

# HEPATOCTYTE GROWTH FACTOR: ITS ROLE IN HEPATIC GROWTH AND PATHOBIOLOGY

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A decade after its discovery in the context of liver regeneration, hepatocyte growth factor (HGF) is now recognized as a multifunctional cytokine involved in growth regulation and differentiation of multiple tissues. The accumulated literature shows that, in addition to liver, HGF has effects on brain growth and development, lung, kidney, intestine, breast, smooth and skeletal muscle, myocardium, and reproductive and genitourinary tissues. The effects of HGF on cellular populations of the above tissues are mitogenic and motogenic. This chapter focuses on general aspects of HGF and its receptor, and on the role of HGF in liver growth and differentiation. The available evidence indicates that HGF has regulatory control of liver embryonic growth

and development, adult liver regeneration and differentiation of hepatocytes, bile duct epithelium, and sinusoidal endothelial cells. Adult liver regeneration occurs as a result of injury to the liver resulting in acute or chronic loss of hepatic parenchyma (1). Injury to the liver can be caused by surgery (such as partial hepatectomy), by chemical toxicity [such as carbon tetrachloride (CCl<sub>4</sub>) administration], by viral infection (e.g., hepatitis B or C virus), accumulation of toxic metabolites from genetic diseases, autoimmune mechanisms, etc. Any injury resulting in loss of hepatic parenchyma triggers hepatic regeneration. HGF has been shown to have potential impact on regeneration induced by any of the above mechanisms (2) (see Chapter 42 and website chapter  W-31).

Studies on the mechanisms of liver regeneration in the early 1960s demonstrated the presence of humoral factor(s) in the circulating blood as the trigger of the regenerative

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response in hepatocytes (3). Subsequent investigations utilizing primary cultures of rat hepatocytes in chemically defined media supported this observation by showing that sera from hepatectomized animals contained a substance that stimulated hepatocyte replication *in vitro* (4,5). With the revolutionary advances in the techniques for protein purification and microsequencing as well as complementary DNA (cDNA) cloning, a serum protein was purified that was directly mitogenic in primary cultures of hepatocytes (6–9) and its gene was molecularly cloned in the late 1980s. This substance was named originally hepatopoietin A (5) and subsequently hepatocyte growth factor (HGF) (10,11). During the years that led to the discovery of the circulating factor in serum as HGF, other nonhumoral substances were found to induce DNA replication in hepatocytes *in vitro* and *in vivo*. These can be classified as “direct mitogens” and include epidermal growth factor (EGF), transforming growth factor- $\alpha$  (TGF- $\alpha$ ), and acidic fibroblast growth factor (aFGF) (12–15). In addition, since the discovery of HGF, many other factors have been found to be critical in liver regeneration and to increase in plasma after partial hepatectomy. These substances include tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (17–19), interleukin-6 (IL-6) (16), and norepinephrine (20). Since these substances are not direct mitogens for hepatocytes, they could not have been detected by the assay that led to the discovery of HGF, as that assay was geared toward isolation of direct mitogens. The simultaneous elevation in the plasma, following partial hepatectomy, of many substances (TNF- $\alpha$ , IL-6, norepinephrine) having regulatory effects on liver regeneration and hepatocyte growth suggests that the plasma circulating regenerative stimulus described above is more complex than originally thought and probably includes other substances besides the mitogen HGF. These substances, even though not directly mitogenic in culture, may have stimulatory effects *in vivo* that would not have been detected by the cell culture bioassay.

## GENERAL ASPECTS OF HEPATOCYTE GROWTH FACTOR STRUCTURE AND FUNCTION

### Biochemical and Structural Properties of Hepatocyte Growth Factor Protein

HGF purified from rabbit serum, rat platelets, or human plasma is a heparin-binding heterodimeric glycoprotein consisting of a heavy chain ( $\alpha$ ) and a light chain ( $\beta$ ) with molecular masses of 58,000 to 69,000 and 30,000 to 34,000, respectively (6–8) as determined by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). HGF isolated from the conditioned medium of a human embryonic lung fibroblast cell line or from human placenta exists predominantly as an unprocessed single-chain pro-HGF polypeptide with a molecular mass of 87,000 to

92,000 (21,22). Analysis of the cDNA nucleotide sequence of HGF revealed that the two polypeptide chains of HGF are encoded in a single open reading frame to yield the pre-pro-HGF molecule coding for 728 amino acids (10,11). The signal peptide of 31 amino acids at the amino terminus of pre-pro-HGF is removed in the endoplasmic reticulum to yield the pro-HGF precursor (11).

Comparison of the amino acid sequence of HGF to those of other known proteins showed homology to factors involved in the blood coagulation cascade or in fibrinolysis. Several of these factors contain varying numbers of polypeptide structures known as kringles, which are composed of a stretch of approximately 90 amino acids forming a double-looped structure held together by three disulfide bonds. For example, factor XII and urokinase-type plasminogen activator (u-PA) each contain one kringle domain; prothrombin and tissue-type plasminogen activator (t-PA) each contain two kringle domains, while plasminogen contains five (11,23). Although each kringle generally has a unique amino acid sequence, a short seven amino acid sequence (Asp-Tyr-Cys-Arg-Asp-Pro-Asp) is common in most kringle domains at a specific location. By comparing the amino acid sequences of HGF and these other kringle-containing proteins, the highest degree of homology (39%) was found to exist between HGF and plasminogen (11). The  $\alpha$  chain of HGF contains four kringle domains with substantial amino acid sequence similarity to the kringles 1, 4, and 5 of plasminogen. The  $\beta$  chain of HGF is also unusual in that it possesses homology to the serine protease domains present in several of the enzymes mentioned above. HGF does not, however, have any known enzymatic (proteolytic) activity likely due to substitution of two out of three amino acids required in the catalytic triad (serine by tyrosine, and histidine by glutamine).

### Pathways of Activation of Hepatocyte Growth Factor

HGF is synthesized as a precursor (pro-HGF) molecule, and this is the predominant form by which HGF is present in the matrix of most tissues (21,22). Conversion of the single chain pro-HGF to the heterodimeric form is an essential step for HGF function. *In vitro* mutagenesis studies in which an amino acid at the cleavage site between the  $\alpha$  and  $\beta$  chains was altered by introducing a single nucleotide substitution in the coding region of the HGF cDNA at this region clearly have demonstrated that pro-HGF binds to the HGF receptor but does not elicit a mitogenic stimulus (24). This argues for a mechanism of HGF activation whereby HGF is cleaved by proteolysis to the mature heterodimeric form (see below). As predicted from the deduced amino acid sequence of pro-HGF as well as by direct N-terminal amino acid sequencing of the  $\beta$  chain of HGF, the cleavage site is at Arg494-Val495, which, when

cleaved, generates the  $\alpha$  and  $\beta$  chains of mature active heterodimeric HGF.

The first described pathway involved in HGF activation involves u-PA. This enzyme is known for its capacity to convert inactive plasminogen into its active form, plasmin. The cleavage site employed for conversion of plasminogen to plasmin has the same sequence (Arg-Val-Val) as that employed for activation of HGF. Incubation of pro-HGF with purified u-PA resulted in HGF activation (25,26). In addition, activation of pro-HGF by tissue homogenates from regenerating liver was substantially inhibited by addition of anti-u-PA antibodies (27). The precise stoichiometry of the reaction is not clear. Studies by Comoglio's group (28) suggest that u-PA and HGF may be forming a one-to-one complex with each other of relatively longer life than commonly seen in enzymatic reactions. On the other hand, this may reflect the artificiality of *in vitro* reactions utilizing u-PA. Results of the interaction of purified u-PA with its substrates in pure reaction are difficult to extrapolate to the true *in vivo* situation, in which u-PA carries out its enzymatic function bound to its cellular receptor. Typically, u-PA itself exists in two forms—single-chain u-PA (scu-PA) and two-chain u-PA (tcu-PA)—each of which possesses activity. Either of them has their activity dramatically enhanced by binding to a specific glycoprotein known as the u-PA receptor (u-PA-R). The latter is a glycosylphosphatidyl inositol (GPI) linked glycoprotein with no apparent function other than that of binding u-PA and enhancing its activity, though some studies have suggested that u-PA-R has signaling capabilities by virtue of its lateral association with other membrane bound signaling molecules, such as integrins and caveolin (29–32). u-PA-R is concentrated in specific locations on plasma membrane or in submembrane vesicles known as caveolae. Recent studies have also demonstrated association or close proximity of the HGF receptor with either u-PA or u-PA-R on the plasma membrane of hepatocytes during liver regeneration (33). Hepatocytes in culture do produce u-PA and activate pro-HGF (34). Significantly, antibodies against the u-PA-R diminish this effect. HGF itself has been shown to increase urokinase gene expression in cultures of epithelial cells (35,36).

Another pathway involved in activation of HGF is that involving a protein with substantial homology to coagulation factor XII (37). This protein was named HGF activator (HGFA) but subsequent studies demonstrated that factor XII itself can also activate HGF to a certain extent (37). Relatively specific inhibitors of HGFA were also recently described, themselves subject to proteolytic activation, suggesting that a complex regulatory pathway employing HGFA may be involved (38). HGFA is produced by hepatocytes and other cell types and appears to function as a soluble protein in tissue fluids (39). Expression of HGFA mRNA in hepatocytes increases following liver or kidney injury (39). Other studies have shown a

coordinated regulation of HGF, HGFA and *c-met* in fetal intestinal mucosa (40).

### Structure–Function Analysis of the Hepatocyte Growth Factor Molecule

Detailed site-directed mutagenesis studies have been carried out by several laboratories to determine the role of each kringle domain in the HGF molecule. At the N-terminus of pro-HGF, deletion of the amino acids forming a hairpin loop (before kringle one) abrogates binding of HGF to its receptor, and thus abolishes its biologic activity. A novel fold seen at the amino-terminal domain and prior to kringle one also confers binding of HGF to heparin (41). Removal of kringles one or two also has a similar effect, indicating that they too are required for interaction of HGF with its cell surface receptor. Deletion of kringle three or four, although substantially reducing HGF bioactivity, did not totally diminish it. As expected, when the entire  $\alpha$  chain of HGF was deleted, complete loss of bioactivity occurred due to the inability of HGF to associate with its receptor. On the other hand, eliminating the entire  $\beta$  chain of HGF did not affect the association of HGF with its receptor, but it did completely block mitogenic stimulation of hepatocytes (24,42,43). This has led some to speculate that the  $\beta$  chain of HGF is responsible for activating the HGF receptor. However, at the present time, a controversy regarding the role of the  $\beta$  chain of HGF in receptor activation exists. For example, some studies suggest that deletion of the  $\beta$  chain of HGF, although abolishing its mitogenic activity, has no effect on HGF-induced motogenicity (44). The proteins known as HGF/NK1 and HGF/NK2 are derived from splicing variation of the full-length HGF messenger RNA (mRNA) and retain the amino-terminal hairpin loop as well as the first kringle (NK1) or the first two kringles (NK2). Several reports have described agonistic effects of these molecules on the HGF receptor (45–49). Transgenic mice overexpressing the HGF splicing variants NK1 and NK2 (see below) have some properties of the phenotype seen with complete HGF transgenics (45). Cooperative effects between the  $\alpha$  chain and the  $\beta$  chain added as separated proteins on stimulation of the HGF receptor have also been described (50). The preponderance of evidence suggests that even though some residual functions of HGF may be expressed in the absence of the  $\beta$  chain, the complete biologic effects of HGF are only seen with molecular forms in which the  $\beta$  chain is preserved.

### Hepatocyte Growth Factor Gene Organization

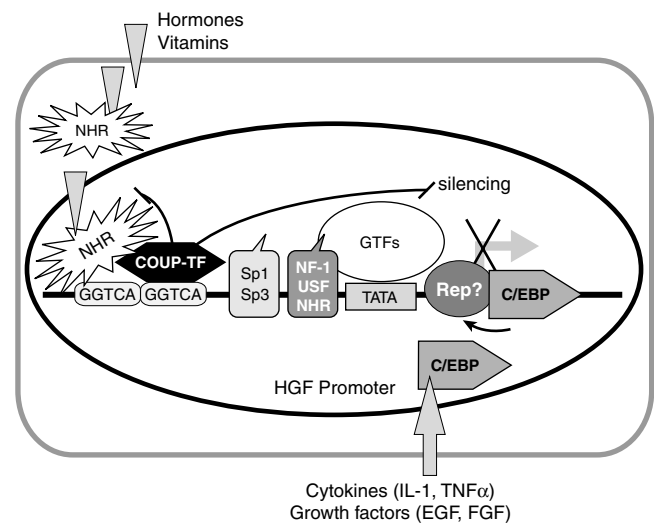
Southern blot analysis has revealed the presence of a single copy gene for human HGF, which is located on the long arm of chromosome 7 at q21.1 (51,52). [This region of

chromosome 7 (7q21-31) harbors the gene for the HGF receptor as well (53).] The structural organization of the human HGF gene has been determined by several overlapping clones isolated from lambda phage genomic libraries and is estimated to span approximately 70 kilobases (kb) of genomic DNA comprising 18 exons interrupted by 17 introns. The first exon contains the 5'-untranslated region of the mRNA and codes for the signal peptide (54,55). The  $\alpha$  chain, which consists of four kringle domains, is encoded by the next ten exons. Each kringle domain of the  $\alpha$  chain is encoded by two exons, in similarity to the genes encoding other kringle-containing proteins. The spacer region between the  $\alpha$  and  $\beta$  chains is found in the 12th exon, while the remaining six exons code for the serine protease-like domain of the  $\beta$  chain. The transcription initiation site for the HGF gene was determined to be at 76 base pairs (bp) upstream of the translation start codon.

### Hepatocyte Growth Factor Gene and Its Transcriptional Control

HGF gene encompasses approximately 70 kb of genomic DNA and contains 18 exons interrupted by 17 introns, and is under tight transcriptional regulation (56). Understanding the molecular mechanisms governing the transcriptional control of this gene is an important area of investigation for several reasons. First, under normal conditions the HGF gene is permanently silenced in most epithelial cells such as hepatocytes and bile duct epithelial cells in the liver, while it is transcribed in the stromal compartment of the liver such as in Ito cells (or stellate cells) and endothelial cells. HGF expression in the mesenchymal cells is inducible by extracellular cues such as hormones and cytokines, and regulation is mainly at the transcriptional level. Second, HGF is aberrantly expressed in some epithelial tumors and in other pathologic conditions such as cirrhosis of the liver (56). Therefore, functional studies on the HGF gene promoter have been carried out to identify the *cis*-acting DNA elements and their cognate transcription factors using *in vitro* and *in vivo* analyses of the transgenic mouse models. The *in vivo* studies have revealed that the proximal promoter (~100 bp, which has a TATA-like box at -30 bp) and its upstream 5'-flanking sequences (at least 600 bp) are absolutely essential for the basal and inducible expression of the HGF gene (57). Moreover, the *in vivo* studies also show that the HGF promoter and its upstream region contain the necessary elements to dictate the cell-type-specific pattern of HGF gene expression (57). Fine mapping of the promoter region using *in vitro* and *in vivo* DNase I footprinting, gel shift, and functional assays has identified several important regulatory elements. A *cis*-acting element at position -16 to +11 bp in the mouse HGF promoter bp was identified as a novel regulatory site through which members of the CCAAT enhancer binding protein (C/EBP) family of transcription factors, especially C/EBP $\beta$  (also known as

NF-IL-6), bind and confer responsiveness to serum, TNF- $\alpha$ , IL-1, IL-6, and EGF (58). Cotransfection studies using expression vectors encoding any of the three isoforms of the C/EBP transcription factor ( $\alpha$ ,  $\beta$ , and  $\delta$ ) resulted in a marked induction of HGF promoter activity (58). This C/EBP site (TTGCAA) is located in the core promoter and overlaps a unique palindrome site (ACCGGT) to which a repressor factor (yet to be identified) binds and represses the HGF gene promoter (59). Another *cis*-acting element in the upstream region of the HGF promoter at position -872 to -860 bp was characterized as an estrogen responsive element (ERE) (an RGGTCA, IR3) to which the nuclear orphan receptor chicken ovalbumin upstream promoter transcription factor (COUP-TF) binds avidly. Estrogen receptor (ER) can compete with COUP-TF on the HGF promoter through binding to the same imperfect ERE element (60). Further characterization of the HGF promoter identified an enhancer element as an Sp1 site (-328 to -297 bp), which has a CTCCC motif to which Sp1 and Sp3 bind and activate the HGF promoter (61). Additional studies on the HGF promoter have uncovered a composite multifunctional regulatory element at position -260 to -230 bp from the transcription start site to which members of the nuclear factor 1 (NF1) and upstream stimulatory factor (USF) bind to the HGF promoter and regulate its transcription. Gel mobility shift and electrophoretic mobility shift assays as well as mutational analyses revealed that the binding sites of



**FIGURE 43.1.** Schematic representation of the hepatocyte growth factor (*HGF*) gene promoter region. The diagram depicts the various *cis* elements and their cognate transcription factors that have been functionally implicated in the regulation of HGF gene transcription. *C/EBP*, CCAAT enhancer-binding protein; *COUP-TF*, chicken ovalbumin upstream transcription factor; *EGF*, epidermal growth factor; *FGF*, fibroblast growth factor; *GTF*, general transcription factors; *IL-1*, interleukin 1; *NHR*, nuclear hormone receptor; *NF-1*, nuclear factor-1; *Rep?*, an unknown repressor; *TNF $\alpha$* , tumor necrosis factor  $\alpha$ ; *USF*, upstream stimulatory factor.

the two different transcription factor families overlap one another and that NF1 suppresses HGF gene promoter activity, while USF has an activating function. Interestingly, during activation of the HGF gene in liver regeneration after partial hepatectomy, it was noted that the binding activity of USF to the HGF promoter element increases, while that of the NF1 decreases. Other studies have shown that the wild-type but not the mutant p53 binds to and activates the HGF promoter (62). Interestingly, the HGFR gene promoter is also upregulated by the wild-type but not the mutant form of p53 (63), which may indicate a common pathway for concerted regulation of the receptor and its ligand expression (see Transitional Regulation of the Hepatocyte Growth Factor Receptor (*c-met*) Gene, below). Studies on the HGF promoter point to a complex pattern of gene regulation involving multiple positive and negative elements through which extracellular and intracellular cues can exert their modulatory functions. A diagram of the HGF promoter and its functional elements and their cognate regulatory factors are shown in Fig. 43.1.

### Hepatocyte Growth Factor Gene Expression and Generation of Splicing Variants

The HGF mRNA and protein have been detected in a variety of adult and embryonic tissues (64–66). Among these are blood, brain, liver, lung, kidney, placenta, and spleen. In general, HGF mRNA is expressed in nonepithelial cells such as fibroblasts in connective tissues, Ito cells and Kupfer cells of the liver, macrophages and endothelial cells in the lung, mesangial cells in the kidney, and leukocytes and megakaryocytes (67–70). The major transcript for human and rat HGF mRNA extracted from placenta, liver, or MRC-5 cells has been reported as 6 kb in size (10,11), which encodes 134 nucleotides of the 5'-untranslated region, 2,184 nucleotides of the HGF coding region, and 3.6 kb of the 3'-untranslated region (10). In addition to the 6-kb transcript, a truncated variant of the HGF transcript (1.5 kb) was detected in placenta that codes for the N-terminal portion of HGF, including either the first kringle or the first two kringles with a putative molecular weight of about 30,000 (71). The proteins encoded by these altered transcripts have been called NK1 and NK2, correspondingly. It was determined that these shorter HGF transcripts are generated by an alternative splicing event. Analysis of conditioned medium from MRC-5 cells as well as cloning and sequencing the truncated cDNA prepared from these cells has revealed that the truncated HGF protein forms exist naturally (72,73). NK2 is unable to stimulate DNA synthesis in target cells normally activated by full-length HGF. However, it competed efficiently with full-length HGF for binding to the HGF receptor (72). The significance of the presence of these abbreviated HGF forms is not clear. Transgenic mice for NK1 exhibit the full biologic

spectrum of the complete HGF transgenics (45). Weaker biologic effects are seen with NK2 transgenics. These effects include enhancement of metastasis in induced melanoma tumors (45). On the other hand, combined bitransgenic mice for both HGF and NK2 have diminished effects compared to the HGF transgenics, suggesting a true *in vivo* role of competition between HGF and the NK2 variant (45). Weaker effects of NK2 as compared to NK1 have also been described in cell cultures (47). Heparin induces dimerization of either NK1 or NK2 and dramatically enhances their biologic effects (49).

Another naturally occurring variant of HGF also exists in relatively high abundance. This variant was isolated from cDNA libraries prepared from MRC-5 cells (21), human leukocyte (74), and human placenta, and results from alternative splicing. This deleted form of HGF differs from full-length HGF by lacking five amino acids (Phe-Lys-Pro-Ser-Ser) in the first kringle domain (21). It is biologically more potent than the undeleted form (43).

Factors that modulate HGF gene expression have been studied *in vitro* utilizing either embryonic lung fibroblast cells or human foreskin fibroblasts, which normally produce relatively substantial amounts of HGF mRNA and protein. Agents that so far are known to upregulate HGF mRNA expression in this system include the cytokine IL-1, and the phorbol ester TPA (tetradecanoylphorbol acetate), a skin tumor promoter (75,76). Factors that negatively modulate HGF gene expression in the cultured fibroblasts include TGF- $\beta$ 1 and the glucocorticoid analogue dexamethasone (77,78). *In vivo*, HGF mRNA expression has been induced experimentally in damaged tissues as well as in distal organs by a variety of injuries such as partial hepatectomy, CCl<sub>4</sub> treatment, nephrectomy, ischemia, or pneumonectomy (79–81). What triggers HGF mRNA expression after such treatments is not clear, but it appears that IL-1 may play an important role in this process. Norepinephrine, a substance that exerts regulatory effects on both HGF and EGF receptors, also increases HGF in embryonic human lung fibroblasts (82,83). This effect of norepinephrine, however, appears to be mediated through the  $\beta$ -adrenergic receptor. It is also not clear whether the increase in HGF mRNA expression in various damaged tissues is controlled at the transcriptional level or regulated posttranscriptionally. For regulation of HGF gene expression, see Hepatocyte Growth Factor Gene and its Transcriptional Control, above.

## THE HEPATOCYTE GROWTH FACTOR RECEPTOR

### General Aspects of *c-met* Structure and Function

The receptor for HGF has been identified as *c-met*, a proto-oncogene product that is a transmembrane protein with tyro-

sine kinase activity in its cytoplasmic portion. Ironically, an oncogene was cloned and sequenced before HGF itself had been cloned and was initially discovered based on its ability to transform normal fibroblast cell lines if introduced to these cells. This transforming oncogene was isolated from the genomic DNA prepared from a human osteosarcoma cell line (HOS) that had been treated with the carcinogen (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, MMNG). Genomic DNA isolated from this treated cell line caused normal, non-tumorigenic NIH-3T3 fibroblasts to become transformed and produce tumors. The activated oncogene responsible for this transformation was named the *Met* oncogene (84,85). It was shown that the activation of the oncogene involved a chromosomal rearrangement that linked a sequence known as the translocated promoter region (tpr) on chromosome 1 to the C-terminal portion of the *Met* gene located on chromosome 7 (53). This translocation resulted in a truncated receptor (65 kd, also called p65tpr-Met) having a constitutively active tyrosine kinase. The cellular counterpart of the activated *Met* oncogene was later isolated, and its cDNA was cloned and sequenced from human and mouse cDNA libraries (86,87). The deduced amino acid sequence of this cDNA revealed structures characteristic of polypeptide growth factor receptors consisting of an extracellular ligand-binding domain rich in cysteine, a transmembrane domain, and an intracellular C-terminal domain with a protein tyrosine kinase (88). The normal counterpart of the activated *Met* oncogene is called the *c-met* proto-oncogene and is now known to be expressed in most epithelial cells, some mesenchymal cells, central nervous system neurons, and muscle cells (89).

An exhausting search for the ligand of this putative growth factor receptor was conducted using every purified and well-characterized polypeptide growth factor available; however, all failed to stimulate the tyrosine kinase activity of *c-met*. In the late 1980s and early 1990s when information on the biochemical characterization of the HGF protein and its broad spectrum of target cells became available, it prompted investigators to test whether HGF is the ligand for *c-met*, and in fact HGF activated the tyrosine kinase of *c-met* (90,91).

The mature *c-met* receptor is a heterodimer held together by disulfide bonds and consists of a large polypeptide chain with a molecular mass of 145,000 d containing the transmembrane domain and the intracellular tyrosine kinase domain (called the  $\beta$  chain), and an  $\alpha$  chain that is approximately 45,000 d (92). Both polypeptide chains of *c-met* are derived from a single chain precursor posttranslationally modified by proteolytic cleavage at a potential cleavage site (Lys303-Arg-Lys-Lys-Arg-Ser308). Similar cleavage sites are also present in the insulin and insulin-like growth factor I receptors. Site-directed mutational analysis of the cleavage site in *c-met* utilizing a hybrid soluble form of *c-met* in which the extracellular domain of the receptor was fused to the constant region of immunoglobulin G

(IgG) heavy chain has shown that substitution of Arg and Lys with Ala totally abolishes the conversion of the *c-met* precursor to a heterodimer (93). Single amino acid substitutions with Ala at positions 304, 306, or 307 also had similar effects. The uncleavable *c-met* binds to mature heterodimeric HGF and to single chain pro-HGF (93). In these studies, however, the effects of modification of the proteolytic cleavage site on the tyrosine kinase activity of *c-met* receptor could not be examined, and it remains to be clarified whether proteolytic processing to the heterodimeric form is required for *c-met* activation and signal transduction. Studies on a human colon carcinoma cell line have shown that the *c-met* precursor was not cleaved to a heterodimer despite its conserved cleavage site (94). This single chain *c-met* retained its tyrosine kinase activity and was constitutively autophosphorylated *in vivo*. Interestingly, the precursor for the insulin receptor was also not processed in the same cell line, suggesting that a common defect may exist in the secretory pathway of this cell line. It is not clear whether a lack of *c-met* processing contributed to the transformation of these cells or whether altered processing merely resulted from other unrelated cellular defects in the cells. C-terminally truncated versions of *c-met* (both membrane bound and soluble forms) that lack the tyrosine kinase domain have also been detected in the culture supernatants from several carcinoma cell lines, but it is unclear whether these forms of *c-met* actually have a physiologic role. It has been postulated that they may interfere with receptor signal transduction by directly competing with the full-length receptor for binding to HGF (95).

### Transcriptional Regulation of the Hepatocyte Growth Factor Receptor (*c-met*) Gene

In contrast to the HGF gene, the HGFR gene is transcribed in both epithelial and mesenchymal cells; however, similar to the HGF gene, the expression of the HGFR gene is inducible and tightly regulated through transcription mechanisms. The HGFR spans approximately 120 kb in size and consists of 21 exons interrupted by 20 introns (96). The HGFR promoter region lacks a TATA box but is guanosine-cytosine (GC) rich and contains several functional SP sites to which SP1 and SP3 proteins bind (97,98). Similar to the HGF gene, the HGFR gene is strongly induced by various extracellular stimuli such as TPA, IL-1, IL-6, TNF- $\alpha$ , EGF, and HGF (99,100).

In the murine HGFR promoter region, potential regulatory elements such as AP-1, Myb, Myc, IL-6-RE, WT1/EGR1 C/EBP, p53, NF $\kappa$ B, HNF3, ERE, AhRARNT, MyoD, ETS, GATA, and several others exist (97). The functionality of some of these sites and their cognate transcription factors have been confirmed. p53, for example, was shown to induce the HGFR promoter in response to ultraviolet (UV) light irradiation and DNA

damage (63). AP-1 was found to mediate the stimulatory effects of HGF on the HGFR promoter in hepatic cells (98). Ets (101) and Pax3 (102) have also been shown to functionally upregulate the HGFR gene promoter. The functional role of other sites/factors awaits future analysis.

It appears that the promoters of the HGF and HGFR genes are modulated by a complex and combinatorial effect of various genetic and perhaps epigenetic (i.e., DNA methylation) phenomena. Although these two promoters are different, they share some common pathways for regulation at the extracellular levels (i.e., both genes are inducible by cytokines such as IL-1 and TNF- $\alpha$ ) and both promoters have common functional *cis*-acting elements (such as p53, C/EBP, Sp1, and AP-1).

### Hepatocyte Growth Factor Receptor and Neoplasia

The gene encoding the *c-met* proto-oncogene has been estimated to be at least 100 kb in size. Northern blot analysis of RNA prepared from normal human, mouse, and rat tissues has shown that *c-met* mRNA and protein are expressed at relatively low levels in a variety of tissues such as breast, liver, lung, kidney, large and small intestine, placenta, skin, stomach, and thyroid as well as in most human carcinoma cell lines (103). These studies have shown that many cell lines and tissues contain a single 9-kb transcript (104); although some tumor cell lines such as a human gastric carcinoma cell line, in which the gene for *c-met* is amplified and overexpressed, contain *c-met* mRNA species with sizes of 9, 8, 7, 5, and 3.4 kb (104). Transfection studies of NIH-3T3 fibroblasts (which normally do not express *c-met* but do express HGF) with a cDNA encoding normal *c-met* resulted in an autocrine loop that led to the transformation of these cells and tumor formation when injected to nude mice (105). Investigation of tissues from human colorectal adenoma and carcinoma primary tumors recently revealed that the *c-met* proto-oncogene is significantly overexpressed in 70% of these tumors as compared to the adjacent normal mucosa (106). A major transcript of 8 kb and a minor transcript of 5 kb were detected in most of these tumors. Amplification of the *c-met* proto-oncogene has also been reported for gastric carcinoma cell lines and for gastric carcinoma tissues, indicating that *c-met* gene amplification may contribute to neoplasia (107). Overexpression of both *c-Neu* and *c-met* has been observed in cholangiocarcinogenesis. Increased expression of both mRNA and protein was seen for these genes in the metaplastic intestinal epithelium, which appears as a precancerous lesion in relation to this process (108). As mentioned, the activated oncogene *tpo-Met* results from gene rearrangement. The size of the transcript encoding the rearranged Met product in MMNG-treated HOS cells is reported as 5 kb. Utilizing a very sensitive reverse-transcriptase polymerase chain reaction (RT-PCR) assay, the expression of the rearranged mRNA

for *tpo-Met* was detected at very low levels in several human carcinoma cell lines derived from pancreas, colon, bladder, and stomach, as well as in the biopsy samples of human gastric mucosa showing cancer or precursor lesions (109). Recently, mutations in the kinase domain of *c-met* were described in familial forms of renal neoplasia (papillary tumors). The mutation pattern in members of the same family strongly suggested that the mutations were associated with the pathogenesis of the neoplasms (110).

Several studies suggest that *c-met* is contributing to the phenotype or to the genesis of hepatocellular carcinomas. Mutations in the kinase domain of *c-met* occur in childhood hepatoblastomas, whereas such mutations were not present in hepatocellular carcinomas of adults. The mutations may be the cause for the appearance of these tumors early in childhood in hepatitis B virus (HBV) carriers (111). Other studies have shown overexpression of *c-met* in variable percentages of hepatocellular cancer (112–116). These findings are not unique to liver cancers and suggest that overexpression of *c-met*, though perhaps contributory to the phenotype, is not a stable feature of the pathogenesis of hepatocellular cancer in adults.

It should be noted that the role of HGF and its receptor in pathogenesis of liver tumors often appears contradictory. There is no evidence from the existing studies in the literature that HGF per se is expressed in hepatocellular carcinomas. On the other hand, hepatocellular carcinomas were seen in transgenic mice in which HGF was overexpressed in hepatocytes. One study demonstrated severe cytologic abnormalities preceding development of hepatocellular carcinomas in mice in which mouse HGF was expressed under the influence of the metallothionein promoter (117). In another study, overexpression of human HGF under the influence of the albumin promoter also enhanced the appearance of hepatocellular carcinomas in mice treated with diethylnitrosamine. The increase in liver neoplasia was more dramatic in the female mice (118). In contrast to these observations, mice bitransgenic for both *c-myc* and HGF under the influence of the albumin promoter showed a decrease in neoplasia when compared to the *c-myc* transgenic mice (119). The effect was associated with enhanced apoptosis in mice in which hepatocytes were expressing HGF. Studies with hepatocellular carcinoma cell lines showed that HGF often fails to stimulate DNA synthesis, and in many instances it may actually cause a decrease in cell proliferation (120,121), and other studies related this effect to prolonged and sustained induction of p21 (122). Injection of HGF in rats bearing preexisting tumors induced by diethylnitrosamine resulted in suppression of DNA synthesis in more than 80% of the early neoplasms, whereas an increase in DNA synthesis was noted in about 5% of the tumors (123). The latter studies suggest that the effect of exogenous HGF and its receptor on existing liver neoplasms may often result in suppression of proliferation of the neoplastic cells. Clearly the effects of stimulation of

HGF receptor are not uniform in liver tumors and may be more suppressive than stimulatory when HGF is administered as an exogenous agent. These pathways are obviously different from when HGF is expressed as an internal factor, as with transgenic mice in which HGF is expressed in hepatocytes. It should be noted that HGF transgenic mice have a much smaller tumor burden and overall much less cytologic effects compared to similar transgenic mice with TGF- $\alpha$  (124). The mixed stimulatory and inhibitory effects of HGF depending on its mode of administration are also compatible with the original isolation of HGF as tumor cytotoxic factor based on its cytotoxic effects on sarcomas and other neoplastic cells (125,126).

### **Hepatocyte Growth Factor Receptor Activation and Its Substrates**

Binding of the active heterodimeric form of HGF to the Met protein causes dimerization of the receptors and activation of the tyrosine kinase catalytic site. Sites of the HGF molecule responsible for the binding to the receptor are discussed above. The cytoplasmic tail of the receptor is composed of a multifunctional docking site made of the tandemly arranged degenerate sequence Y1349VHVXXXY1356VNV. Two adjacent tyrosine residues (Y1234 and Y1235) are also essential for the activation of the catalytic site of the receptor (127). Unique to the Met receptor (and its homologues c-Ron and c-Sea) is that there is only one docking site for the interaction with downstream substrates (128). As is typical for tyrosine kinase receptors, activation of the catalytic site is associated with docking of many proteins with SH2 and SH3 homology domains. Such signal transducers include phosphatidylinositol-3 (PI3) kinase, phospholipase C $\gamma$ , pp60c-src, and the GRB-2-Sos complex (129). Activation of the HGF receptor also induces activation of STAT3 (signal transducer and activator of transcription), though not as rapidly as IL-6 or EGF (130,131). Following activation and downstream signaling events, the Met receptor protein is subject to polyubiquitination and degradation in proteasomes (132). Studies have shown that a major direct target of the Met receptor is the adaptor molecule Gab-1, a docking protein with functional similarity to the IRS1 substrate of the insulin receptor (133,134). Gab-1 is also a target of phosphorylation by the EGF receptor (135). The precise interaction of Gab-1 with the Met receptor has not been fully characterized, and it appears that Grb2 is involved (134). It should be noted that while there are many distinct differences in the effects of HGF and those of other receptor tyrosine kinase ligands, such as EGF and FGF1, there is no obvious unique signaling pathway associated with the HGF receptor that is not seen with the other receptor tyrosine kinases. Any differences described are incremental and quantitative rather than qualitative. This reflects our lack of full understanding of the signaling pathways of receptor tyrosine kinases in general. A good example of this issue are the different effects induced in cells bearing

the EGF receptor by the EGF-R ligands EGF and TGF- $\alpha$ . Use of site-directed mutagenesis and transgenic models has provided some understanding of the contributions of the different signaling pathways to the end results of HGF action. As mentioned above, the two tyrosines (Y1349 and Y1356) in the cytoplasmic tail are essential for the function of the receptor. Mutation of the two tyrosines results in loss of biologic function, as shown by loss of the transforming activity in susceptible cell lines (134). In whole animals, mutation of both tyrosine residues of the catalytic site in the mouse genome resulted in embryonic death, with placenta, liver, and limb muscle defects, mimicking the phenotype of met null mutant mice (see below). In contrast, disrupting the consensus site for Grb2 binding allowed development to proceed to term without affecting placenta and liver. There was, however, a striking reduction in limb muscle and a generalized deficit of secondary fibers (136). These data show that the different components of the Met signaling pathway are differentially crucial for different end points of the action of HGF, with late myogenesis being more critical to the association of Grb2 binding than other phenomena. Activation of *c-met* receptor may also be affected by lateral effects mediated by other adjacent tyrosine kinases, at least in neoplastic cells. Overexpression of TGF- $\alpha$  leads to tyrosine phosphorylation of the HGF receptor in several cell lines (137). The effect is blocked by antibodies to TGF- $\alpha$  or the EGF receptor. The mechanisms for the phenomenon are not clear, and it has not been shown to occur in normal epithelial cells; thus, it may be related to changes associated with the neoplastic phenotype (138).

### **GENERAL BIOLOGIC ASPECTS OF HEPATOCYTE GROWTH FACTOR**

#### **Hepatocyte Growth Factor Uptake and Clearance *In Vivo***

Following injection into the penile vein, HGF disappears from the blood in a biphasic fashion consisting of a rapid phase (half-life of 4 minutes) followed by a slow phase where high levels of HGF remain in the peripheral blood (terminal half-life of 85 minutes). The major organ for HGF uptake in the rat is liver and, to a lesser extent, the kidney (139,140). In contrast to the large amount of <sup>125</sup>I-HGF in the peripheral blood after systemic injection, administration of radiolabeled HGF into the portal vein resulted in the appearance of much less radioactivity in the peripheral blood (more than 70% of the injected HGF did not leave the liver), implicating liver as a major site of HGF uptake. This is further supported by the drastic reduction in plasma clearance of HGF when it was injected into rats after treatment with carbon tetrachloride (141). A small portion of HGF (less than 5%) was detected in the bile soon after HGF administration, indicating that HGF either remained in the liver or more likely reentered the blood compartment (139). When HGF sequestration is expressed

per gram of tissue, spleen and adrenal glands were also efficient in taking up HGF (139,140). Clearance of HGF complexed to heparin is much slower than that of the native protein alone (142,143). Whether the uptake of HGF is mediated through *c-met* or through other binding sites such as mannose or galactose receptors, asialoglycoprotein receptors, or other cell-associated heparin-like molecules is unclear. Studies by Liu et al. have shown that hepatic uptake of HGF was reduced in the presence of excess unlabeled ligand, indicating a saturation of binding/removal phenomenon (184). Receptor-mediated HGF internalization in perfused rat liver was shown to be only partially inhibited by agents known to interfere with receptor-mediated peptide uptake, suggesting that internalization of HGF may occur by both receptor- and nonreceptor- (low-affinity sites) mediated mechanisms (140). *In vitro* studies using isolated rat hepatocytes as well as *in vivo* experiments with rats have revealed the presence of two binding sites for HGF: one sensitive to the presence of excess heparin and one resistant, probably representing the HGF receptor (144). In addition, other studies have shown that a relatively large amount of HGF in the rat liver is sequestered in the extracellular matrix in the subendothelial space, which could be eluted by *in situ* perfusion using 1 M NaCl (145). The cell types that are responsible for HGF uptake in the liver or other organs, however, remain to be determined. Since the liver is the major site for HGF uptake, it is possible that a disturbance in HGF clearance from the circulation by liver or kidney may account for high levels of HGF noted in various liver diseases such as fulminant hepatic failure and chronic renal failure (see below). In addition to heparinoids and glycosaminoglycans, recent studies have shown a remarkable affinity of HGF for binding to thrombospondin and different types of collagen forms, including collagen IV (146).

### Biologic Effects on Cellular Targets

There are currently more than 13,000 publications in the Medline stored literature related to effects of HGF on different cellular types. Targets of the biologic effects of HGF include all normal epithelial cells, many neoplastic cell lines, skeletal, and smooth muscle, and several neuronal cell types. The effects of HGF on epithelial cells include stimulation of duct formation *in vivo* and *in vitro* and support of branching morphogenesis. In general, in addition to its mitogenic effects, HGF is also a strong motogen. HGF was independently purified and characterized from the culture medium of MRC-5 human embryonic lung fibroblasts, based on its ability to induce motility and migration of a variety of normal epithelial and carcinoma cells and was named scatter factor (SF) by Stoker et al. in the 1980s (147–150). After the amino acid sequence of SF became available from its cDNA nucleotide sequence, it was noted that these two activities (mitogenesis of HGF and motoge-

nesis of SF) are caused by the same molecular entity. In general, HGF is produced by mesenchymal and stromal cells, and it stimulates the growth and/or motility of a variety of epithelial cells in a paracrine and possibly an endocrine fashion. HGF was found to have strong trophic effects for motor neurons of the spinal cord (151). HGF is also expressed in many neurons of the central nervous system, including frontal lobe and temporal lobe (152). A distinct pattern of HGF expression was seen in the hippocampus, in which one is populated by neurons expressing HGF, whereas an adjacent portion contains neurons expressing the HGF receptor (153). The functions of HGF in the adult central nervous system are not clear at this point.

In liver, HGF is produced by the stellate cells (Ito cells) (154,155), and it has effects on cells expressing the Met receptor, such as hepatocytes, bile duct cells, and sinusoidal endothelial cells (see Chapters 30 and 65). HGF stimulates motogenesis and mitogenesis in primary cultures of rat, mouse, and human hepatocytes (156–158) and bile duct epithelium (157). Other studies have demonstrated angiogenic effects of HGF, but to date there are no studies available on the direct effects of HGF on sinusoidal endothelial cells. HGF stimulates both motogenesis and mitogenesis in cultures of hepatocytes in collagen gels (159), resulting in formation of hepatocellular plates. The mitogenic effects of HGF are suppressible by both complex matrix (type I collagen gels, Matrigel) and TGF- $\beta$ 1 (159). The same agents, however, do not affect the mitogenic effects of HGF (158). HGF and EGF have synergistic effects promoting growth of hepatocytes in long-term cultures allowing for clonogenic growth of hepatocytes (160). Of interest, addition of HGF alone in such cultures in the presence of collagen gels stimulated arrangement of the hepatocytes in acinar and ductular configurations (160). In three dimensional cultures, HGF (as well as EGF) was essential for the formation of histiotypic organization of the hepatocytes and these effects were antagonized by TGF- $\beta$ 1 (161). HGF has stimulatory effects on liver growth and regeneration (see Hepatocyte Growth Factor Role in Liver Regeneration, below). Complex metabolic effects of HGF on hepatocytes include stimulation of fatty acid and triglyceride synthesis and lipoprotein secretion (162).

### Role of Hepatocyte Growth Factor in Embryogenesis

Complementary patterns of expression of HGF and its receptor are seen in embryogenesis (163) (see Chapter 2). In general, HGF is expressed by mesenchymal cell types, whereas expression of the receptor is seen in developing epithelial cell populations. Transient HGF and *c-met* expression is seen in areas of muscle formation and in developing motor neurons. In kidney, mesenchymal cells expressing HGF develop expression of the Met receptor at the time when the kidney mesenchyme undergoes epithelial conver-

sion (164). Deletion mutants of HGF or its receptor Met resulted in similar phenotypes. Both conditions were associated with embryonic lethality (165–167). In both conditions there were defects in development of liver, placenta, and skeletal muscle. Livers were overall atrophic with a decreased number of hepatocytes. The reasons for the hepatic effects are not clear, but they attest to the overall trophic and growth-stimulating effects of HGF in liver. Of interest, HGF and *c-met* expression are maximally enhanced in liver during embryogenesis at a time when hepatocytes are arranged in acinar and hemiacinar configurations (160). Previous studies have shown that this stage is essential for hepatic development and that it precedes formation of the hepatic plates. In view of the above-mentioned effects of HGF inducing acinar configurations in hepatocyte cultures in collagen gels (160) and similar effects on tubulogenesis in other cell types, it is tempting to speculate that the presence of HGF is crucial for the completion of these complex stages of histologic morphogenesis.

### HEPATOCTYTE GROWTH FACTOR–RELATED MOLECULES

In addition to plasminogen, which shares significant sequence and structural homology with HGF, a cDNA clone encoding another product with similar structure and overall sequence homology to that of HGF has also been described. The putative product of this cDNA clone was named HGF-like protein (HGFL). It contains four kringle domains in the  $\alpha$  chain and a serine protease-like domain in the  $\beta$  chain, and has 40% overall amino acid sequence homology to HGF. Its mRNA was detected in high levels in the liver, and to a lesser degree in lung, adrenal, and placenta (168,169). In contrast to HGF, which is synthesized primarily by mesenchymal cells, HGFL is produced predominantly by hepatocytes, and it is secreted into the plasma as the inactive single chain precursor. Also, in contrast to HGF, whose cleavage appears to be mediated by two predominant pathways, cleavage of HGFL to its active heterodimeric form is mediated by a variety of proteases (168). The gene for the HGF-like molecule was localized to human chromosome 3, and it consists of the same numbers of introns and exons as HGF, although the overall size of HGF-like gene is approximately one-tenth of that of HGF. Comparison of a partial amino acid sequence of a plasma protein known as macrophage stimulating factor (MSP) with that of the HGFL molecule suggests that the HGFL gene product is identical to a protein known as MSP (170) that has been reported to stimulate the responsiveness of mouse peritoneal resident macrophages to chemoattractants and to activate their phagocytic activity. Activities associated with HGFL and its receptor *c-Ron* (see below) include stimulating proliferation of mammary duct epithelial cells and keratinocytes, maturation of megakaryocytes, motility

of keratinocytes, and bone resorption and contraction of osteoclasts. Even though the effects of HGF and HGFL overlap in some epithelial systems, HGFL has no essential role in embryogenesis. Disruption of the HGFL gene was compatible with embryogenesis (171). Adult mice homozygous for the HGFL deletion developed microvesicular steatosis in hepatocytes. The receptor of HGFL was identified as the product of the human proto-oncogene *c-Ron* (172). The protein encoded for the HGFL receptor is very similar to the Met protein, including similar tyrosine kinase activation domains and docking sites composed of two tyrosines (see above). Homozygous deletion of the mouse *stk* receptor (the murine homologue of the human *c-ron*) is associated with early embryonic lethality. Embryos fail to implant and do not progress beyond the blastocyst stage (173). The discrepancy between the severe effects on embryogenesis following deletion of the HGFL receptor and the complete absence of effects following deletion of the ligand HGFL raises the possibility that other ligands for *c-Ron* might also exist. Such ligands, however, have not been identified as yet. The remarkable similarities of the structures of HGF and HGFL and those of their receptors suggest that the two receptor/ligand systems evolved in parallel from another ancestral receptor/ligand gene set, not identified so far. Homologues of both *c-met*, *c-Ron*, and of the avian homologue *c-Sea* were recently identified in the teleost puffer fish (*Fugu rubripes*), suggesting that the HGF and HGFL ligand/receptor systems emerged early on in vertebrate evolution (174).

### ROLE OF HEPATOCTYTE GROWTH FACTOR IN THE LIVER REGENERATION AND CHRONIC LIVER INJURY

More information is available on the expression of HGF in animals with experimentally induced liver injuries and liver diseases than for any other diseased tissue or organ. Most of the liver-related studies have focused on the role of HGF in liver regeneration, its effects in chronic liver injury, and changes related to HGF and its receptor in fulminant hepatitis.

#### Hepatocyte Growth Factor Role in Liver Regeneration

In experimental animals treated with hepatotoxins that induce hepatocyte necrosis (such as treatment with  $\text{CCl}_4$ ), or other kinds of liver injuries such as ischemia, physical crushing, or two-thirds partial hepatectomy (PHx), the levels of HGF protein and HGF activity in the plasma increase very rapidly during the early phase of liver regeneration, implying that HGF may be involved in the overall process, leading the hepatocytes to enter the cell cycle (175,176). The amount of the HGF transcript also rises not only in the

livers of injured animals but also in the lung, spleen, and kidney, suggesting endocrine as well as paracrine functions for HGF in liver regeneration (79–81,177). The role of inflammatory cytokines and norepinephrine as signaling agents for this phenomenon was discussed above. As mentioned, HGF was originally isolated from plasma of partially hepatectomized rats and was considered to be the agent primarily responsible for the transmission of the mitogenic signals to hepatocytes during liver regeneration. Subsequent studies using anti-HGF antibodies demonstrated a rise of HGF in the plasma within 1 to 2 hours after partial hepatectomy (79). More prolonged changes in plasma HGF after partial hepatectomy were also noted after human liver resection (178), persisting up to 2 weeks after partial hepatectomy. The origin of the HGF rising in the plasma is not clear. Direct measurements of HGF clearance during liver regeneration did not demonstrate significant differences in HGF clearance between normal and regenerating liver (139).

In view of the direct mitogenic effects of HGF on hepatocytes in culture and the early rise of HGF in the plasma, it was logical to postulate a direct link between this event and stimulation of hepatocyte DNA synthesis. Direct infusion of moderate doses of HGF systemically or into the portal circulation in normal rats or mice has direct mitogenic effects on hepatocytes. DNA synthesis is induced in zone 1 (periportal) hepatocytes (179). This effect is enhanced by preinfusion of TNF- $\alpha$  (17). When HGF is infused in larger amounts, it causes a dramatic increase in liver weight associated with enhanced mitogenesis in hepatocytes. This was observed both in normal as well as IL-6-deficient mice (180). Withdrawal of the infused HGF is followed by marked hepatocyte apoptosis and a decrease in overall hepatic DNA back to normal levels (181). Infusion of smaller amounts of HGF following prior treatment of the animals with small amounts of collagenase (179) also leads to enhancement of the effect of HGF. The peculiar effect of collagenase raises the possibility that infusion of HGF *in vivo* is likely to be less effective in a normal matrix environment but would be more effective in an environment of matrix remodeling. This would imply that matrix remodeling may be part of the early stages of the regenerative process, causing enhanced sensitivity of hepatocytes to mitogenic stimuli such as HGF. This hypothesis is also supported by findings in hepatocyte cultures. Stimulation of DNA synthesis by either EGF or HGF is suppressed when hepatocytes are maintained in collagen type I gels and extracts from the EHS mouse sarcoma cell line (Matrigel) (159,182). Additional studies had shown that, during isolation of hepatocytes by collagenase perfusion of the liver, hepatocytes enter into the cell cycle, as evidenced by increased expression of cell cycle marker proteins (182,183). This suggests that the effect of matrix degradation and release of matrix bound products (which include HGF) may be sufficient to induce hepatocytes to enter into

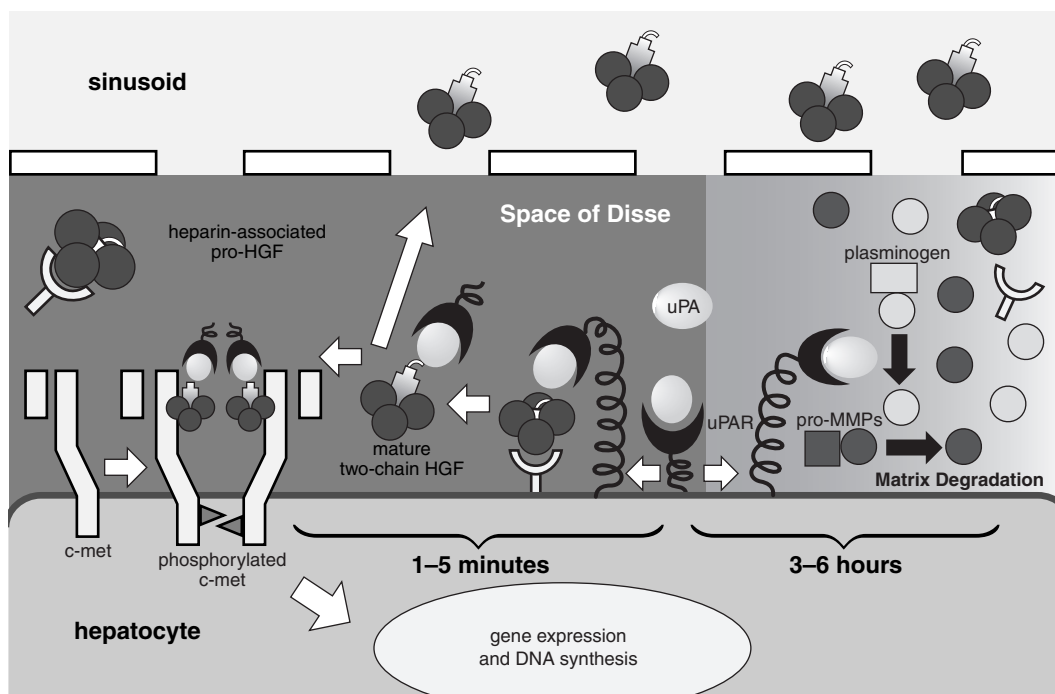
the cell cycle. As mentioned above, HGF is present in large amounts in the matrix of normal liver (145). Infusion of radiolabeled HGF demonstrates accumulation of HGF in periportal sites (184). Processes associated with matrix degradation have the potential of releasing HGF and making it available to hepatocytes either directly in their local environment, or via release in the plasma and reuptake by the hepatocytes. Pathways leading to matrix remodeling involve urokinase or membrane-type matrix metalloproteinases (MT-MMPs) initiated protease cascades (185,186). Urokinase is also involved in activation of HGF (see Pathways of Activation of Hepatocyte Growth factor, above). Urokinase activity is rapidly upregulated following partial hepatectomy, primarily due to translocation of the urokinase receptor from cytoplasm to the plasma membrane (27). Direct evidence that urokinase is actually involved in activation of HGF during liver regeneration was provided by the demonstration that single-chain HGF processing to the active two-chain form by regenerating liver homogenates is inhibited by addition of antiurokinase antibodies (27). Further studies have shown that increased urokinase activity immediately after PHx leads to activation of all the expected subsequent steps in matrix remodeling, such as activation of plasminogen to plasmin and activation of MMP-9 (187,188), increased expression of MMP-9 on hepatocytes, as well as degradation of biomatrix proteins. Of relevance to these findings, mice with homozygous deletions of the plasminogen gene have defective regenerative responses following chemical injury (189). In addition to the activation of the urokinase-initiated matrix remodeling cascade, there is also activation of the pathway initiated by MT-MMP. The latter pathway proceeds to activation of MMP-2 and also involves tissue inhibitor of metalloproteinase-2 (TIMP-2). Activation of this pathway occurs later than the activation of the urokinase pathway (187). While the above studies demonstrate that there is active matrix remodeling occurring during liver regeneration, they do not directly prove that HGF rising in the plasma is derived from hepatic matrix. Given the high concentrations of HGF in hepatic matrix, however, and the active remodeling of matrix in periportal sites, this is an attractive possibility that warrants further investigation. Other substances associated with biomatrix are also elevated in the plasma with the same kinetics as HGF. These include hyaluronic acid and TGF- $\beta$ 1 (190). Elimination of the mito-inhibitory TGF- $\beta$ 1 protein from the ambient environment of the hepatocytes via matrix remodeling may be the reason why only hepatocytes, of the many potential epithelial cell targets, respond to the elevated levels of plasma HGF during the early stages of liver regeneration. It also should be noted that HGF itself may be a secondary later contributor to the activation of the matrix remodeling cascades via effects on hepatocyte gene expression. It has been shown that HGF induces MMP gene expression in cultures of epithelial cells (191). A schematic picture of the interaction between urokinase,

matrix remodeling, and HGF release and local activation in the early stages of liver regeneration is shown in Fig. 43.2.

Other converging observations also support the importance of HGF released from preexisting stores during the early stages of liver regeneration. Tyrosine phosphorylation of the HGF receptor occurs at 30 and 60 minutes after partial hepatectomy, coinciding with the rise of HGF in the plasma (33). Mice deficient in urokinase, the initiator of both matrix remodeling and HGF activation, show retarded and decreased regenerative activity following partial hepatectomy (192). The importance of the plasma HGF as well as HGF present in the ambient environment of hepatocytes in the early stages of liver regeneration was directly demonstrated by infusion of anti-HGF antibodies prior to partial hepatectomy. It was noted that hepatocyte proliferation was suppressed for more than 72 hours, whereas proliferation of nonparenchymal cells was not affected (193).

The signal transduction pathways associated with activation of HGF receptor have been extensively studied in many different cell types (see Chapter 35). In the context of liver regeneration and hepatocyte mitogenesis, key events associated with proliferation of hepatocytes include activa-

tion of transcription factors NF $\kappa$ B, AP-1, and STAT3. Defective activation of these transcription factors is seen in knockout mice for IL-6 and TNF- $\alpha$  receptor I (16,18). Mice deficient for HGF or its receptor do not survive embryogenesis. Thus, the contribution of HGF to the signaling events associated with hepatocyte proliferation at the early stages of liver regeneration cannot be directly assessed, other than by use of blocking antibodies. Studies with hepatocyte cultures, however, have demonstrated that HGF alone (as well as EGF alone) can stimulate a complete mitogenic pathway in resting quiescent rat or human hepatocytes, including activation of AP-1, STAT3, and NF $\kappa$ B (130,131). Since HGF is a complete mitogen for hepatocytes both *in vivo* (179) and *in vitro* (159), and since the HGF receptor is activated within 30 to 60 minutes after partial hepatectomy (33), it is highly likely that HGF contributes to the activation of these specific signaling events or other signaling events associated with mitogenesis in hepatocytes. It is also highly likely that key signaling events related to hepatocyte mitogenesis may be subject to the control of multiple cytokines, creating overlapping and redundant pathways via which entry of hepatocytes into the cell cycle is guaranteed, even in the absence of some of the



**FIGURE 43.2.** Schematic cartoon of hepatocyte growth factor (HGF)-related immediate early events occurring in the hepatocyte microenvironment following partial hepatectomy. The scheme shows proteolytic activation of inactive pro-HGF and matrix degradation via the urokinase-type plasminogen activator (u-PA) u-PA receptor (u-PAR) system, plasmin, and metalloproteinases (MMPs) after partial hepatectomy. Activation of HGF appears to be an early event, since phosphorylation of the HGF receptor occurs by 30 minutes. The events related to HGF thought to occur in the first 5 minutes are depicted on the **left** of the schematic cartoon. Matrix degradation and proteolysis events occurring later are shown on the **right**.

key extracellular signals. Mice deficient in IL-6 or TNF- $\alpha$  receptor I eventually restore most if not all of the hepatic mass. The same observations relate to rats subjected to blockade of the  $\alpha_1$ -adrenergic (norepinephrine) receptor (20) or following infusion of TGF- $\beta$ 1 (194) or administration of *N*-acetylaminofluorene (AAF) (195). The only blockade that appears to completely block the regenerative response is the permanent inactivation of NF $\kappa$ B (196). The inability to permanently block liver regeneration by eliminating specific nonmitogenic cytokines clearly associated with the regenerative response suggests that there is considerable redundancy in the pathways generated by the mitogens (HGF and EGF) and nonmitogenic cytokines. It also suggests that the multiplicity of signaling pathways activated in hepatocytes is crucial not only for the initiation of the mitogenic signals but also for the timing and the precision of the regenerative response. As mentioned above, with the exception of the elimination of the intracellular NF $\kappa$ B signaling, all defects associated with extracellular signals (homozygous deletions in IL-6, TNF- $\alpha$  receptor I, or u-PA, blockade of the norepinephrine  $\alpha_1$ -adrenergic receptor, infusion of TGF- $\beta$ 1, etc.) only delay and do not abolish liver regeneration. This suggests that these extracellular signals are crucial for the timing and precision of liver regeneration but operate in a redundant fashion with receptor tyrosine kinase ligands (HGF, EGF, TGF- $\alpha$ ) and are not absolutely essential for the completion of the response. This may be difficult to sort out, as mentioned above, unless conditional deletion mutant strains of mice for either HGF or its receptor become available in the future.

### Hepatocyte Growth Factor and Chronic Liver Injury

Several reports have shown that administration of HGF during and/or after exposure of the liver to a variety of chronic injury models has ameliorative effects on hepatic histology and retards or prevents fibrosis (197). The effects on injury caused by chemicals may be due to effects of HGF on metabolic activation of the toxic chemical, resulting in less liver injury, when HGF is administered during the time of the administration of the chemical. Convincing results have also been presented, however, showing effects of HGF after the chemical had already induced a certain degree of liver injury and fibrosis (197). Other reports have shown similar effects of HGF in chronic models of injury of kidney (198) and lungs (199). HGF also prevented massive hepatic failure induced by lipopolysaccharide (LPS) and galactosamine, suggesting that the protective effects of HGF against liver injury are more general and not limited to directly toxic chemical agents (200). In other studies, HGF abrogated the severe apoptosis and liver failure induced by the agonist anti-fas antibody both in culture and in whole animals, while inducing the antiapoptotic protein bcl-xL (201). The mechanisms leading to these

effects are not entirely clear, but they raise the possibility that HGF may be used as part of a therapeutic regimen to prevent chronic injury to the liver induced by a variety of agents, such as alcoholism and chronic hepatitis. In a recent report (202) HGF was used in a gene therapy protocol in which vectors expressing HGF were given by intramuscular injection to mice with liver fibrosis induced by chemical injury. The expression of the HGF in the vector led to increased levels of HGF in the plasma and was associated with substantial improvement of the histologic picture of the fibrotic livers, raising the issue of using HGF as part of a gene therapy protocol for treatment of liver fibrosis and prevention of cirrhosis. The 5 amino acid deletion variant of HGF has been specifically investigated in this regard, and it appears to be more effective as an antifibrogenic agent than the complete HGF molecule (203,204).

### Hepatocyte Growth Factor and Fulminant Hepatitis

When HGF was first discovered, it was thought that it could be used as a therapeutic agent in situations such as fulminant hepatitis, in which massive death of hepatocytes is not accompanied by compensatory regenerative activity. It was soon shown, however, that levels of HGF in plasma are very high during fulminant hepatitis. In fact, this condition is associated with the highest levels of HGF than any other disease state (205–207). Elevated levels of plasma HGF are also seen in chronic liver disease and liver failure (207). These findings are in apparent contradiction to the protective effects of HGF against acute and chronic liver injury, discussed above, and suggest that HGF, while apparently useful in chronic injury models, may not be of use for therapy in the catastrophic environment of fulminant hepatic failure. The reasons for the paradox are not clear. While levels of HGF in the plasma appear very high, probably due to massive release of HGF from the hepatic bi-matrix, the status of HGF activation (two chain active versus single chain inactive) has not been investigated. Recent preliminary studies suggest that the apparent inability of HGF to stimulate hepatocyte replication and prevent death of hepatocytes may be due to severe downregulation of the HGF receptor induced by simultaneous elevation of IL-6 and TGF- $\beta$ 1 in the plasma (208).

### CONCLUSION

In the last ten years since their discovery, HGF and its receptor have emerged as major regulators of cell growth, motility, and morphogenesis. Despite the multiple targets of HGF, the embryonic lethality associated with placenta, muscle, and liver dysmorphogenesis in HGF and *c-met* knockout mice clearly suggests that liver is a major overall target of the HGF/*c-met* system. The multiplicity of effects

on several tissues and the new evidence for the emergence of the HGF receptor in early vertebrates suggest that HGF may have evolved as a signaling molecule in response to the need for complex cell movement and morphogenesis associated with vertebrate embryonic growth. In that sense, since liver regeneration may be viewed as an example of the most complex new tissue formation in an adult animal, the crucial need of HGF for liver regeneration is not surprising. More refined animal genetic models may be able to further elucidate the complex effects of the HGF/*c-met* system, not only on liver growth and regeneration but on overall liver function, maintenance, and physiology.

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## REFERENCES

- Grisham JW. A morphological study of deoxyribonucleic acid synthesis and cell proliferation in regenerating liver, autoradiography with <sup>3</sup>H-thymidine. *Cancer Res* 1962;22:842-849.
- Michalopoulos GK, DeFrances M. Liver regeneration. *Science* 1997;276:60-71.
- Moolten FL, Bucher NLR. Regeneration of rat liver: transfer of humoral agents by cross circulation. *Science* 1967;158:272-274.
- Michalopoulos GK, Cianciulli HD, Novotny AR, et al. Liver regeneration studies with rat hepatocytes in primary culture. *Cancer Res* 1982;42:4673-4678.
- Michalopoulos GK, Houck KA, Dolan M, et al. Control of hepatocyte proliferation by two serum factors. *Cancer Res* 1984;44:4414-4441.
- Nakamura T, Nawa K, Ichihara A, et al. Subunit structure of hepatocyte growth factor from rat platelets. *FEBS Lett* 1987;224:311-318.
- Gohda E, Tsubouchi H, Nakayama H, et al. Partial purification and characterization of hepatocyte growth factor from plasma of patients with fulminant hepatic failure. *J Clin Invest* 1988;81:414-419.
- Zarnegar R, Michalopoulos GK. Purification and biological characterization of human Hepatopoietin A: A polypeptide growth factor for hepatocytes. *Cancer Res* 1989;49:3314-3320.
- Zarnegar R, Muga S, Enghild J, et al. NH<sub>2</sub>-terminal amino acid sequence of rabbit hepatopoietin A: a heparin-binding polypeptide growth factor for hepatocytes. *Biochem Biophys Res Commun* 1989;163:1370-1376.
- Miyazawa K, Tsubouchi H, Naka D, et al. Molecular cloning and sequence analysis of cDNA for human hepatocyte growth factor. *Biochem Biophys Res Commun* 1989;163:967-973.
- Nakamura T, Nishizawa T, Hagiya M, et al. Molecular cloning and expression of hepatocyte growth factor. *Nature* 1989;342:440-443.
- McGowan JA, Strain AJ, Bucher NLR. DNA synthesis in primary cultures of adult rat hepatocytes in a defined medium: effects of epidermal growth factor, insulin, glucagon, and cyclic-AMP. *J Cell Physiol* 1981;180:353-363.
- McGowan JA, Bucher NLR. Pyruvate promotion of DNA synthesis in serum-free primary cultures of adult rat hepatocytes. *In Vitro Cell Dev Biol* 1983;19:159-166.
- Mead JE, Fausto N. Transforming growth factor TGF $\alpha$  may be a physiological regulator of liver regeneration by means of an autocrine mechanism. *Proc Natl Acad Sci USA* 1989;86:1558-1562.
- Kan M, Huan J, Mansson P, et al. Heparin-binding growth factor type 1 (acidic fibroblast growth factor): a potential biphasic autocrine and paracrine regulator of hepatocyte regeneration. *Proc Natl Acad Sci USA* 1989;86:7432-7436.
- Cressman DE, Greenbaum LE, DeAngelis RA, et al. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science* 1996;274:1379-1383.
- Webber EM, Bruix J, Pierce RH, et al. Tumor necrosis factor primes hepatocytes for DNA replication in the rat. *Hepatology* 1998;28:1226-1234.
- Yamada Y, Webber EM, Kirillova I, et al. Analysis of liver regeneration in mice lacking type 1 or type 2 tumor necrosis factor receptor: requirement for type 1 but not type 2 receptor. *Hepatology* 1998;28:959-970.
- Yamada Y, Kirillova I, Peschon JJ, et al. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. *Proc Natl Acad Sci USA* 1997;18:94:1441-1446.
- Cruise JL, Knechtle SJ, Bollinge RR, et al.  $\alpha$  1-adrenergic effects and liver regeneration. *Hepatology* 1987;7:1189-1194.
- Rubin JS, Chan ML, Bottaro D, et al. A broad-spectrum human lung fibroblast-derived mitogen is a variant of hepatocyte growth factor. *Proc Natl Acad Sci USA* 1991;88:415-419.
- Hernandez J, Zarnegar R, Strom S, et al. Characterization of the effects of human placental HGF on rat hepatocytes. *J Cell Physiol* 1992;150:116-121.
- Deleted.
- Lokker NA, Mark MR, Luis EA, et al. Structure-function analysis of hepatocyte growth factor: identification of variants that lack mitogenic activity yet retain high affinity receptor binding. *EMBO J* 1992;11:2503-2510.
- Mars WM, Zarnegar R, Michalopoulos GK. Activation of hepatocyte growth factor by the plasminogen activators u-PA and t-PA. *Am J Pathol* 1993;143:949-958.
- Naldini L, Tamagnone L, Vigna E, et al. Extracellular proteolytic cleavage by urokinase is required for activation of hepatocyte growth factor/scatter factor. *EMBO J* 1992;11:4825-4833.
- Mars WM, Liu ML, Kitson RP, et al. Immediate early detection of urokinase receptor after partial hepatectomy and its implications for initiation of liver regeneration. *Hepatology* 1995;21:1695-1701.
- Naldini L, Vigna E, Bardelli A, et al. Biological activation of pro-HGF (hepatocyte growth factor) by urokinase is controlled by a stoichiometric reaction. *J Biol Chem* 1995;270:603-611.
- Reuning U, Magdolen V, Wilhelm O, et al. Multifunctional potential of the plasminogen activation system in tumor invasion and metastasis (review). *Int J Oncol* 1998;13:893-906.
- Koshelnick Y, Ehart M, Stockinger H, et al. Mechanisms of signaling through urokinase receptor and the cellular response. *Thromb Haemost* 1999;82:305-311.
- Chapman HA, Wei Y, Simon DI, et al. Role of urokinase receptor and caveolin in regulation of integrin signaling. *Thromb Haemost* 1999;82:291-297.
- Preissner KT, Kanse SM, Chavakis T, et al. The dual role of the urokinase receptor system in pericellular proteolysis and cell

- adhesion: implications for cardiovascular function. *Basic Res Cardiol* 1999;94:315–321.
33. Stolz DB, Mars WM, Petersen BE, et al. Growth factor signal transduction immediately after two-thirds partial hepatectomy in the rat. *Cancer Res* 1999;59:3954–3960.
  34. Mars WM, Kim TH, Beer-Stolz D, et al. The presence of urokinase in serum free primary rat hepatocyte cultures and its role in activating hepatocyte growth factor. *Cancer Res* 1996;56:2837–2843.
  35. Ried S, Jager C, Jeffers M, et al. Activation mechanisms of the urokinase-type plasminogen activator promoter by hepatocyte growth factor/scatter factor. *J Biol Chem* 1999;274:16377–86.
  36. Webb CP, Hose CD, Koochekpour S, et al. The geldanamycins are potent inhibitors of the hepatocyte growth factor/scatter factor-met-urokinase plasminogen activator-plasmin proteolytic network. *Cancer Res* 2000;60:342–349.
  37. Shimomura T, Miyazawa K, Komiyama Y, et al. Activation of hepatocyte growth factor by two homologous proteases, blood-coagulation factor XIIa and hepatocyte growth factor activator. *Eur J Biochem* 1995;229:257–261.
  38. Shimomura T, Denda K, Kawaguchi T, et al. Multiple sites of proteolytic cleavage to release soluble forms of hepatocyte growth factor activator inhibitor type 1 from a transmembrane form. *J Biochem (Tokyo)* 1999;126:821–828.
  39. Okajima A, Miyazawa K, Naitoh Y, et al. Induction of hepatocyte growth factor activator messenger RNA in the liver following tissue injury and acute inflammation. *Hepatology* 1997;25:97–102.
  40. Matsubara Y, Ichinose M, Yahagi N, et al. Hepatocyte growth factor activator: a possible regulator of morphogenesis during fetal development of the rat gastrointestinal tract. *Biochem Biophys Res Commun* 1998;253:477–484.
  41. Chirgadze DY, Hepple J, Byrd RA, et al. Insights into the structure of hepatocyte growth factor/scatter factor (HGF/SF) and implications for receptor activation. *FEBS Lett* 1998;23:126–129.
  42. Okigaki M, Komada M, Uehara Y, et al. Functional characterization of HGF mutants obtained by deletion of structural domains. *Biochemistry* 1992;31:9555–9561.
  43. Matsumoto K, Takehara T, Inoue H, et al. Deletion of kringle domains or the N-terminal hairpin structure in HGF results in marked decreases in related biological activities. *Biochem Biophys Res Commun* 1991;181:691–699.
  44. Hartmann G, Naldini L, Weidner KM, et al. A functional domain in the heavy chain of SF/HGF binds the *c-met* receptor and induces cell dissociation but not mitogenesis. *Proc Natl Acad Sci USA* 1992;89:11574–11578.
  45. Otsuka T, Jakubczak J, Vieira W, et al. Disassociation of met-mediated biological responses in vivo: the natural hepatocyte growth factor/scatter factor splice variant NK2 antagonizes growth but facilitates metastasis. *Mol Cell Biol* 2000;20:2055–2065.
  46. Day RM, Cioce V, Breckenridge D, et al. Differential signaling by alternative HGF isoforms through *c-met*: activation of both MAP kinase and PI 3-kinase pathways is insufficient for mitogenesis. *Oncogene* 1999;18:3399–3406.
  47. Montesano R, Soriano JV, Malinda KM, et al. Differential effects of hepatocyte growth factor isoforms on epithelial and endothelial tubulogenesis. *Cell Growth Differ* 1998;9:355–365.
  48. Cioce V, Csaky KG, Chan AML, et al. Hepatocyte growth factor (HGF)/NK1 is a naturally occurring HGF/scatter factor variant with partial agonist/antagonist activity. *J Biol Chem* 1996;271:13110–13115.
  49. Schwall RH, Chang LY, Godowski PJ, et al. Heparin induces dimerization and confers proliferative activity onto the hepatocyte growth factor antagonists NK1 and NK2. *J Cell Biol* 1996;133:709–718.
  50. Matsumoto K, Kataoka H, Date K, et al. Cooperative interaction between  $\alpha$ - and  $\beta$ -chains of hepatocyte growth factor on *c-met* receptor confers ligand-induced receptor tyrosine phosphorylation and multiple biological responses. *J Biol Chem* 1998;273:22913–22920.
  51. Zarnegar R, Petersen B, DeFrances MC, et al. Localization of hepatocyte growth factor (HGF) gene on human chromosome 7. *Genomics* 1992;12:147–150.
  52. Fukuyama R, Ichijoh Y, Minoshima S, et al. Regional localization of the HGF gene to human chromosome 7 band q21.1. *Genomics* 1992;11:410–415.
  53. Dean M, Park M, LeBeau MM, et al. The human met oncogene is related to tyrosine kinase oncogenes. *Nature* 1985;318:385–388.
  54. Seki T, Hagiya M, Shimonishi M, et al. Organization of the HGF encoding gene. *Gene* 1991;102:213–219.
  55. Miyazawa K, Kitamura A, Kitamura N. Structural organization and the transcriptional initiation site of the human HGF gene. *Biochemistry* 1991;30:9170–9176.
  56. Zarnegar R. Regulation of HGF and HGFR gene expression. *EXS* 1995;74:33–49.
  57. Bell AW, Jiang JG, Chen Q, et al. The upstream regulatory regions of the hepatocyte growth factor gene promoter are essential for its expression in transgenic mice. *J Biol Chem* 1998;273:6900–6908.
  58. Jiang JG, Zarnegar R. A novel transcriptional regulatory region within the core promoter of the hepatocyte growth factor gene is responsible for its inducibility by cytokines via the C/EBP family of transcription factors. *Mol Cell Biol* 1997;17:5758–5770.
  59. Liu Y, Beedle AB, Lin L, et al. Identification of a cell-type-specific transcriptional repressor in the promoter region of the mouse hepatocyte growth factor gene. *Mol Cell Biol* 1994;14:7046–7058.
  60. Jiang JG, Bell A, Liu Y, et al. Transcriptional regulation of the hepatocyte growth factor gene by the nuclear receptors chicken ovalbumin upstream promoter transcription factor and estrogen receptor. *J Biol Chem* 1997;272:3928–3934.
  61. Jiang JG, Chen Q, Bell A, et al. Transcriptional regulation of the hepatocyte growth factor (HGF) gene by the Sp family of transcription factors. *Oncogene* 1997;14:3039–3049.
  62. Metcalfe AM, Dixon RM, Radda GK. Wild-type but not mutant p53 activates the hepatocyte growth factor/scatter factor promoter. *Nucleic Acids Res* 1997;25:983–986.
  63. Seol DW, Chen Q, Smith ML, et al. Regulation of the *c-met* proto-oncogene promoter by p53. *J Biol Chem* 1999;274:3565–3572.
  64. Tashiro K, Hagiya M, Hishizawa T, et al. Deduced primary structure of rat hepatocyte growth factor and expression of the mRNA in rat tissues. *Proc Natl Acad Sci USA* 1990;87:3200–3204.
  65. Okajima A, Miyazawa K, Kitamura N. Primary structure of rat Hepatocyte growth factor and induction of its mRNA during liver regeneration following hepatic injury. *Eur J Biochem* 1990;193:375–381.
  66. Zarnegar R, Muga S, Rahija R, et al. Tissue distribution of hepatopoietin A: a heparin binding polypeptide growth factor for hepatocytes. *Proc Natl Acad Sci USA* 1990;87:1252–1256.
  67. DeFrances MC, Wolf H, Michalopoulos GK, et al. The presence of hepatocyte growth factor in the developing rat. *Development* 1992;116:387–395.
  68. Kinoshita T, Tashiro K, Nakamura T. Marked increase of HGF mRNA in non-parenchymal liver cells of rats treated with hepatotoxins. *Biochem Biophys Res Commun* 1989;165:1229–1234.
  69. Noji S, Tashiro K, Koyama E, et al. Expression of Hepatocyte growth factor gene in endothelial and Kupffer's cells of damaged

- rat livers as revealed by in situ hybridization. *Biochem Biophys Res Commun* 1990;173:42–47.
70. Schirmacher P, Geerts A, Pietrangelo A, et al. Hepatocyte growth factor/hepatopoietin A is expressed in fat-storing cells from rat liver but not myofibroblast-like cells derived from fat-storing cells. *Hepatology* 1992;15:5–11.
  71. Miyazawa K, Kitamura A, Naka D, et al. An alternatively processed mRNA generated from human hepatocyte growth factor gene. *Eur J Biochem* 1991;197:15–22.
  72. Chan AM-L, Rubin JS, Bottaro DP, et al. Identification of a competitive HGF antagonist encoded by an alternative transcript. *Science* 1991;254:1382–1385.
  73. Cioce V, Csaky KG, Chan AML, et al. Hepatocyte growth factor (HGF)/NK1 is a naturally occurring HGF/scatter factor variant with partial agonist/antagonist activity. *J Biol Chem* 1996;271:13110–13115.
  74. Seki T, Ihara I, Sugimura A, et al. Isolation and expression of cDNA for different forms of hepatocyte growth factor from human leukocyte. *Biochem Biophys Res Commun* 1990;172:321–327.
  75. Gohda E, Kataoka H, Tsubouchi H, et al. Phorbol ester-induced secretion of human hepatocyte growth factor by human skin fibroblasts and its inhibition by dexamethasone. *FEBS Lett* 1992;301:107–110.
  76. Matsumoto K, Okazaki H, Nakamura T. Up-regulation of HGF gene expression by IL-1 in human skin fibroblasts. *Biochem Biophys Res Commun* 1992;188:235–243.
  77. Matsumoto K, Tajima H, Okazaki H, et al. Negative regulation of HGF gene expression in human lung fibroblasts and leukemic cells by transforming growth factor  $\beta$  and glucocorticoids. *J Biol Chem* 1992;267:24917–24920.
  78. Ota S, Tanaka Y, Bamba H, et al. Nonsteroidal anti-inflammatory drugs may prevent colon cancer through suppression of hepatocyte growth factor expression. *Eur J Pharmacol* 1999;367:131–138.
  79. Zarnegar R, DeFrances MC, Kost D, et al. Expression of Hepatocyte Growth Factor (HGF) in regenerating rat liver after partial hepatectomy. *Biochem Biophys Res Commun* 1991;177:559–565.
  80. Kono S, Nagaike M, Masumoto K, et al. Marked induction of HGF mRNA in intact kidney and spleen in response to injury of distant organs. *Biochem Biophys Res Commun* 1992;186:991–998.
  81. Hamanoue M, Kawaida K, Takao S, et al. Rapid and marked induction of HGF during liver regeneration after ischemic or crush injury. *Hepatology* 1992;16:1485–1492.
  82. Cruise JL, Houck KA, Michalopoulos GK. Induction of DNA synthesis in cultured rat hepatocytes through stimulation of  $\alpha$ 1 adrenoceptor by norepinephrine. *Science* 1985;227:749–751.
  83. Broten J, Michalopoulos G, Petersen B, et al. Adrenergic stimulation of hepatocyte growth factor expression. *Biochem Biophys Res Commun* 1999;262:76–79.
  84. Cooper CS, Park M, Blair D, et al. Molecular cloning of a new transforming gene from a chemically transformed human cell line. *Nature* 1984;311:29–33.
  85. Park M, Dean M, Cooper CS, et al. Mechanism of met oncogene activation. *Cell* 1986;45:895–904.
  86. Park M, Dean M, Kaul K, et al. Sequence of Met protooncogene cDNA has features characteristic of the tyrosine kinase family of growth factor receptors. *Proc Natl Acad Sci USA* 1987;84:6379–6383.
  87. Chan AM, King HWS, Deakin EA, et al. Characterization of the mouse met protooncogene. *Oncogene* 1988;2:593–599.
  88. Tempest PR, Stratton MR, Cooper CS. Structure of the met protein kinase activity among human tumor cell lines. *Br J Cancer* 1988;58:3–7.
  89. Vigna E, Naldini L, Tamagnone L, et al. Hepatocyte growth factor and its receptor, the tyrosine kinase encoded by the *c-met* proto-oncogene. *Cell Mol Biol (Noisy-le-grand)* 1994;40:597–604.
  90. Bottaro DP, Rubin JS, Faletto DL, et al. Identification of the hepatocyte growth factor receptor as the *c-met* proto-oncogene product. *Science* 1991;251:802–804.
  91. Naldini L, Vigna E, Narsimhan R, et al. Hepatocyte growth factor stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene, *c-met*. *Oncogene* 1991;6:501–504.
  92. Giordano S, DiRenzo MF, Narsimhan RP, et al. Biosynthesis of the protein encoded by the proto-oncogene. *Oncogene* 1989;4:1383–1388.
  93. Mark MR, Lokker NA, Zioncheck TF, et al. Expression and characterization of hepatocyte growth factor receptor-IgG fusion protein. *J Biol Chem* 1992;267:26166–26171.
  94. Mondino A, Giordano S, Comoglio P. Defective posttranslational processing activates the tyrosine kinase encoded by the Met proto-oncogene (hepatocyte growth factor receptor). *Mol Cell Biol* 1991;11:6084–6092.
  95. Prat M, Crepaldi T, Gandino L, et al. C-terminal truncated forms of Met, the hepatocyte growth factor receptor. *Mol Cell Biol* 1991;11:5954–5962.
  96. Liu Y. The human hepatocyte growth factor receptor gene: complete structural organization and promoter characterization. *Gene* 1998;215:159–169.
  97. Seol DW, Zarnegar R. Structural and functional characterization of the mouse *c-met* proto-oncogene (hepatocyte growth factor receptor) promoter. *Biochim Biophys Acta* 1998;1395:252–258.
  98. Seol DW, Chen Q, Zarnegar R. Transcriptional activation of the hepatocyte growth factor receptor (*c-met*) gene by its ligand (hepatocyte growth factor) is mediated through AP-1. *Oncogene* 2000;19:1132–1137.
  99. Moghul A, Lin L, Beedle A, et al. Modulation of *c-met* proto-oncogene (HGF receptor) mRNA abundance by cytokines and hormones: evidence for rapid decay of the 8 kb *c-met* transcript. *Oncogene* 1994;9:2045–2052.
  100. Chen Q, Seol DW, Carr B, et al. Co-expression and regulation of Met and Ron proto-oncogenes in human hepatocellular carcinoma tissues and cell lines. *Hepatology* 1997;26:59–66.
  101. Gambarotta G, Boccaccio C, Giordano S, et al. Ets up-regulates MET transcription. *Oncogene* 1996;13:1911–1917.
  102. Epstein JA, Shapiro DN, Cheng J, et al. Pax3 modulates expression of the *c-met* receptor during limb muscle development. *Proc Natl Acad Sci USA* 1996;93:4213–4218.
  103. Prat M, Narsimhan RP, Crepaldi T, et al. The receptor encoded by the human *c-met* oncogene is expressed in hepatocytes, epithelial cells and solid tumors. *Int J Cancer* 1991;49:323–328.
  104. Giordano L, Ponzetto C, DiRenzo MF, et al. Tyrosine kinase receptor indistinguishable from in-Met protein. *Nature* 1989;339:155–156.
  105. Rong S, Bodescot M, Blair D, et al. Tumorigenicity of the met proto-oncogene, and the gene for HGF. *Mol Cell Biol* 1992;12:5152–5158.
  106. Liu C, Park M, Tsao MS. Overexpression of proto-oncogene but EGF receptor or *c-erbB-2* in primary human colorectal carcinomas. *Oncogene* 1992;7:181–185.
  107. Kuniyasu H, Yasui W, Kitadai Y, et al. Frequent amplification of the gene in scirrhous type stomach cancer. *Biochem Biophys Res Commun* 1992;189:227–232.
  108. Radaeva S, Ferreira-Gonzalez A, Sirica AE. Overexpression of C-NEU and C-MET during rat liver cholangiocarcinogenesis: a link between biliary intestinal metaplasia and mucin-producing cholangiocarcinoma. *Hepatology* 1999;29:1453–1462.
  109. Soman NR, Corriea P, Ruiz BA, et al. The TPR-Met oncogenic

- rearrangement is present and expressed in human gastric carcinoma and precursor lesions. *Proc Natl Acad Sci USA* 1991; 88:4892–4896.
110. Schmidt L, Duh FM, Chen F, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. *Nat Genet* 1997;16: 68–73.
  111. Park WS, Dong SM, Kim SY, et al. Somatic mutations in the kinase domain of the Met/hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas. *Cancer Res* 1999; 59:307–310.
  112. Ueki T, Fujimoto J, Suzuki T, et al. Expression of hepatocyte growth factor and its receptor *c-met* proto-oncogene in hepatocellular carcinoma. *Hepatology* 1997;25:862–866.
  113. Ljubimova JY, Petrovic LM, Wilson SE, et al. Expression of HGF, its receptor *c-met*, *c-myc*, and albumin in cirrhotic and neoplastic human liver tissue. *J Histochem Cytochem* 1997;45: 79–87.
  114. D'Errico A, Fiorentino M, Ponzetto A, et al. Liver hepatocyte growth factor does not always correlate with hepatocellular proliferation in human liver lesions: its specific receptor *c-met* does. *Hepatology* 1996;24:60–64.
  115. Selden C, Farnaud S, Ding SF, et al. Expression of hepatocyte growth factor mRNA, and *c-met* mRNA (hepatocyte growth factor receptor) in human liver tumors. *J Hepatol* 1994;21: 227–234.
  116. Boix L, Rosa JL, Ventura F, et al. *c-met* mRNA overexpression in human hepatocellular carcinoma. *Hepatology* 1994;19: 88–91.
  117. Sakata H, Takayama H, Sharp R, et al. Hepatocyte growth factor/scatter factor overexpression induces growth, abnormal development, and tumor formation in transgenic mouse livers. *Cell Growth Differ* 1996;7:1513–1523.
  118. Bell A, Chen Q, DeFrances MC, et al. The five amino acid-deleted isoform of hepatocyte growth factor promotes carcinogenesis in transgenic mice. *Oncogene* 1999;18:887–895.
  119. Santoni-Rugiu E, Preisegger KH, Kiss A, et al. Inhibition of neoplastic development in the liver by hepatocyte growth factor in a transgenic mouse model. *Proc Natl Acad Sci USA* 1996;93: 9577–9582.
  120. Ogasawara H, Hiramoto J, Takahashi M, et al. Hepatocyte growth factor stimulates DNA synthesis in rat preneoplastic hepatocytes but not in liver carcinoma cells. *Gastroenterology* 1998;114:775–781.
  121. Shiota G, Kawasaki H, Nakamura T, et al. Inhibitory effect of hepatocyte growth factor on metastasis of hepatocellular carcinoma in transgenic mice. *Res Commun Mol Pathol Pharmacol* 1996;91:33–39.
  122. Shima N, Stolz DB, Miyazaki M, et al. Possible involvement of p21/waf1 in the growth inhibition of HepG2 cells induced by hepatocyte growth factor. *J Cell Physiol* 1998;177:130–136.
  123. Liu ML, Mars WM, Michalopoulos GK. Hepatocyte growth factor inhibits cell proliferation in vivo of rat hepatocellular carcinomas induced by diethylnitrosamine. *Carcinogenesis* 1995; 16:841–843.
  124. Jhappan C, Stahle C, Harkins RN, et al. TGF $\alpha$  overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. *Cell* 1990;61: 1137–1146.
  125. Higashio K, Shima N, Goto M, et al. Identity of a tumor cytotoxic factor from human fibroblasts and hepatocyte growth factor. *Biochem Biophys Res Commun* 1990;170:397–404.
  126. Amicone L, Spagnoli FM, Spath G, et al. Transgenic expression in the liver of truncated Met blocks apoptosis and permits immortalization of hepatocytes. *EMBO J* 1997;16:495–503.
  127. Longati P, Bardelli A, Ponzetto C, et al. Tyrosines 1234–1235 are critical for activation of the tyrosine kinase encoded by the MET proto-oncogene (HGF receptor). *Oncogene* 1994;9: 49–57.
  128. Ponzetto C, Bardelli A, Zhen Z, et al. A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. *Cell* 1994;77: 261–271.
  129. Comoglio PM, Boccaccio C. The HGF receptor family: unconventional signal transducers for invasive cell growth. *Genes Cells* 1996;1:347–354.
  130. Runge DM, Runge D, Foth H, et al. STAT 1 $\alpha$ /1 $\beta$ , STAT 3 and STAT 5: expression and association with *c-met* and EGF-receptor in long-term cultures of human hepatocytes. *Biochem Biophys Res Commun* 1999;265:376–381.
  131. Schaper F, Siewert E, Gomez-Lechon MJ, et al. Hepatocyte growth factor/scatter factor (HGF/SF) signals via the STAT3/APRF transcription factor in human hepatoma cells and hepatocytes. *FEBS Lett* 1997;405:99–103.
  132. Jeffers M, Taylor GA, Weidner KM, et al. Degradation of the Met tyrosine kinase receptor by the ubiquitin-proteasome pathway. *Mol Cell Biol* 1997;17:799–808.
  133. Birchmeier W, Brinkmann V, Niemann C, et al. Role of HGF/SF and *c-met* in morphogenesis and metastasis of epithelial cells. *Ciba Found Symp* 1997;212:230–240.
  134. Bardelli A, Longati P, Gramaglia D, et al. Gab1 coupling to the HGF/Met receptor multifunctional docking site requires binding of Grb2 and correlates with the transforming potential. *Oncogene* 1997;15:3103–3111.
  135. Lehr S, Kotzka J, Herkner A, et al. Identification of tyrosine phosphorylation sites in human Gab-1 protein by EGF receptor kinase in vitro. *Biochemistry* 1999;38:151–159.
  136. Maina F, Casagrande F, Audero E, et al. Uncoupling of Grb2 from the Met receptor in vivo reveals complex roles in muscle development. *Cell* 1996;87:531–542.
  137. Presnell SC, Stolz DB, Mars WM, et al. Modifications of the hepatocyte growth factor/*c-met* pathway by constitutive expression of transforming growth factor- $\alpha$  in rat liver epithelial cells. *Mol Carcinogen* 1997;18:244–255.
  138. Jo M, Stolz DB, Esplen JE, et al. Cross-talk between epidermal growth factor receptor and *c-met* signal pathways in transformed cells. *J Biol Chem* 2000;275:8806–8811.
  139. Appasamy R, Tanabe M, Murase N, et al. Pharmacokinetics of HGF blood clearance, organ uptake and biliary excretion in normal and partially hepatectomized rat. *J Lab Invest* 1993; 68:270–276.
  140. Liu KX, Kato Y, Narukawa M, et al. Importance of the liver in plasma clearance of hepatocyte growth factor in rats. *Am J Physiol* 1992;263:G642–G649.
  141. Liu KX, Kato Y, Yamazaki M, et al. Decrease in the hepatic clearance of hepatocyte growth factor in carbon tetrachloride-intoxicated rats. *Hepatology* 1993;17:651–660.
  142. Kato Y, Liu KX, Nakamura T, et al. Heparin-hepatocyte growth factor complex with low plasma clearance and retained hepatocyte proliferating activity. *Hepatology* 1994;20:417–424.
  143. Schwall RH, Chang LY, Godowski PJ, et al. Heparin induces dimerization and confers proliferative activity onto the hepatocyte growth factor antagonists NK1 and NK2. *J Cell Biol* 1996;133:709–718.
  144. Zarnegar R, DeFrances MC, Oliver L, et al. Identification and partial characterization of receptor binding sites for HGF on rat hepatocytes. *Biochem Biophys Res Commun* 1990;173: 1179–1185.
  145. Masumoto A, Yamamoto N. Sequestration of a hepatocyte growth factor in extracellular matrix in normal adult rat liver. *Biochem Biophys Res Commun* 1991;174:90–95.
  146. Lamszus K, Joseph A, Jin L, et al. Scatter factor binds to throm-

- bospondin and other extracellular matrix components. *Am J Pathol* 1996;149:805–819.
147. Stoker M, Gherardi E, Perryman M, et al. Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. *Nature* 1987;327:239–242.
  148. Gherardi E, Gray J, Stoker M, et al. Purification of scatter factor, a fibroblast-derived basic protein that modulates epithelial interactions and movement. *Proc Natl Acad Sci USA* 1989;86:5844–5848.
  149. Gherardi E, Stoker M. Hepatocytes and scatter factor. *Nature* 1990;346:228.
  150. Weidner KM, Behrens J, Vandekerckhove J, et al. Scatter factor: molecular characteristics and effect on the invasiveness of epithelial cells. *J Cell Biol* 1990;111:2097–2108.
  151. Ebens A, Brose K, Leonardo ED, et al. Hepatocyte growth factor/scatter factor is an axonal chemoattractant and a neurotrophic factor for spinal motor neurons. *Neuron* 1996;17:1157–1172.
  152. Jung W, Castren E, Odenthal M, et al. Expression and functional interaction of hepatocyte growth factor-scatter factor and its receptor *c-met* in mammalian brain. *J Cell Biol* 1994;126:485–494.
  153. Achim CL, Katyal S, Wiley CA, et al. Expression of HGF and cMet in the developing and adult brain. *Brain Res Dev* 1997;102:299–303.
  154. Schirmacher P, Geerts A, Jung W, et al. The role of Ito cells in the biosynthesis of HGF-SF in the liver. *EXS* 1993;65:285–299.
  155. Schirmacher P, Geerts A, Pietrangelo A, et al. Hepatocyte growth factor/hepatopoietin A is expressed in fat-storing cells from rat liver but not myofibroblast-like cells derived from fat-storing cells. *Hepatology* 1992;15:5–11.
  156. Strain AJ, Ismail T, Tsubouchi H, et al. Native and recombinant human hepatocyte growth factors are highly potent promoters of DNA synthesis in both human and rat hepatocytes. *J Clin Invest* 1991;87:1853–1857.
  157. Joplin R, Hishida T, Tsubouchi H, et al. Human intrahepatic biliary epithelial cells proliferate in vitro in response to human hepatocyte growth factor. *J Clin Invest* 1992;90:1284–1289.
  158. Petersen B, Yee CJ, Bowen W, et al. Distinct morphological and mitogenic effects induced by TGF- $\beta$  1, HGF and EGF on mouse, rat and human hepatocytes. *Cell Biol Toxicol* 1994;10:219–230.
  159. Michalopoulos GK, Bowen W, Nussler AK, et al. Comparative analysis of mitogenic and morphogenic effects of HGF and EGF on rat and human hepatocytes maintained in collagen gels. *J Cell Physiol* 1993;156:443–452.
  160. Block GD, Locker J, et al. Population expansion, clonal growth and specific differentiation patterns in primary cultures of hepatocytes induced by HGF/SF, EGF and TGF $\alpha$  in a chemically defined (HGM) medium. *J Cell Biol* 1996;132:1133–1149.
  161. Michalopoulos GK, Bowen WC, Zajac VF, et al. Morphogenetic events in mixed cultures of rat hepatocytes and non-parenchymal cells maintained in biological matrices in the presence of hepatocyte growth factor and epidermal growth factor. *Hepatology* 1999;29:90–100.
  162. Kaibori M, Kwon AH, Oda M, et al. Hepatocyte growth factor stimulates synthesis of lipids and secretion of lipoproteins in rat hepatocytes. *Hepatology* 1998;27:1354–1361.
  163. Sonnenberg E, Meyer D, Weidner KM, et al. Scatter factor/hepatocyte growth factor and its receptor, the *c-met* tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. *J Cell Biol* 1993;123:223–235.
  164. Tsarfay I, Rong S, Resau JH, et al. The Met proto-oncogene mesenchymal to epithelial cell conversion. *Science* 1994;263:98–101.
  165. Schmidt C, Bladt F, Goedecke S, et al. Scatter factor/hepatocyte growth factor is essential for liver development. *Nature* 1995;373:699–702.
  166. Uehara Y, Minowa O, Mori C, et al. Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. *Nature* 1995;373:702–705.
  167. Bladt F, Riethmacher D, Isenmann S, et al. Essential role for the *c-met* receptor in the migration of myogenic precursor cells into the limb bud. *Nature* 1995;376:768–771.
  168. Degen SJ, Stuart LA, Han S, et al. Characterization of the mouse cDNA and gene coding for a hepatocyte growth factor-like protein: expression during development. *Biochemistry* 1991;30:9781–9791.
  169. Han S, Stuart LA, Degen SJ. Characterization of the DNF15S2 locus on human chromosome 3: identification of a gene coding for four kringle domains with homology to hepatocyte growth factor. *Biochemistry* 1991;30:9768–9780.
  170. Skeel A, Yoshimura T, Showalter SD, et al. Macrophage stimulating protein: purification, partial amino acid sequence, and cellular activity. *J Exp Med* 1991;173:1227–1234.
  171. Bezerra JA, Carrick TL, Degen JL, et al. Biological effects of targeted inactivation of hepatocyte growth factor-like protein in mice. *J Clin Invest* 1998;101:1175–1183.
  172. Waltz SE, Toms CL, McDowell SA, et al. Characterization of the mouse Ron/Stk receptor tyrosine kinase gene. *Oncogene* 1998;16:27–42.
  173. Muraoka RS, Sun WY, Colbert MC, et al. The Ron/STK receptor tyrosine kinase is essential for peri-implantation development in the mouse. *J Clin Invest* 1999;103:1277–1285.
  174. Cottage A, Clark M, Hawker K, et al. Three receptor genes for plasminogen related growth factors in the genome of the puffer fish *Fugu rubripes*. *FEBS Lett* 1999;443:370–374.
  175. Lindroos P, Zarnegar R, Michalopoulos GK. Hepatocyte growth factor (hepatopoietin A) rapidly increases in plasma before DNA synthesis and liver regeneration stimulated by partial hepatectomy and carbon tetrachloride administration. *Hepatology* 1991;13:743–750.
  176. Kinoshita T, Hirao S, Matsumoto K, et al. Possible endocrine control by hepatocyte growth factor of liver regeneration after partial hepatectomy. *Biochem Biophys Res Commun* 1991;177:330–335.
  177. Yanagita K, Nagaike M, Ishibashi H, et al. Lung may have an endocrine function producing hepatocyte growth factor in response to injury of distal organs. *Biochem Biophys Res Commun* 1992;182:802–809.
  178. Tomiya T, Tani M, Yamada S, et al. Serum hepatocyte growth factor levels in hepatectomized and nonhepatectomized surgical patients. *Gastroenterology* 1992;103:1621–1624.
  179. Liu ML, Mars WM, Zarnegar R, et al. Collagenase pretreatment and the mitogenic effects of hepatocyte growth factor and transforming growth factor- $\alpha$  in adult rat liver. *Hepatology* 1994;19:1521–1527.
  180. Patijn GA, Lieber A, Schowalter DB, et al. Hepatocyte growth factor induces hepatocyte proliferation in vivo and allows for efficient retroviral-mediated gene transfer in mice. *Hepatology* 1998;28:707–716.
  181. Nagoshi S, Yasuda H, Suda J, et al. Hepatocyte apoptosis and hepatic expression of transforming growth factor- $\beta$ 1 mRNA during involution of hyperplastic rat liver induced by hepatocyte growth factor. *J Gastroenterol Hepatol* 1998;13:786–793.
  182. Rana B, Mischoulon D, Xie Y, et al. Cell-extracellular matrix interactions can regulate the switch between growth and differentiation in rat hepatocytes: reciprocal expression of C/EBP  $\alpha$  and immediate-early growth response transcription factors. *Mol Cell Biol* 1994;14:5858–5869.
  183. Kost DB, Michalopoulos GK. Effect of epidermal growth factor on the expression of protooncogenes *c-myc* and *c-Ha-ras* in

- short-term primary hepatocyte culture. *J Cell Physiol* 1990;144:122–127.
184. Liu ML, Mars WM, Zarnegar R, et al. Uptake and distribution of hepatocyte growth factor in normal and regenerating adult rat liver. *Am J Pathol* 1994;144:129–140.
185. Dano K, Romer J, Nielsen BS, et al. Cancer invasion and tissue remodeling—cooperation of protease systems and cell types. *APMIS* 1999;107:120–127.
186. Seiki M. Membrane-type matrix metalloproteinases. *APMIS* 1999;107:137–143.
187. Kim TH, Mars WM, Stolz DB, et al. Extracellular matrix remodeling at the early stages of liver regeneration in the rat. *Hepatology* 1997;26:896–904.
188. Kim TH, Mars WM, Stolz DB, et al. Expression and activation of pro-MMP-2 and pro-MMP-9 during rat liver regeneration. *Hepatology* 2000;31:75–82.
189. Bezerra JA, Bugge TH, Melin-Aldana H, et al. Plasminogen deficiency leads to impaired remodeling after a toxic injury to the liver. *Proc Natl Acad Sci USA* 1999;96:15143–15148.
190. Michalopoulos GK. Hepatocyte growth factor (HGF) and its receptor (Met) in liver regeneration, neoplasia and disease. In: Jirtle RL, ed. *Liver regeneration and carcinogenesis*. New York: Academic Press, 1995:27–49.
191. Dunsmore SE, Rubin JS, Kovacs SO, et al. Mechanisms of hepatocyte growth factor stimulation of keratinocyte metalloproteinase production. *J Biol Chem* 1996;271:24576–24582.
192. Roselli HT, Su M, Washington K, et al. Liver regeneration is transiently impaired in urokinase-deficient mice. *Am J Physiol* 1998;275:G1472–1479.
193. Burr AW, Toole K, Chapman C, et al. Anti-hepatocyte growth factor antibody inhibits hepatocyte proliferation during liver regeneration. *J Pathol* 1998;185:298–302.
194. Russell WE, Coffey RJ Jr, Ouellette AJ, et al. Type  $\beta$  transforming growth factor reversibly inhibits the early proliferative response to partial hepatectomy in the rat. *Proc Natl Acad Sci USA* 1988;85:5126–5130.
195. Solt DB, Medline A, Farber E. Rapid emergence of carcinogen-induced hyperplastic lesions in a new model for the sequential analysis of liver carcinogenesis. *Am J Pathol* 1977;88:595–618.
196. Iimuro Y, Nishiura T, Hellerbrand C, et al. NF $\kappa$ B prevents apoptosis and liver dysfunction during liver regeneration. *J Clin Invest* 1998;101:802–811.
197. Matsuda Y, Matsumoto K, Yamada A, et al. Preventive and therapeutic effects in rats of hepatocyte growth factor infusion on liver fibrosis/cirrhosis. *Hepatology* 1997;26:81–89.
198. Mizuno S, Kurosawa T, Matsumoto K, et al. Hepatocyte growth factor prevents renal fibrosis and dysfunction in a mouse model of chronic renal disease. *J Clin Invest* 1998;101:1827–1834.
199. Yaekashiwa M, Nakayama S, Ohnuma K, et al. Simultaneous or delayed administration of hepatocyte growth factor equally represses the fibrotic changes in murine lung injury induced by bleomycin. A morphologic study. *Am J Respir Crit Care Med* 1997;156:1937–1944.
200. Kosai K, Matsumoto K, Funakoshi H, et al. Hepatocyte growth factor prevents endotoxin-induced lethal hepatic failure in mice. *Hepatology* 1999;30:151–159.
201. Kosai K, Matsumoto K, Nagata S, et al. Abrogation of Fas-induced fulminant hepatic failure in mice by hepatocyte growth factor. *Biochem Biophys Res Commun* 1998;244:683–690.
202. Ueki T, Kaneda Y, Tsutsui H, et al. Hepatocyte growth factor gene therapy of liver cirrhosis in rats. *Nat Med* 1999;5:226–230.
203. Masunaga H, Fujise N, Shiota A, et al. Preventive effects of the deleted form of hepatocyte growth factor against various liver injuries. *Eur J Pharmacol* 1998;342:267–279.
204. Yasuda H, Imai E, Shiota A, et al. Antifibrogenic effect of a deletion variant of hepatocyte growth factor on liver fibrosis in rats. *Hepatology* 1996;24:636–642.
205. Tsubouchi H, Horono S, Gohda E, et al. Clinical significance of human hepatocyte growth factor in blood from patients with fulminant hepatic failure. *Hepatology* 1989;9:875–881.
206. Tsubouchi H, Hirono S, Gohda E, et al. Human hepatocyte growth factor in blood of patients with fulminant hepatic failure. I. Clinical aspects. *Dig Dis Sci* 1991;36:780–784.
207. Tsubouchi H, Niitani Y, Hirono S, et al. Levels of the human hepatocyte growth factor in serum of patients with various liver diseases determined by an enzyme-linked immunosorbent assay. *Hepatology* 1991;13:1–5.
208. Mizuguchi T, Hui T, Kamohara Y, et al. IL-6 and TGF- $\beta$  1 downregulate *c-met* expression through suppression of Sp-1 DNA binding activity. Abstract 928, *Hepatology* 1999;30:392A.

