since few of them are effective on all types of HGF-producing cells, studies using intrahepatic HGF-producers, such as fat-storing cells, sinusoidal endothelial cells and Kupffer cells, will be necessary to identify the factors that regulate hepatic HGF gene expression after liver injury. In vivo studies on the effect of HGF inducers will also be needed.

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References


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