جامعة الزقازيق
كلية طب بنها
قسم الباثولوجيا الإكلينيكية

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Hepatocyte Growth Factor

Previous studies have shown that liver regeneration is associated with the appearance of hepatic mitogenes in the peripheral blood. The search for the nature of these mitogenes has identified only two substances in peripheral blood capable of stimulating hepatocytes proliferation . These substances were labeled as hepatopoietins A and B.

It was be subsequently shown that hepatopoietins A is identical to the hepatocyte growth factor (HGF) identified later.

(HGF) was first identified in the serum of partially hepateoctomized rats and purified from rat platelets and human plasma *(Gohda et al., 1988).* HGF exhibits multiple biological activities on a wide variety of cells and has mitogenic, motogenic (enhancement of cell motility), morphogenic, and anti-apoptotic activities. These biological activities are important for organization of tissue structures during development and regeneration. Several lines of evidence indicate an important role for HGF in development and morphogenesis of epithelial organs, including the liver , placenta, lung ,tooth , mammary gland and kidney Essential roles of HGF in the development of mammalian fetal tissues were also defined by disruption of HGF or the *c-Met* gene in mice. These mice with targeted mutation of the HGF or c-Met gene died at embryonic day 13-15 due to impaired organogenesis of the placenta and liver. *(Matsumoto K et al .,1998)* .Acting as a motogen , HGF induces cell movement and while acting as morphogen, it induces the formation of tissue-like structures for various cell types *(Montesaiw Jt. et 01.1991).*

HGF is the stimulus for DNA synthesis in hepatocytes . Furthermore

,HGF has other biological activities such as dissociation of epithelial cells , growth inhibition of some tumour cells and tubule formation by kidney epithelial cells . *(Montesano et al., 1991).*

The serum levels of HGF are elevated not only in human patients with fulminante hepatitis , but also in those with chronic hepatitis and cirrhosis. HGF messenger RNA (mRNA) is expressed in various tissues. (Tashiro k . *et al., 1991)* Serum human hepatocyte growth factor levels were increased in correlation with derangements of thrombin time , total bilirubin and other parameters reflecting hepatocellular dysfunction in patients with chronic liver disease .This may indicate that increased serum hHGF levels were correlated with AST and ALT , could be the result of hepatocellular dysfunction . Furthermore , the levels were correlated with , parameters of hepatocellular necrosis , when hepatocellular dysfunction was not so severe . It was strongly suggested that the increase of serum hHGF levels in patients with liver disease was produced by hepatocellular necrosis and dysfunction .

Serum hHGF levels were markedly higher in patients with fulminant hepatic failure than in those with chronic hepatic failure irrespective of severity of hepatic failure .The difference of serum hHGF levels between two types would be due to the difference in the extent of hepatocellular necrosis. This difference of serum hHGF levels may provide a useful tool to differentiate fulminant and chronic types of hepatic failure . ***(Kinoshta. T** et al., 1990).*

The native form of HGF (pro - HGF) is synthesized as a single chain precuser protein consisting of 728 Amino Acids and requiring protease activator such as urokinase plasminogen activator.

Damaged hepatocytes taking up HGF may provide protease that activate single-chain HGF molecule into an active heterodimer (Mature HGF), This active hetrodimer is consisting of heavy α-chain (69Kilo Dalton) and light β-chain (34Kilo Dalton) . *(Nakmura et al., 1989).*

The heavy α -chain consists of 4 kringle domains similar to the kringle domains described for other proteins . Kringles are double-loop polypeptide structures in which a smaller loop is held together with disulfide bonds within a larger loop. The kringle domains present in HGF have substantial sequence similarity to kringle domains of plasminogen and prothronbin. The light β -chain has the structure of pseudo -protease (of the serine protease superfamily) The formation of two chains is essential for the biological activity of human HGF , since mutants which are not cleaved into both chains have no biological activity .The formation of two chains is derived by cleavage of the single chain HGF molecule next to Arginine (in position 494) and before Valine (in position 495) *(Kinoshita T, Hirao s, et al .,1990).*

Several functions have been assigned to kringles in other proteins, which in general are related to the abilities of kringle-bearing proteins to hind or intercalate with other proteins.

The amino Acid sequence of human HGF is more than 90% identical to that of rat HGF , thus HGF has no species specificity with regard to biological activities *(Nakamura T et al., 1989)*

HGF is internalized into the cells through a pathway mediated by high affinity HGF receptor known as c-mct protein.

HGF bound to the c-met receptor is rapidly internalized in the cells , degraded and finally released from the cells.

HGF promotes proliferation of the hcpatocytes by its interaction with the cell surface receptors (c- met protein).

1- SOURCES OF HEPATOCYTE GROWTH FACTOR:-

HGF is present in measurable amounts in normal plasma . Increased amounts of HGF present in serum led to the isolation of HGF form platelets. HGF mRAN was found in Northern blots from the adult rat lung , kidney , brain , liver , thymus and submandibular gland . HGF mRNA was also found in human fetal liver , placenta and embryonic lung. Previous studies localized HGF mRNA in nonparenchymal cells. The main cell type producing HGF in the liver is the cell of Ito. Also endothelial cells and Kupffer cells are the sources of HGF in normal and damaged livers . Ito cells appear to be the main type producing HGF mRNA in the normal liver. HGF was also localized in several other tissues using HGF-specific antibodies. In the liver, as mentioned above, HGF was localized in nonparenchymal cell types . HGF was not found in hepatocytes from the normal or regenerating liver *(Zarnegar R et al, 1989)*

In the central nervous system, **HGF** was found in large neurons of brain cortex and cerebellum and motor neurons of the spinal cord. In normal placenta, HGF was localized in the syncytiotrophoblast. HGF was also found throughout the gastrointestinal mucosa, in all squamous epithelia (including uterine cervix and Hassall's corpuscles of the thymus) and iii all lining glanduliir epithelia, including breast ducts, tracheobronchial columnar epithelium and prostate glands *(Wolf HK, zarnegar el at., 1991).* Human ovum also appeared to contain **HGF** .

In the kidney, **HGF** .was found primarily in the distal tubules and collecting ducts.

The presence of **HGF** in many tissues feeding into the portal circulation suggests that trophic effects for the liver through the portal circulation may be exercised through **HGF** from sites other than intrahepatic

If human hepatocyte growth factor is produced only by mesenchymal cells but sequestrated and active only on parenchymal and epithelial cells, it may be a very important factor for mesenchymal - parenchymal cells interaction which is a very interesting point in different liver diseases.

2- EFFECTS OF HGF ON DIFFERENT CELL TYPES

a) Mitogenic Effects

Given the fact that HGF was isolated using the bioassay of the stimulation of DNA synthesis in primary cultures *of* hepatocyes, the effects of HGF on hepatocytes are the best characterized. HGF is mitogenic for the rat and for human hepatocytes maintained in scrum free, chemically defined media and in the absence of other mitogenic factors. The effect is seen in doses as small as 1 ng/ml, with a maximal effect seen in the range of 5 to 10 ng/ml. On a molar basis, **HGF** is the most potent mitogen for hepatocytes *(Zarnegar R, et al, 1989).*

The effect of HGF is inhibited by transforming growth factor β (TGFP), and enhanced by norepinephrine *(Lindroos P.M., et al 1991).* Additive effects are seen between **HGF** and epidermal growth factor (EGF).

In addition to **DNA** synthesis, **HGF** also stimulates protein synthesis in hepatocytes. This effect is not substantially inhibited by **TGFβ** .

In addition to mitogenic effect on hepatocytes, several recent papers describe mitogenic effects of **HGF** on other cell types. **HGF** Stimulates **DNA** synthesis in renal proximal tubular epithelial cells, melanocytes, and keratinocytes . In addition, HGF suppresses the proliferation of tumors cells such as hepatoma cells and melanoma cells. HGF may play a major role in tissue repair and organogenesis through these pleiotropic activities . So, HGF may prove to be a useful treatment to accelerate liver regeneration . to suppress the growth of hepatoma and to prevent the onset of hepatitis or intrahepatic cholestasis induced by drugs **(Tajima H, et al ., 1991).**

HGF did not stimulate **DNA** synthesis in fibroblasts. The effects on tubular epithelium are additive with **EGF** and **aFGF** and inhibited by **TGF β**. The effects on melanocytes are also inhibited by **TGF β**.

b) Effects On Cell Motility

Several epithelial and endothelial cell lines " scatter " on the addition of **HGF** to the medium. The scattering effect is best summarized as the dissociation and migration, and it is precisely measured in specific assays. The effect is seen in variable concentrations. The concentrations causing scattering effects and mitogenic effects do not always coincide, and it has been stated that **HGF** exercises only scattering effects in some cell lines in the absence of mitogenic activity ***(Stoker M, et at.,1987).***

Scattering effects are induced only on epithelial and endothelial cells, not on fibroblasts or mesenchymal cells. It should also be mentioned that **HGF** has both mitogenic and "scattering" effects on hepatocytes in identical concentrations ***(Zarnegar R ,et al ,.1989).*** The addition of **HGF** in hepatocyte cultures induces the formation of long processes and cell migration. These morphological changes appear before and during DNA synthesis and arc much more pronounced than similar changes induced by **EGF**.

c) Tumor Cytotoxic Effects

High concentrations of HGF induce inhibited growth or arc cytotoxic to some carcinoma and sarcoma cell lines- In addition , **HGF** suppresses the proliferation of tumors cells such as hepatoma cells and melanoma cells. ***(Tojima H, et al ., 1991)***

3- HGF C-met protein Receptor:

It is a proto-oncogene receptor for hepatocyte grown factor (HGF***)***

The c-met proto-oncogne is a receptor like tyrosine kinase composed of disulfide - linked subunits of 50 KD (α subunit) and 145 KD (β subunit) ***(S-Giordono et al .,1989).***

In the fully processed c-met receptor the u subunit is extracellular, and the β subunit has extracellular binding domains,trans membrane portions and intracytoplasmic tyrosine kinase domains as well as sites of tyrosine phosphorylation ***(Bottaro DP.Rubin JS. Et al .,1991)***

HGF binding to c-met receptor induces rapid phosphorylation of the intracytoplasmic tyrosine residues on the c-met β-chain ***(Naldini et al., 1991)***

The HGF receptors encoded by c-met is distributed in many tissues including the liver, skin , kidney , lungs , uterus and brain .

The widespread distribution of the c-met protein receptor demonstrates the importance of HGF not only for liver regeneration but also for growth and differentiation in many other tissues.

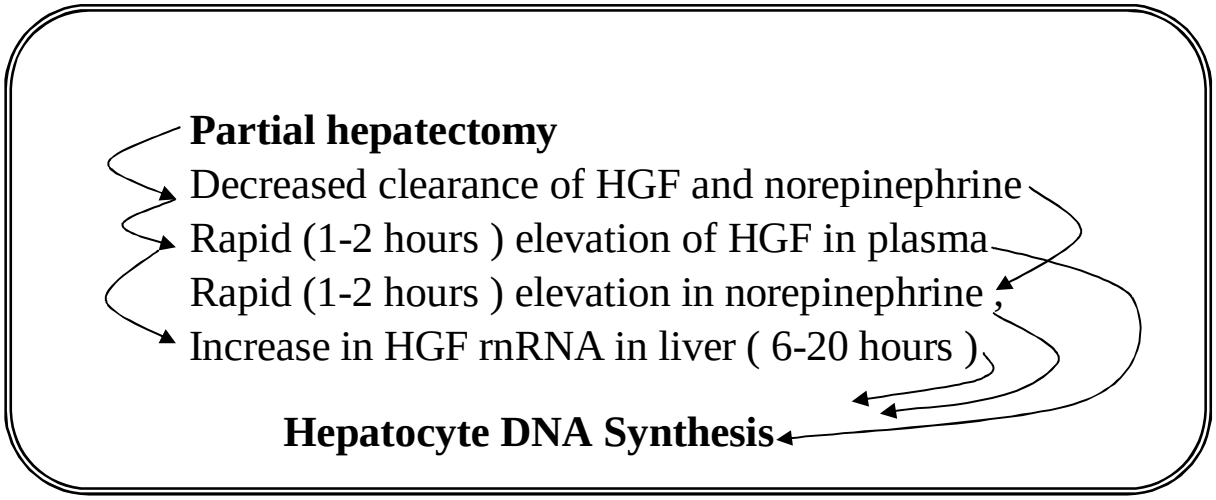
Recent studies have localized the **HGF** gene on human chromosome 7. The gene encoding the c-met receptor of HCF is also localized on the same chromosome and in close proximity ***(Zarnegar at el ., 1991)***.

4- HGF and liver regeneration

The elevation of HGF protein in the plasma before the elevation of **HGF**mRNA in the liver clearly suggests that the **HGF** increase in the plasma is not the result of increased synthesis from hepatic sources. Although platelets contain large amounts of **HGF**, the induction of severe

thrombocytopenia in rats did not interfere with liver regeneration, suggesting that platelets are not involved With the generation of any relevant autogenic signals for this process. Given the wide tissue distribution of **HGF**, it is conceivable that **HGF** is released in the plasma from a variety of sources. If circulating **HGF** is normally cleared by the liver, acute reduction in liver mass (alter hepatic resection) or loss of the capacity to clear **HGF** (after toxins) may explain the acute rise of **HGF** before **DNA** synthesis. Such acute elevation of plasma values also known to occur for norepinephrine. Norepinephrine is not a direct mitogenic substance for hepatocytes but it amplifies the mitogenic effect of **HGF** and **EGF** on the hepatocytes ***(Lindroos P.M. et al, 1991)*** . Us rise in the plasma after two-thirds partial hepatectomy follows similar kinetics with the rise of **HGF** . Because circulating norepinephrine is known to be . degraded by the hepatic monoamine oxidase, the removal ot two-thirds

of the liver results in a dramatic decrease in the capacity to degrade norepinephrine, resulting in increased concentration in the plasma. Norepinephrine is one of the strongest co-mitogens for hepatocytes in culture. We have proposed that the simultaneous elevation of **HGF** and norepinephrine very soon after partial hepatcetomy provides the mitotic stimulus leading to liver regeneration . ***(Lindroos P.M. et al., 1991)***



The above findings are important in that they provide the first evidence for the emergence of a mitogenic signal for the liver that precedes hepatocyte **DNA** synthesis ***(Kan M, et al., 1989).***

Tsnbouehi el al .,(1993) have pointed out that there are two mechanisms involved in liver regeneration after injury .One is a paracrine mechanism and the other is an endocrine mechanism .

The sharp changes in **HGF** concentrations in the plasma after two-thirds partial hepatectomy or during hepatic failure reported by ***Tomiya et al., (1992)*** suggest the possibility that **HGF** production and release by several tissues may have an endocrine role for the liver. Paracrine effects might also be of importance by way of **HGF** produced by the cells of Ito .

HGF In Renal Rengeneration And Renal Disease

Hepatocyte growth factor (**HGF**) has mitogenic , motogenic, morphogenic. and anti-apoptotic activities on renal cells and is a potential renotropin for renal protection and repair, in chronic renal failure/fibrosis, HGF in the kidney declines in a reciprocal manner to the increase in transforming growth factor-[beta] (TGF-[beta]). Neutralization of HGF by the antibody leads to acceleration of renal failure/fibrosis while HGF administration leads to remarkable attenuation, thus indicating the importance of HGF versus TGF-[beta], counterbalance in both pathogenesis and therapeutics in cases of chronic renal failure A recent study in laboratory animals indicated that decreased expression of HGF is associated with the onset of chronic renal failure/fibrosis which supplements of HGF can prevent.***(Matsumoto K .et al., 2000)***,

a) Biological actions in the kidney:-

HGF has mitogenic action on proximal tubular cells in primary culture and on renal epithelial cell lines. HGF protects renal epithelial cells from apoptotic cell death induced by serum starvation and by cisplatin ***(Matsumoto K. et al., 2000)***. The morphogenic activity of HGF to induce branching tubulogenesis is especially characteristic.

Importantly, HGF induces branching tubulogenesis of primary cultured renal proximal tubular cells while other growth factors, including fibroblast growth factor-1 and -7, epidermal growth factor, or insulin-like growth factor-, failed to induce this morphogenesis

(Matsumoto K. et al., 2000). Transforming growth factor-[beta]1 (TGF-[beta]l), by contrast, blocked HGF-induced branching tubulogenesis and proliferation of proximal tubular cells. In tubular regions, HGF is expressed in interstitial cells, probably endothelial cells and macrophages . Thus HGF is a stromal signal for renal tubular protection, growth, and morphogenesis.

In the glomerulus , HGF that is produced by mesangial and endothelial cells is negatively regulated by TGF β1 and angiotensin II, ***(Matsumoto K. et al., 2001).***

b) Renotropic role of HGF in acute renal injury:-

HGF levels rapidly increased in patients with acute renal failure and with acute renal rejection after renal transplantation . It is noteworthy that c-Met/HGF receptor mRNA expression is also upregulated in response to acute renal injury ***(Ishibashi K et al ., 1992).***

Rapid increases in HGF levels in the kidney and blood , as well as an increase in the c-Met HGF receptor mRNA level, reflect compensatory responses to renal injuries , to prevent acute tubular death and to induce renal regeneration. Evidence for this hypothesis was obtained by experiments in which HGF was administered to laboratory animals. Recombinant HGF stimulates renal regeneration after unilateral nephrectomy , acute renal injuries induced by cisplatin, and acute renal ischemia . Compared to controls, rats given HGF after acute renal injuries had more satisfactory renal functions, much less histological damage, and reduced mortality;

onset of acute renal failure was prevented by HGF ***(Kawaida K , et al ., 1994)*** . These results strongly suggest that HGF enhances renal regeneration and affords protection to the kidney subjected to acute renal injury.

c) HGF in chronic renal failure/fibrosis.

Fibrotic organ diseases such as liver cirrhosis, lung fibrosis, and chronic renal failure/fibrosis are devastating to organ function and arc progressive diseases characterized by hyperaccumulation of extracellular matrix (ECM) components and withdrawal of parenchymal (or epithelial and endothelial) cells. The long-term overproduction of transforming growth factor-[beta] (TGF-[beta]) is a key event leading to the pathology of various experimental and human fibrotic disorders ***(peters H , et al ., 1997)*** . TGF-[beta] potently stimulates ECM deposition, inhibits matrix degradation, and induces growth arrest and apoptosis of epithelial and endothelial cells. In experimental animals, intravenous administration and transgenic expression of TGF-[beta] showed the kidney to be highly susceptible to glomerular and interstitial fibrosis -The same seems to apply to chronic renal disease in humans, where excessive TGF-fbeta] has been noted in cases of glomerulonephritis, diabetic nephropathy, and hypertensive glomerular injury ***(Border WA et al, 1998)*** Recent studies clearly demonstrated that endogenous HGF protects the nephrotic kidney from fibrosis .In the ICR strain-derived glomerulonephritis (ICGN). Administration of recombinant HGF to ICGN mice strongly suppressed TGF-[beta] expression and prevented the onset of renal fibrosis and chronic renal failure.

All these findings mean that the reciprocal balance between TGF-[beta] and HGF is closely associated with the pathogenesis of renal fibrosis. Not only an increase in TGF-[beta] levels, but also a decrease in renal HGF expression may be responsible for the occurrence of renal fibrosis ***(Matsumoto K. el al., 2001)***.

HGF Gene Therapy For Liver Cirrhosis

Liver cirrhosis is the irreversible end result of chronic liver disease an is characterized by fibrous scarring and hepatocellular regeneration, it is a major cause of morbidity and mortality worldwide and is induced by factors such as chronic hepatitis virus infection, drug abuse, bilharzial infection and alcohol abuse. The ideal strategy for the treatment of liver cirrhosis should include prevention of fibrogenesis, stimulation of hepatocyte mitosis and reorganization of the liver architecture. Hepatocyte growth factor (HGF) gene therapy has been investigated in a rat model of liver cirrhosis. In rats with lethal liver cirrhosis produced by dimethylnitrosamine, repeated transfection of the HGF gene into skeletal muscle induced a high plasma level of HGF and tyrosine phosphorylation of the c-Met/HGF receptor. Hepatocyte growth factor gene transduction inhibited fibrogenesis and hepatocyte apoptosis and also produced resolution of fibrosis in the cirrhotic liver. Hepatocyte growth factor gene therapy

may have the potential to be useful for the treatment of patients with liver cirrhosis.

1- Role Of Transform in a Growth Factor In Liver Fibrosis:-

Although the molecular mechanisms of hepatic fibrosis are not fully understood, several studies have demonstrated that over expression of transforming growth factor (TGF)-[beta]1 plays a pivotal role in the progression of fibrosis ***(Friedman sl. et al., 1993)*** It has been reported that several lines of TGF-beta]l transgenic mice have high plasma levels of TCF-(beta)l and develop liver fibrosis. Transforming growth factor-[beta] 1 induces the phenotypic transition of hepatic stellate cells into proliferating myofibroblast-like cells, which enhance production of extracellular matrix (ECM)

components, and attenuates the degradation of ECM proteins ***(Nakamura et al.,1986)***. Chronic hepatitis virus infection is the most common aetiological factor in human liver cirrhosis. Transforming growth factor-detail mRNA expression is closely correlated with the fibrogenic activity in liver tissues of chronic liver disease induced by hepatitis B or C viruses (HBV and HCV, respectively).

2-Hepatocyte growth factor gene transfection and transduction:-

Several approaches, including studies on transgenic animals as well as *in vivo* infusion of HGF into animals, have revealed that HGF plays an essential role in both the development and regeneration of liver and have shown that HGF has anli-apoptotic and cytoprotective effects on hepatocytes . In addition, ***(Matsuda. et al ., 1997)*** reported that HGF. Showed antifibrogenic activity in a rat liver fibrosis model.

A number of transfection methods and vector systems have been used to deliver foreign genes into the liver. For example, retroviral vectors have been used for transfection of the rat liver in combination with partial hepatectomy. However, the transfection efficiency remains relatively low. Adenoviral vectors appear to be more efficient for transfection of hepatocytes *in vivo*, but these vectors cause cytotoxicity because of the high immunogenicity of adenoviral proteins. A new gene transduction method using liposomes containing haemagglutinating virus of Japan (HVJ-liposomes) has been developed. Recently, it was reported that more than 60% of hepatocytes expressed an exogenous gene when transfected with HVJ- liposomes ***(Hirano .T .,et al ,. 1998).*** Furthermore, repeated *in vivo* transfection was shown to prolong exogenous gene expression in the rat liver and did not induce cytotoxic T lymphocyte (C'TL) activity against transfected hepatocytes.

3-Hepatocyte growth factor gene therapy for liver cirrhosis:-

We have developed a novel form of gene therapy for rat liver cirrhosis by transfer of the HGF gene into skeletal muscle *(Ueki T. et al .,1999).* Dimethylnitrosamine (DMN)-induccd cirrhosis in rats is characterized by the collapse of parenchymal cells and the formation of regenerative nodules separated by fibrous septa, similar to the pathological cirrhotic changes found in humans. If left untreated, the cirrhotic rats die within 7 weeks due to liver dysfunction. In rats with

DMN-induced liver cirrhosis, hepatic TGF-[beta]1 expression showed an increase during the progression of cirrhosis. Of particular interest was our finding that expression of TGF-[beta]l was strongly suppressed after treatment***(Ueki T. el at, 1999).*** Consequently, the transition from hepatic stellate cells (o myofibroblast-like cells in the liver was inhibited . Moreover, HGF gene therapy prevented the apoptosis of hepatocytes after administration of DMN. Transduction of the HGF gene suppressed the increase of TGF-[beta]l after exposure to DMN. Thus, HGF gene therapy may have improved fibrosis in the cirrhotic liver by inhibiting TGF-[beta] 1 expression, but the molecular mechanisms by which HGF suppresses TGF-[beta]l remains to be addressed.

Hepatocyte growth factor stimulated hepatocyte mitosis in the cirrhotic rat liver, which played an important role in the progress of liver regeneration. Moreover, histological examination revealed that the lobular and vascular architecture was well organized after gene therapy, while measurement of portal pressure revealed that portal hypertension was markedly improved ***(Ueki T. et al ., 1999).*** These findings indicate that HGF gene therapy results in the prevention of fibrogenesis, stimulation of liver regeneration and reorganization of the hepatic architecture, suggesting that it should be taken into

consideration as a potential treatment for liver cirrhosis.

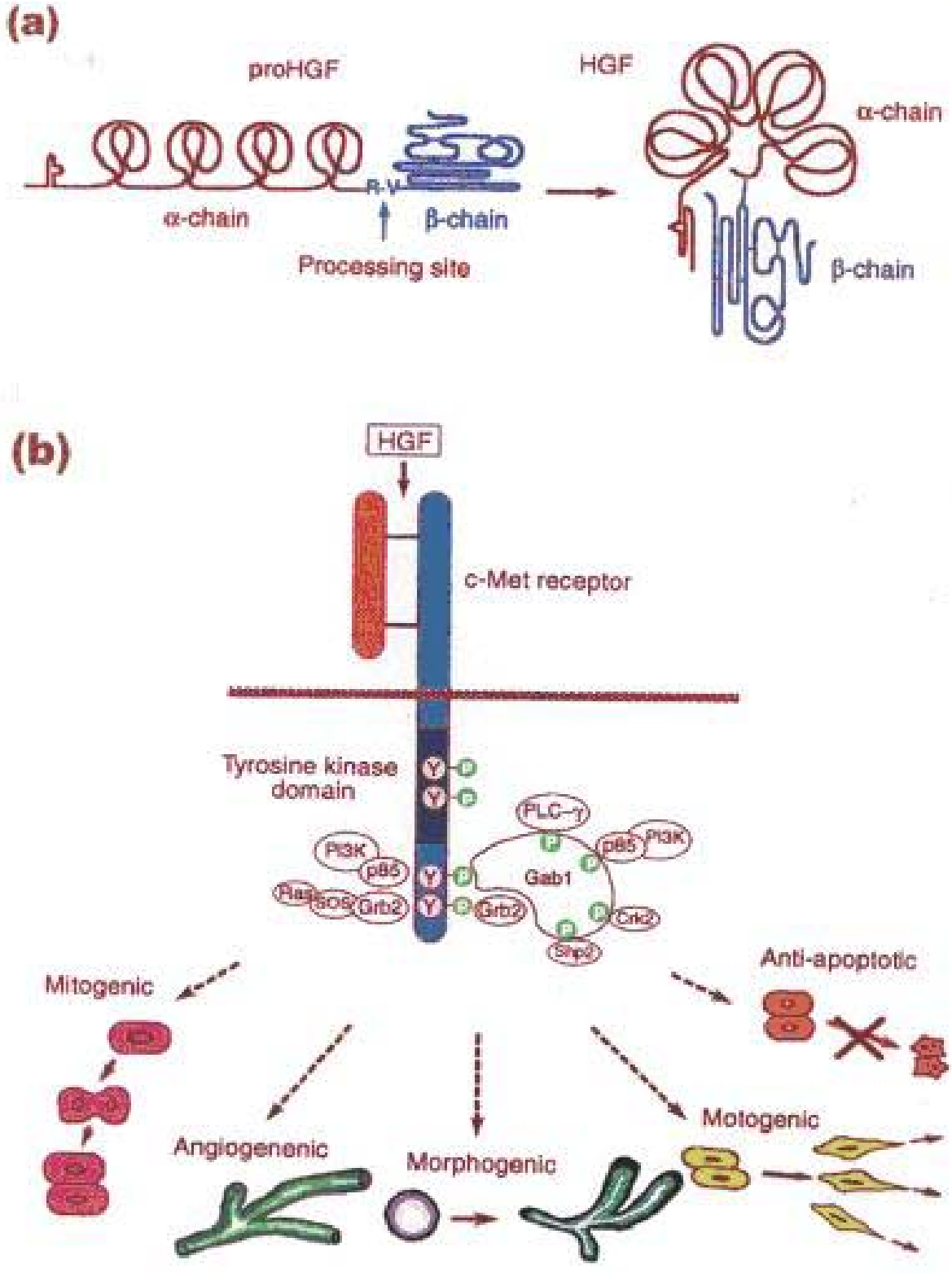
It is of note that transfection of rats with 40[mu]g HGF DNA rescued all animals from fatal liver cirrhosis and that their cirrohosis was completely suppressed. it is theoretically inadvisable to place damaged hepatocytes at risk as targets of transfection .

The direct damaged hepatocytes at risk as targets of transfection .The direct injection of liposomes containing DNA and the envelope protein of HVJ into skeletal muscle is a useful way to deliver a protein into the systemic circulation. This method can be applied to living animals because of its simplicity, safety and lack of toxicity. A sustained plasma level of HGF was achieved by repetitive transfection of the HGF gene and there was no significant inflammation or activation of the cellular and humoral immune system ***(Ueki T. et al, 1999).***

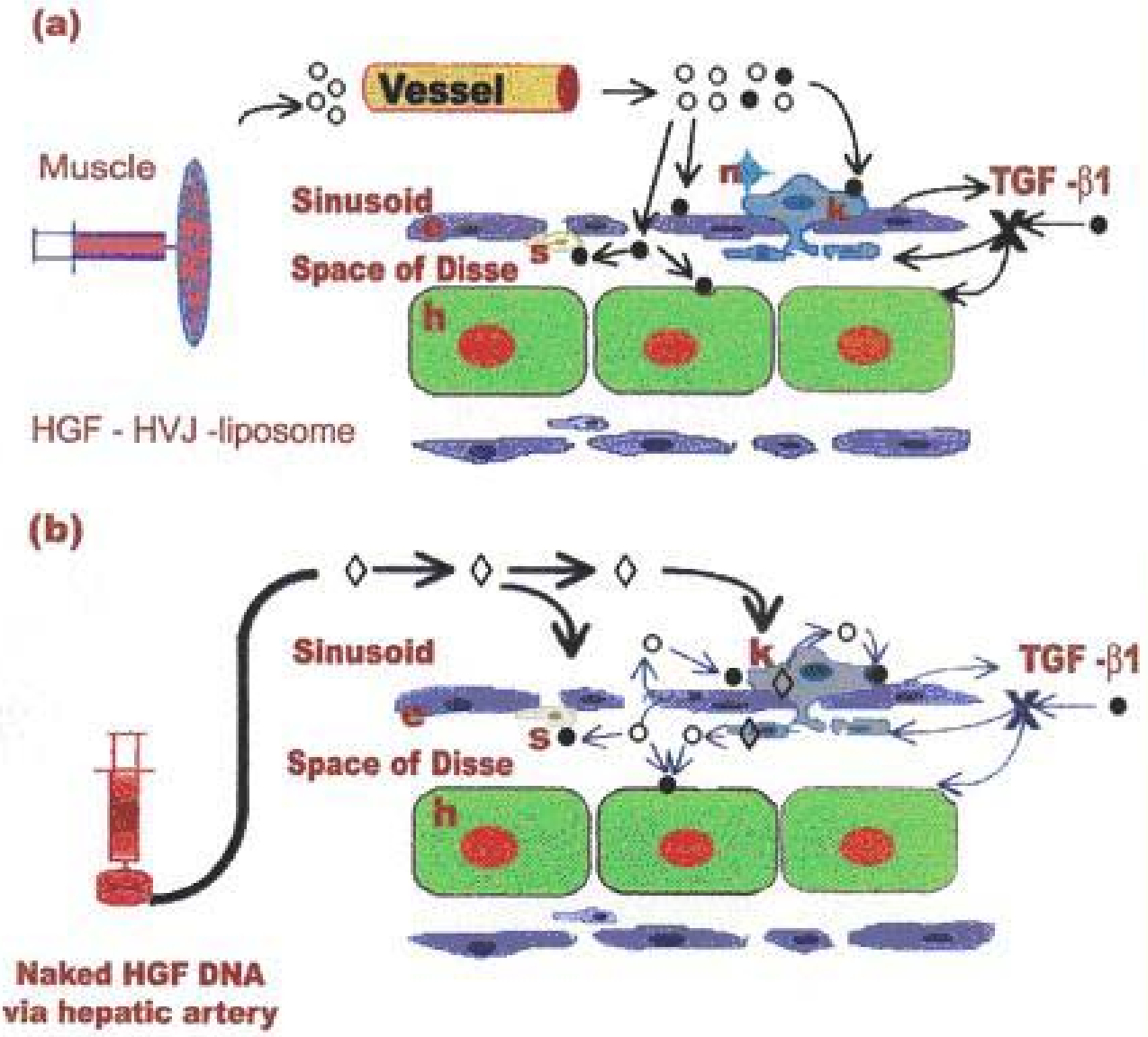
The first successful trial of transgene expression in muscle involved the direct injection of DNA encoding various marker proteins and resulted in gene expression in the muscle fibres for more than 2 months. However, HVJ-liposome-mediated gene transfer to muscle has shown a much higher efficiency than that obtained by naked DNA injection. So the extension of this approach to large animals should be investigated further.

Repeated *in vivo* transfection using HVJ-liposomes is simple and safe and can be done without causing substantial inflammation or activation of cellular and humoral immunity. Another new approach, infusion of naked plasmid DNA via the hepatic artery, is also safe and can be done without eliciting a strong host immune response. Using these newly

developed methods, HGF gene therapy may eventually be applied clinically for the treatment of patients with liver cirrhosis which is otherwise fatal and unresponsive to conventional therapy ***(Fujimoto J. et al., 2000) .***



Schematic structures. (*A*) Prohepatocyte growth factor (HGF) and mature HGF. (*B*) Typical biological activities of HGF mediated by c-Met/HGF receptor and intracellular signal transducers which associate with tyrosine-phosphorylated c-Met.



Proposed mechanisms of hepatocyte growth factor (HGF) gene therapy. Injection of HGF-HVJ-liposomes into muscle leads to release ofpro-HGF into the blood stream. Pro-HGF is converted to the active form (mature HGF) in the injured liver by activators, such as urokinase plasminogen activator. (a) Mature HGF has biological effects on hepatocytes, endothelial cells, Kupffer cells, natural killer cells and stellate cells, (b) Naked HGF plasmids can be delivered via the hepatic artery and efficient HGF gene expression can be obtained in the liver using this approach. Nonparenchymal cells produce Pro-HGF and secrete it into the sinusoid and the space of Disse. After conversion into the mature form, HGF acts on liver cells.

e = endothelial cell.
K= Kupffer cell.
S=stellate cell.
O=pro-HGF.
h= hepatocyte.
N= natural killer cell.
◊=plasmid-HGF vector.
●= mature HGF.