Review

Hepatocyte growth factor activators, inhibitors and antagonists and their implication in cancer intervention

C. Parr and W.G. Jiang
Metastasis Research Group, University Department of Surgery, University of Wales College of Medicine, Heath Park, Cardiff, UK

Summary. Hepatocyte growth factor (HGF), otherwise known as scatter factor (SF), has been demonstrated over the past decade to elicit a number of functions that may be tumorigenic, and enhance the invasive/metastatic nature of cancer cells. Clinical studies have also demonstrated that HGF/SF, together with its receptor, cMET, is closely related to the disease progression and prognosis of patients with cancer. The past few years have seen the identification of numerous inhibitors and antagonists to the action of HGF/SF. These factors have demonstrated a possible role in minimising the action of HGF/SF on cancer cells, and may be of therapeutic value in the future. This article overviews the activators, inhibitors, and antagonists to HGF/SF and discusses the possible implications in cancer therapy.

Key words: Hepatocyte growth factor, cMET proto-oncogene, HGF/SF antagonists, HGF/SF activator, HGF/SF activator inhibitor

Introduction

Since the discovery of HGF/SF (hepatocyte growth factor/scatter factor) in the eighties, there has been vigorous interest to determine its roles in cancer development and cancer metastasis. HGF/SF, via its action on its specific receptor, cMET, displays an array of biological functions in cancer cells, such as the enhancement of cell migration, invasion and matrix degradation, and induction of angiogenesis. Its role in cancer development and progression has also been confirmed in clinical studies, where the level of HGF/SF and its receptor are associated with the disease progression and prognosis in patients bearing cancer. Recent efforts have also led to the discovery of a few agents/factors that act as inhibitors to the action of HGF/SF in cancer cells. This article will first briefly examine the action of HGF/SF on cancer cells and go on to discuss the activators and potential inhibitors for HGF/SF.

HGF/SF elicits wide spectrum of effects on cancer cells and plays an important role in cancer progression

The discovery of HGF/SF indicated its importance on hepatocyte and epithelial cells. Subsequent developments and identification of the HGF/SF receptor has strongly indicated a role for HGF/SF and its receptor in cancer and cancer progression (Fig. 1) (Jiang and Hiscox, 1997a; Jiang et al., 1999a,b).

HGF/SF affects tumour cell proliferation, growth, and cell cycle regulation

Although the initial interest in HGF/SF was on cell growth in hepatocytes, it was soon realised that HGF/SF also governed the behaviour of cells other than that of liver cells. The factor has been shown to enhance the growth of a number of cancer cell types, including ovarian cancer cells (Corps et al., 1997), endometrial epithelial cells (Sugawara et al., 1997), neonatal hepatocytes (Pagan et al., 1997), hematopoietic progenitor cells (Weimar et al., 1995), gastric cancer cells (Kaji et al., 1996), renal cell carcinoma cells (Kobayashi et al., 1994), pancreatic cancer cells (Direnzo et al., 1995; Hasegawa et al., 1995) and prostate cancer cells (Humphrey et al., 1995). HGF/SF regulated growth of cells appears to depend upon the type of tumour cells, as it exerts an inhibition of the growth of the melanoma cells, squamous cell carcinoma (SCC) cells, hepatocellular carcinoma (HCC) cells, and colon cancer cells (Tajima et al., 1991; Jiang et al., 1993a). HGF/SF has been reported to induced apoptosis in ovarian epithelial cells (Gulati and Peluso, 1997) and regulate cell cycle progression in tumour cells (Liu et al., 1997).

HGF/SF and cancer invasive/metastatic behaviour

The presence of metastatic disease in cancer patients
HGF/SF inhibitors in cancer

is the most significant factor affecting their survival (Stracke and Liotta, 1995). HGF/SF has been shown to affect a number of events in the metastatic process (Fig. 1).

1. HGF/SF inhibits the assembly of adherens junctions

Pasdar et al. (1997) showed that HGF/SF treated cells failed to form stable cell-cell adhesion junctions due to an increased stability of the newly synthesized soluble E-cadherin and an altered phosphorylation pattern of E-cadherin within the cell. HGF/SF has the ability to dissociate epithelial colonies and thus its effects on cellular adhesion molecules such as the caderhins has been studied. Cadherin function is modulated by intracellular proteins, termed catenins, that act as molecular linkers between the cytoplasmic domain of the cadherin protein and the cytoskeletal network within the cell (Jiang, 1996; Jiang et al., 1996a-c).

2. HGF/SF may promote the dismantling of cell-cell adhesion complexes

It has been postulated that HGF/SF may inhibit cadherin function by altering the phosphorylation of these cadherin-associated proteins thus affecting their binding to the intracellular portion of E-cadherin. Hiscox and Jiang (1998) have reported that HGF/SF enhances the tyrosine phosphorylation status of β-catenin and also results in the dissociation of β-catenin from E-cadherin. HGF/SF-induced tyrosine phosphorylation of β-catenin, which may down-regulate cadherin-mediated cell-cell adhesion by such a mechanism, has also been observed by others (Shibamoto et al., 1994; Tannapfel et al., 1994; Hiscox and Jiang, 1998). β-catenin also plays a central regulatory role in cell-cell adhesion and cell growth (Jiang and Hiscox, 1997b).

3. HGF/SF and cell-matrix adhesion

HGF/SF regulates matrix related parameters in cancer cells. The factor increases the adhesion of c-met positive B-lymphoma cells to fibronectin and also promotes their migration and invasion (Weimar et al., 1997). Since these interactions are mediated via β1, α4β1 and α5β1-integrins, it is possible that HGF/SF enhances the expression of such molecules on the cell surface. Evidence has shown that HGF/SF does indeed alter the integrin expression pattern on several cell types, increasing the expression of α2β1 on HepG2 liver cancer cells with a subsequent increase in the adhesive capabilities of these tumour cells toward both matrix and endothelial cells (Kawakamikimura et al., 1997). HGF/SF has also been shown to affect the initial recruiting of integrins, cytoskeletal proteins, pp125FAK and paxillin into focal adhesion complexes via the tyrosine kinase activity of c-met (Matsumoto et al., 1994; Jiang, 1996; Jiang et al., 1996a-c). Thus HGF/SF affects both cell-matrix receptors (integrins) as well as intracellular post-receptor events.

4. HGF/SF, matrix degradation and invasion

HGF/SF stimulates the invasion of tumour cells

---

**Fig. 1.** HGF/SF plays a multiple role in the development and progression of tumours. It influences the growth of malignant cells and affects a number of steps in the metastatic cascade.
through the matrix. The constitutively active met hybrid, TPR/c-Met, is known to activate the uPA gene (Besser et al., 1997), to stimulate the production of tPA and uPA (Morimoto et al., 1994), to increase the expression and secretion of plasminogen activator inhibitor type 1 (PAI-1) and tissue factor in the human hepatoma cell line, Hep G2 (Wojta et al., 1994). HGF/SF has also been shown to stimulate the production of matrix metalloproteinases (MMPs) by cancer cells (Bennett et al., 1997) and stimulate collagenase-1 and stromelysin-1 production in a dose-dependent and matrix dependent manner (Dunsmore et al., 1996).

5. HGF/SF and tumour intra- and extravasation, endothelial interaction and homing

HGF/SF increases the adhesion of tumour cells to the endothelium by regulating a number of events involved in this interaction (Kawakamikimura, 1997). HGF/SF increases the expression of CD44 on endothelial cells, a molecule which may play a critical role in tumour-endothelial interaction and the establishment of metastasis (Hiscox and Jiang, 1997b). Furthermore, HGF/SF also reduces the communication between the endothelial cells (Moorby et al., 1995; Jiang et al., 1997).

6. HGF/SF and tumour motility

HGF/SF acts as a motility enhancer in a variety of cancer cells, including ovarian cancer cells (Corps et al., 1997), colorectal cancer cells (Jiang et al., 1993b, 1996), breast cancer cells (Jiang et al., 1996b), melanoma cells (Jiang et al., 1993b) and lung cancer cells (Singhkaw et al., 1995; Harvey et al., 1996).

7. HGF/SF and angiogenesis

HGF/SF is a known potent angiogenic factor and acts via the stimulation of endothelial cell proliferation and motility in vitro, altering the adhesive property of endothelial cells, to facilitate the formation of capillary tubules, and the migration of endothelial cells in the matrix (Fig. 1) (Bussolino et al., 1992; Comoglio et al., 1993; Grant et al., 1993; Corps et al., 1997; Vanelle et al., 1998). In vivo studies using HGF/SF implants have revealed ingrowth of new blood vessels in mouse subcutaneous tissue and rat corneas (Rosen et al., 1990; Bussolino et al., 1992). Stimulation of human omentum mesentary endothelial (HOME) cells with HGF/SF in the presence of active tissue plasminogen activator (tPA) results in the outgrowth of tubule-like structures, suggesting a co-operative role for these factors in eliciting angiogenic responses (Morimoto et al., 1994).

HGF activator (HGFA)

Discovery of HGFA

HGF/SF was discovered as a single chain protein, or pro-HGF. This form of HGF/SF is inactive and unable to elicit any biological activities. This inactive single chain form of HGF/SF, pro-HGF/SF, requires site specific proteolytic cleavage, into the active heterodimeric form of HGF/SF before it can elicit any biological action.

Shimomura et al. (1992) reported the purification of a HGF/SF-converting enzyme present in fetal bovine serum, by monitoring HGF/SF conversion on SDS-page. Following this, the protein was purified from human serum. It was revealed that this serum protease was capable of converting pro-HGF/SF into the active form of HGF/SF in vitro, thus it was termed HGF Activator (HGFA) (Miyazawa et al., 1993). HGFA has since been shown to be the key mediator in the localised activation of HGF/SF in injured tissue (Miyazawa et al., 1996).

Biochemical characterisation and structure of HGFA

The chromosomal location of the HGFA gene has been determined as 4p16 (Miyazawa et al., 1998), and the complete sequence of the HGFA gene was found to cover about 7.5 kb of DNA, consisting of 14 exons separated by 14 introns. Initiation of transcription occurs at a site 75 bp upstream of the translational start codon, and the coding region is composed of multiple putative domains. The first translation product of HGFA is the HGFA precursor.

Many serine proteases are generated from their precursors, via limited proteolysis, upon the initiation of blood coagulation. HGFA appears to follow this trend, as HGFA also exists as a precursor form in the plasma. The HGFA precursor is made up of a single polypeptide chain, consisting of 655 amino acids, has a molecular weight of around 96kDa, and has no HGFSF converting ability (Shimomura et al., 1993). The cDNA sequence for this novel serine protease revealed that the active form of HGFA is derived from the COOH-terminal region of a precursor protein, and is composed of multiple domains.

Thrombin converts the HGFA precursor into the active form of HGFA

The HGFA precursor is inactive in plasma and does not possess the ability to convert single chain HGF/SF, as it requires activation to fulfill the function of HGF/SF activator. A presence in human serum initiates the activation of the HGFA precursor, as human serum revealed a high degree of HGF/SF converting activity. Shimomura et al. (1993), examined the ability of various serine proteases, from the blood coagulation and fibrinolysis mechanisms, to act as activators of the HGFA precursor. They identified thrombin as the most effective protease for cleavage of the precursor. This cleavage occurred, via limited proteolysis, at the bond between Arg407 and Ile408, in vitro. Thrombin therefore, links HGFA to the blood coagulation cascade, as upon initiation of blood coagulation, the serine protease thrombin is generated from its precursor, pro-thrombin. Once active, the HGFA protease then has the
ability to convert pro-HGF/SF into the active form of HGF/SF. It is by this method, that active HGF/SF is generated in the injured tissues (Miyazawa et al., 1994).

The bioactive HGFA molecule is created through activation of HGFA precursor molecule, resulting from the removal of the NH2-terminal end. Evidence suggests that the purpose of this discarded region may have been to aid in the binding of the precursor to negatively charged substances. This removed part of the precursor contains structural domains which appear to be involved in the interaction with anionic surfaces, and consists of type I and type II fibronectin domains, two EGF domains and a kringle domain. It has been shown in vitro that the inactive HGFA precursor can be effectively cleaved by thrombin, in the presence of negatively charged substances. This cleavage of the HGFA precursor results in the generation of several fragments, although the 2 major fragments produced are 66kDa and 34kDa in size. The 66kDa fragment represents the inactive NH2-terminal region of the precursor which may have been involved in the binding of the precursor to the cell surface for activation by thrombin (Furie and Furie, 1988). Whereas, the 34kDa fragment represents the active form of HGFA and is composed of the COOH-terminal region.

HGFA homologues

It was observed that the amino acid sequences of the HGFA peptide domains revealed a high degree of similarity to the corresponding region of other serine proteases. These proteases included blood coagulation factor XII (Hageman Factor), tissue-type Plasminogen Activator (tPA) and Urokinase. These serine proteases have in the past been reported to convert pro-HGF/SF into the active form of HGF/SF in vitro (Naldini et al., 1992; Mars et al., 1993). The degree of similarity between these factors and the HGFA precursor were as follows: Factor XII (47%), tPA (40%) and urokinase (38%) (Miyazawa et al., 1993). Factor XII is itself a precursor to the active serine protease Factor XIIa and shares a high degree of similarity to the HGFA precursor. His447, Asp497 and Ser598 are also present in the same corresponding position in each of the above serine proteases, and are present in the catalytic triad of the proteases and make up the active site.

HGF/SF has a very high sequence similarity to plasminogen, for this reason, the proteases that activated plasminogen were examined for their ability to activate HGF/SF. Reports suggested that pro-HGF/SF could be converted to HGF/SF by the serine proteases that activate plasminogen, these serine proteases included urokinase, tPA and plasmin (Gak et al., 1992; Naldini et al., 1992). Mizuno et al. (1992), however, reported conflicting results and showed that these enzymes did not induce any detectable level of HGF converting ability. Mizuno et al. (1994), then went on to purify and characterise a new serine protease that did reveal HGF/SF converting ability and designated it the HGF-converting enzyme. This enzyme is a disulphide-linked heterodimer with a molecular mass of about 90kDa, and is composed of a 65kDa heavy chain and a 32kDa light chain.

Serine proteases that share sequence similarities with HGFA (tPA, urokinase and Factor XIIa) had in the past been reported as possessing the ability to convert pro-HGF to the heterodimeric active form of HGF/SF. However, Shimomura et al. (1995), also reports that t-PA and urokinase are not the major HGF/SF converters, as the ability of urokinase and tPA to convert pro-HGF to active HGF/SF was over a 1000 times lower than that of HGFA and Factor XIIa.

The full nucleotide sequence for the HGFA precursor shares 39% homology with the multiple putative domains of blood coagulation Factor XII. The bioactive Factor XIIa is also similar to HGFA in that it is first synthesised as an inactive precursor and requires activation to elicit any biological function. Factor XII is activated via proteolytic processing with plasma kallikrein to generate Factor XIIa, however, plasma kallikrein did not possess the ability to convert the HGFA precursor into the active form (Shimomura et al., 1992). There is substantial similarity between the structural formations of HGFA and factor XIIa, as factor XII is composed of all the same structures as the HGFA precursor, plus has similar exon/intron arrangements. This could suggest that both HGFA and Factor XII genes could have originally been from the same ancestral gene, but are now different due to evolutionary divergence.

Recently, a novel plasma hyaluronan-binding protein (PHBP) was purified from human plasma, the amino acid sequence of which revealed significant homology to HGFA (31%) (Choi-Miura et al., 1996). The structural differences between HGFA and PHBP are that the HGFA precursor is composed of a characteristic structural domain, type I and II fibronectin domains, two EGF domains, a kringle domain and a serine protease domain. Also, PHBP is located on chromosome 10 (Sumiya et al., 1997), unlike HGFA, which is located on chromosome 4. The gene for PHBP also revealed significant similarity to FXII, tPA and urokinase, for these reasons PHBP may play a role in activation of HGF/SF or plasminogen.

The multiple functions of HGFA

The leading contenders for the major protease for HGF/SF conversion were HGFA, the HGF/SF converting enzyme and Factor XIIa. Human serum normally possesses a high pro-HGF/SF-converting ability. This serum was analysed in the presence of a monoclonal antibody (F1-4) known to inhibit the action of HGFA. The results revealed that the ability of human serum to convert pro-HGF into the active form was almost completely lost. Therefore, neither the HGFA-converting enzyme nor Factor XIIa are the major pro-
HGF/SF activators in human serum, as Shimomura et al. (1995), demonstrated that HGFA is the main protease responsible.

Prothrombin is activated to create the mature form of thrombin which plays a central role in blood coagulation. Thrombin is also responsible for the activation of the HGF precursor (Shimomura et al., 1993), thereby linking the HGFA precursor activation to the blood coagulation cascade. Factor XIa, which shares significant homology to HGFA, is the protease responsible for the initiation of the blood coagulation cascade. Reports suggest the conversion of the inactive HGFA precursor to the active form of HGFA is initiated in response to blood clotting following tissue injury. Once the HGFA protease is active it can convert pro-HGF to the active heterodimeric form of HGF/SF, thereby activating a multi-functional cytokine that facilitates tissue regeneration.

Once activated, HGFA also has the ability to interact with heparin, which may serve to aid the localisation of HGF/SF cleavage, and linking activation to blood coagulation. Proteolytic activation of HGF/SF occurs in the injured tissues, thus it seems evident that the activation system for HGF/SF can be regulated locally (Miyazawa et al., 1994).

Okajima et al. (1997), suggests that HGFA is an acute phase protein of the acute inflammatory response, as the HGFA mRNA level increases, following tissue injury resulting in acute inflammation. The precise role of HGFA in relation to the inflammatory response is unclear.

HGFA may also play a role in the conversion of pro-HGF to HGF/SF during the process of mammalian development, as HGF/SF has been shown to be a necessity for mouse development (Uehara et al., 1995).

**HGFA is responsible for HGF/SF activation**

During tissue damage, HGF/SF activation functions as a mechanism for localising the biological functions and role of HGF/SF in tissue regeneration. Furthermore, in cancer states, HGF/SF activation is thought to be a method of aiding tumour progression. Enzymatic activity, with the ability to convert pro-HGF/SF to HGF/SF, was observed in the injured liver. Other studies report that the level of active HGF/SF increased in the liver and kidney upon treatment with heparo- toxin and nephrotoxin, respectively (Fig. 2).

HGF/SF in its normal state can be found as the inactive precursor form of pro-HGF/SF. For HGF/SF to evoke its biological function as a mitogen, motogen and morphogen, it first has to be converted from the inactive single chain form of pro-HGF/SF to the heterodimeric form of active HGF/SF. Pro-HGF requires activation as it has no biological effect on its own. Although at one stage pro-HGF/SF was considered to possess a mitogenic ability on cultivated hepatocytes, but in the presence of leupeptin, an inhibitor for any protease activation of pro-HGF, this mitogenic function was lost (Mizuno et al., 1992).

Pro-HGF/SF is synthesised as a 728 amino acid precursor (Nakamura et al., 1989), then cleaved intracellularly through removal of the NH2-terminal end (31 amino acid residues) as a signal peptide (Yoshiyama et al., 1991). Pro-HGF/SF is secreted in this precursor form and undergoes proteolytic processing in human serum.

It has been shown that for HGF/SF to elicit its biological effects, it requires proteolytic activation from the single chain pro-form (Fig. 2) (Gak et al., 1992; Hartmann et al., 1992). This conversion requires site specific extracellular hydrolysis of the Arg494-Val495 peptide bond to yield a heavy of 69 kDa chain (α-chain) and a light chain of 34 kDa (β-chain), which are joined together by a disulfide bond. The β-subunit of the HGF/SF molecule contains a serine protease-like domain, although it has no protease activity because it lacks the presence of triad residues at the active site, which are conserved in the active serine proteases (Nakamura et al., 1989).

The HGF-converting enzyme was described by Mizuno et al. (1994), as a serine protease with the ability to convert pro-HGF/SF to HGF/SF, for 

![Fig. 2. Activation of HGF/SF. Pro-HGF/SF is activated by HGF activator (HGFA), which itself is activated by other proteases.](image-url)
Therefore, linking the localised process. This process of events would explain how the therefore, serine means of mediating the biological influence of HGF/SF, resulting in the tissue regeneration, at the surface or extracellular matrix of the tissue, that requires activation to cell membrane, as once the HGFA precursor increases due to blood coagulation resulting from tissue injury. This would suggest HGFA is activated in response to tissue damage (Miyazawa et al., 1994).

The mature HGFA has a strong affinity for heparin, as does the inactive HGF/SF. Pro-HGF/SF is capable of binding to cell surfaces or the extracellular matrix to for activation, this binding is enhanced by its affinity to heparin-like glycosaminoglycans (Naldini et al., 1991). Therefore, linking the localised HGF/SF activation to blood coagulation and the need for active HGF/SF following tissue damage (Miyazawa et al., 1996).

This ties in with the ability for active HGFA to convert pro-HGF/SF on the cell membrane, as the HGFA protease is mature it is aquires the ability to interact with heparin (Miyazawa et al., 1996), thereby ensuring local activation of pro-HGF/SF by HGFA. Also, Moriyama et al., (1995), report the joint expression of HGF/SF, HGFA and the HGF/SF receptor (c-met) in glioma cells, suggesting a self regulatory mechanism. Therefore, it appears the HGFA/SF is activated on the cell surface or extracellular matrix of the tissue, that requires regeneration, at the site of tissue damage.

HGFA and thrombin are the proteases responsible for HGF/SF and HGFA activation, respectively. For this reason, it appears that following tissue damage, the inflammatory response and the blood coagulation cascade are initiated and produce thrombin. This thrombin then converts the secreted HGFA precursor into active HGFA, in the serum and at the site of the tissue injury. Once active, HGFA has the ability to induce the localised conversion of pro-HGF/SF to the site of the damage, the active form of HGF/SF then plays its role in the tissue regeneration process. This process of events would explain how the active form of HGF/SF was cleaved exclusively in injured tissues.

Controlling the activation of HGFA may act as a means of mediating the biological influence of HGF/SF, therefore, serine protease inhibitors were employed to examine possible methods of decreasing HGFA activity. Serine protease inhibitors, naturally present in human serum, include antithrombin III, c-1 inhibitor and e2-antiplasmin and are capable of inhibiting the blood clotting ability of Factor X:1a (Tans and Roosing, 1987).

The HGF/SF converting ability of Factor X:1a and HGFA was examined in the presence of these protease inhibitors. The inhibitors suppressed the influence of Factor X:1a, but none of these inhibitors could prevent HGFA converting pro-HGF/SF to HGF/SF (Shimomura et al., 1995). However, the HGF/SF converting ability observed in injured tissues was inhibited, in vitro, by an anti HGFA antibody known as P1-4. Using this method, Miyazawa et al. (1996), confirmed HGFA as the mediator of HGF/SF activation in injured tissues. One study reports that regulation of pro-thrombin secretion could play a role in mediating tissue regeneration as the active form, thrombin, cleaves the HGFA precursor to create the active form of HGFA (Yagi et al., 1995).

During tissue injury there are two methods of regulation of HGF/SF activity, 1) the localised proteolytic activation by HGFA; 2) enhanced production of HGF/SF mRNA, via activation of HGF/SF expression at the transcriptional level, therefore to understand the mechanism involved we need to know the factors involved in inducing gene transcription to completely understand the role HGF/SF plays in tissue regeneration.

The limiting step of the HGF/SF signalling pathway in vivo, appears to be: 1) the extracellular activation of HGF/SF by HGFA; 2) the inhibition of this protease by HAI-1 and HAI-2 (see later); 3) or maintaining the balance of the levels of active HGFA and HAI-1/2 to control HGFA/SF activation.

**HGFA expression and localisation**

Miyazawa et al., (1993), initially detected HGFA in the liver with northern blot analysis. Since then, the parenchymal cells of the rat liver have also been reported as a major site of HGFA mRNA synthesis, following tissue injury or acute inflammation (Okajima et al., 1997).

It was observed that no notable mRNA signal for HGFA was detectable in colon carcinoma cell lines or in normal and malignant colonic mucosa (Kataoka et al., 1998). However, Moriyama et al. (1995), reports the expression of the HGFA gene in human normal brain tissue and in glioma cells. Whereas, in situ hybridisation histochemistry was performed to reveal the presence of HGFA mRNA in white matter astrocytes of brain tissue (Yamada et al., 1997).

It was reported that the active form of HGFA was generated exclusively in the injured tissues (Miyazawa et al., 1996). When injury was induced on the liver, HGF/SF conversion occurred at this site but not at the kidney, lung or spleen. Whereas, when the rat was treated with nephrotoxin, the kidney was injured resulting in an increase in the presence of active HGFA to the level required for HGF/SF conversion. During this time, HGF/SF conversion was not detected in the liver, lung or spleen. Another study reports that in normal gastric mucosa HGFA is not expressed, but following ulceration damage HGFA is expressed in the submucosa of the stomach (Kinoshita et al., 1997).

Miyazawa et al. (1996), suggested that heparin may increase the ability of HGFA to activate HGF/SF in the
Patterns of HGFA expression

In the fetal rat gastrointestinal tract, a pattern was observed between HGFA and the HGF/SF receptor (c-met) mRNA expression. It was observed that HGFA mRNA was only present in epithelial cells expressing c-met mRNA (Matsubara et al., 1998).

Rat HGFA mRNA expression, following liver injury, was shown to increase to as much as double during the first 6 hours following the initial injury, followed by a decrease over the following 24 hour period. The same increase in liver HGFA mRNA was observed following renal injury and also during acute inflammation (Okajima et al., 1997). This group also proposed that the increase in HGFA mRNA level was due to the tissue injury initiating the inflammatory response of which HGFA was reported as a possible acute phase protein. Following tissue injury, HGFA activity could possibly be enhanced due to increased HGFA mRNA production in the liver, via the inflammatory response, and increased activation of the HGFA precursor, via thrombin in the blood coagulation cascade. The cytokines IL-1 and IL-6 are known to mediate the mRNA level of acute phase proteins during the inflammatory response. However, Okajima et al. (1997), revealed that neither IL-1 or IL-6 had any effect on the HGFA mRNA level.

Implications in cancer

The role of HGF/SF in tumours is still not completely understood. It was the recognition of the HGF/SF receptor as being c-met, discovered as an activated oncogene (Cooper et al., 1984), that increased the efforts to fully understand the role that HGF/SF plays in cancer. Pro-HGF/SF is secreted by mesenchyme cells and activated in the extracellular environment by HGFA. HGFA therefore plays a key role in the regulation of HGF/SF-induced action during tumour progression.

In Europe and North America, breast cancer is the second largest cause of cancer related death in women. HGF/SF has been shown to enhance the metastatic potential of breast cancer cells in vitro. Studies have also revealed that the levels of HGFA are elevated in breast tumour tissue (Hiscox et al., 1998), which may result in increased conversion of pro-HGF/SF to the heterodimeric active form of HGF/SF, thereby promoting tumour metastasis. Also, it has been noted that increased expression of plasminogen activator, which shows significant homology to the HGFA precursor, in breast cancer cells correlates with tumorigenic progression (Spiryatos et al., 1992).

It was therefore important to discover if there was a mediator available for the production of HGFA, because cancer studies could then look at HGFA as a possible target of inhibition. If HGFA activity could be downregulated then the influence of HGF/SF on cancer cells could be suppressed.

Recently, an inhibitor for HGFA was discovered (Shimomura et al., 1997), this inhibitor was identified as a novel kunitz-type serine protease inhibitor and termed HAI.

HGF activator inhibitors (HAI)

As already discussed, HGFA was found as an inactive precursor in human plasma, but was detected in its active form in human serum, this suggests that the factor responsible for inhibiting HGFA action is not present in the serum. Reports show that the action of HGFA is not inhibited by plasma protease inhibitors, because of the presence of the active form of HGFA in serum. Therefore, it seemed reasonable to assume that an inhibitor to HGFA could be produced in the tissues.

Discovery of HAI

Shimomura et al. (1997), examined human cell lines for an inhibitor to HGFA action and observed an inhibitory factor in the conditioned media of various cell lines. This protein was then purified from a human MKN45 stomach carcinoma cell line and cloned to reveal a novel Kunitz-type serine protease inhibitor. The newly discovered HGFA inhibitor was designated HGF activator inhibitor (HAI) (Shimomura et al., 1997).

However, soon after the discovery of HAI, Kawaguchi et al. (1997), identified a second inhibitor of HGFA action from the conditioned media of the stomach carcinoma cell line MKN45. This newly discovered HGFA inhibitor was purified, cloned and found to be another new Kunitz-type serine protease inhibitor. To distinguish between these two very similar HGFA inhibitors, HAI was re-designated HAI-type1 (HAI-1) for the initial discovery, and HAI-type 2 (HAI-2) for this newly identified HGFA inhibitor.

HAI-1 and HAI-2 belong to a family of Kunitz-type
serine protease inhibitors. The initial discovery of a Kunitz-type serine protease inhibitor was upon the recognition of bovine pancreatic trypsin inhibitor (BPTI), also known as aprotinin (Laskowski and Kato, 1980).

HAI-1

HAI-1 is classed as a Kunitz-type serine protease inhibitor due to the similarity it shares with this family of inhibitors in the residue regions 250-300 and 375-425, as these regions contain the Kunitz domains. The primary HAI-1 translation product is made up of 513 amino acid residues. HAI-1 is composed of an NH2-terminal putative signal peptide (1-35 residues), kunitz domain 1 (250-300 residues) and kunitz domain 2 (375-425 residues), each kunitz domain contains three disulphide bonds. The region between these kunitz domains (319-353 residues) shares a high similarity to the low density lipoprotein (LDL) receptors binding domain. There is also a hydrophobic region about 20 amino acids at the COOH-terminal end (Shimomura et al., 1997).

The NH2-terminal is 35 residues from a signal peptide, and there is a transmembrane domain in the c-terminal region (Yamada et al., 1998). The mature form of the HAI-1 molecule has a molecular mass of around 40kDa. However, it was calculated that the expected molecular mass of the primary translated product of HAI-1 should be 56,893, but it is secreted as 40kDa; therefore, this mature secreted form appears to be cleaved at the c-terminal region (Yamada et al., 1998).

Recently, Shimomura et al. (1999), identified several new soluble forms of HAI-1. These soluble forms differ in form depending upon their site of cleavage, as the primary translation product contains multiple sites for proteolytic processing.

HAI-2

HAI-2 has similar structural domains to HAI-1. The size of mature HAI-2 is smaller with a molecular mass of about 14kDa, although the primary translation product was expected to be 25,415Da. Therefore, as with HAI-1, the purified form of HAI-2 from the conditioned medium is the proteolytically truncated form of the membrane form. HAI-2 does not have the same length NH2-terminal, and there is no LDL ligand binding domain between the two kunitz domains (Kawaguchii et al., 1997).

The primary translation product of of HAI-2 is composed of 252 amino acid residues. The NH2-terminal is composed of the putative signal peptide (1-27 residues), kunitz domain 1 (38-88 residues), kunitz domain 2 (133-183 residues), and a hydrophobic region (198-221 residues) at the COOH-terminal end (Kawaguchii et al., 1997).

Itoh et al. (1999), detected a new HAI-2 variant in mice. This HAI-2 variant was obtained from mouse kidney by RT-PCR, and it was revealed that this variant was shorter than HAI-2 because it lacked the first kunitz domain. However, they also reported that this form of HAI-2 was not present in human tissue.

Roles of HAI-1 and HAI-2 structural domains

The structures known as Kunitz domains, are also present in several mammalian serine protease inhibitors, and appear to be responsible for the inhibitory action of the protein concerned.

HAI-1, when proteolytically truncated into its extracellular active form, has a molecular mass of 40kDa, this means that it consists of approximately 360 amino acids. Due to HAI-1's original size of 513 amino acids and location of the structural domains, it would suggest that the cleavage occurs in the second kunitz domain to release the truncated form. This truncated form is composed of the NH2-terminal, the Kunitz domain 1, and the LDL receptor-like domain. On this evidence it could be proposed that Kunitz-domain 1 is the domain required to exert the inhibitive qualities of HAI-1 on the HGFA protease.

Qin et al. (1998), proved this in HAI-2 with biochemical analysis by introducing point mutations into each of the kunitz domains, and monitoring their ability to inhibit HGFA activity. A point mutation in kunitz domain 2 had no effect on the HAI-2 proteins ability to inhibit HGFA action. However, a point mutation in kunitz domain 1 did suppress the ability of HAI-2 to inhibit HGFA. Therefore, it can be said that kunitz domain 1 (NH2-terminal end), is the kunitz domain responsible for inhibition of HGFA converting ability.

As only the presence of Kunitz domain 1 is required for HGFA suppression, then maybe Kunitz domain 2 has an alternative responsibility. Kunitz domain 2 may act as an inhibitor for another serine protease, as reports suggest that HAI-2 is identical to the placental protein Bikunin (Qin et al., 1998). Also, Bikunin has also been reported as being a gene homolog to a protein overexpressed in pancreatic cancer known as KOP (Muller-Pillasch et al., 1998). Placental bikunin contains the kunitz domain 2, and it seems evident that this domain is responsible for inhibiting trypsin, tissue kallikrein, plasma kallikrein and plasmin (Delaria et al., 1997). Therefore, this may suggest that HAI-2, Bikunin and KOP genes are from the same ancestral gene, and that the role of Kunitz domain 2 is dependant upon the protein inhibitor it is expressed in. It may act as an inhibitory factor for one serine protease but may not elicit any effect on another.

The LDL receptor-like domain contains a negatively charged domain, the reason for which is unknown, although it may aid in formation of a protease inhibitor complex during the inhibition of HGFA by HAI-1 because the HGFA precursor revealed high affinity for negatively charged substances.

The presence of a hydrophobic sequence in the
COOH-terminal of the primary translation products, of both HAI-1 and HAI-2, suggests that they are produced in a membrane-associated form, and then proteolytically cleaved into a truncated form and secreted into the extracellular environment (Kawaguchi et al., 1997). However, the newly discovered soluble HAI-1 forms were identified in a transmembrane form integrated into the cells plasma membrane. These soluble HAI-1 forms vary in size depending on the site of proteolytic cleavage. The transmembrane associated form reveals multiple sites for proteolytic processing. There are at least two proteases, one of which is a metalloprotease, which can cleave at a specific site to release a soluble form of HAI-1 (Shimomura et al., 1999).

Structural differences occur between HAI-1 and HAI-2, these include the different N-linked sugar chains that are joined to the protein portion, and also HAI-1 was adsorbed by a hydrophobic column, whereas, HAI-2 was not. This indicates that HAI-2 is more hydrophilic than HAI-1.

Slight differences also occur in the Kunitz domains, as Kunitz domain 1 of HAI-1 and HAI-2 share 54% homology. However, the homology between the kunitz domain 1 and 2 of HAI-2 is only 41% (Qin et al., 1998).

Similarities of HAI-1 and HAI-2 to other molecules

Kunitz domains 1 and 2 in HAI-1 show 47% homology to kunitz domain 1 of the precursor of human β-amyloid (APP), and the kunitz domain 2 of the human APP homolog protein, respectively. APP effectively acts as an inhibitor to several serine proteases (Sinha et al., 1990), however, APP shows no inhibitory action toward HGFA (Kawaguchi et al., 1997).

Muller-Pillasch et al. (1998), discovered a novel putative transmembrane protein that was over expressed in pancreatic cancer, and designated it KOP (kunitz domain containing protein overexpressed in pancreatic cancer). This new protein is located on chromosome 19q13.1, contains two kunitz-type serine protease inhibitors, and importantly was found to be a gene homolog to bikunin. Bikunin has previously been suggested as being identical to HAI-2, therefore it appears the KOP gene is also homologous to HAI-2. Comparison of the cDNA sequences of KOP, placental bikunin and HAI-2 has revealed that all 3 proteins are identical (Qin et al., 1998).

The roles played by HAI-1 and HAI-2

The inhibitory action of HAI-1/2 on HGFA could lead to consequences resulting from the suppression of HGFA action. Tissue repair requires the influence of HGFSF to initiate mitogenesis, therefore HGFA plays an important role as pro-HGFSF activator. HAI-1/2 effectively suppress HGFA action to inhibit the requirements of HGFSF during tissue repair.

Tumourigenesis is a multistep process which, to be understood, requires identification of all genes involved. For this reason, recognition of new proteases and protease inhibitors is an important step in understanding the mechanisms involved in cancer. A disruption of the balance between these proteases and their inhibitors has been reported to be a key factor in tumour progression (Declerck and Imren, 1994).

Inhibition of HGFA by HAI-1 and HAI-2

It is evident that both HAI-1 and HAI-2 are the inhibitors responsible for the inhibition of the proteolytic activity of HGFA in vitro (Kawaguchi et al., 1997; Shimomura et al., 1997). Shimomura et al. (1997), revealed that 50% inhibition of the activity induced by 450ng/ml of HGFA can be achieved with HAI-1 at a concentration of 250ng/ml. An equimolar complex is formed following 30 minutes incubation of HGFA with HAI-1. Whereas, it was reported that 300ng/ml of HAI-2 was sufficient to induce a 50% inhibition of 2 μg/ml of HGFA (Kawaguchi et al., 1997).

HAI-1/2 bind to the HGFA serine protease, to prevent it from binding to the single chain form of HGFSF and cleaving it into the active heterodimer of HGFSF. The inhibiting abilities of HAI-1/2 are reliant upon the presence of kunitz domain 1. However, it also appears that different forms of HAI-1/2 have differing inhibitory action against HGFA.

HAI expression

The primary translational product of HAI-1/2 suggests that these inhibitors are produced in a membrane associated form, due to the presence of a hydrophobic region in the COOH-terminal end of the sequence. To be secreted in the appropriate form, these products require cleavage at the c-terminal region. When HAI-1 and HAI-2 are secreted from the cells, they are in the proteolytically truncated form of 40 kDa and 14 kDa, respectively. However, the new soluble forms of HAI-1 are released from a transmembrane associated form, via proteolytic processing of a specific cleavage site by a specific protease.

HAI-1/2 expression in human liver was low, whereas with HGFA protease mRNA, the degree of expression in the liver was observed in high levels, as HGFA is secreted from liver (Kawaguchi et al., 1997). This shows that HAI-1/2 levels in the adult liver and lung are low, which is to be expected as HGFA/SF plays a key role in liver and lung tissue regeneration. Therefore, a high level of HGFA is required for the conversion of pro-HGFA/SF to HGFA/SF at this site.

HAI-1 mRNA has been detected in high levels in adult placenta, pancreas, kidney, prostate and small intestine, furthermore, HAI-1 mRNA has also been observed in fetal kidney and lung. However, in adult lung the level of expression was low (Shimomura et al., 1997). HAI-1 mRNA is expressed in colorectal mucosal tissue (Kataoka et al., 1998), and on the surface of colon epithelial cells.
KOP, the similar/identical gene to HAI-1 and HAI-2, was also seen to be highly expressed in placenta and pancreatic tissue, whereas low levels were detected in heart, lung, brain, liver, and skeletal muscle (Müller-Pillasch et al., 1998). However, HAI-1 mRNA was observed at a detectable level in the white matter of all brain tissue astrocytes examined (Yamada et al., 1998).

HAI-1 was mainly found to be expressed in human simple columnar epithelial cells of ducts, tubules and mucosal surface of various organs. HAI-1 was not detected in hepatocytes, endocrine cells, stromal mesenchymal cells and inflammatory cells (Kataoka et al., 1999).

Patterns of HAI-1 and HAI-2 mRNA have been examined. It appears to be that the expression levels between HAI-1 and HAI-2 were of similar distribution in the tissue examined, except in adult testis, where HAI-2 expression was far greater than that of HAI-1 (Kawaguchi et al., 1997). These results suggest that HAI-1 and HAI-2 work in unison to specifically inhibit HGFA action.

Patterns of expression

There appears to be an inverse correlation between tumour progression and HAI-1 mRNA expression. HAI-1 was expressed in the normal colorectal mucosa, although the degree of expression appeared to be decreased in adenocarcinoma tissues of the colorectum. Also, it was noted that the carcinoma cell lines expressing HAI-1 did not express any detectable level of HGFA mRNA (Kataoka et al., 1998).

The liver hepatocytes, a rich source of HGFA mRNA, normally show little or no signal for HAI-1 mRNA. However, during hepatitis the scattered hepatocytes did reveal the presence of HAI-1. Also, an enhanced expression of HAI-1 was also observed during regeneration of kidney tubule epithelial cells following infarction (Kataoka et al., 1999).

Evidence suggests that the expression of HAI-1 mRNA in colon carcinoma tissues is down regulated (Kataoka et al., 1998), this would aid tumour progression as a decreased level of HAI-1 would limit the suppression effect on HGFA. Therefore, HGFA activity increases resulting in increased conversion of pro-HGF to HGF/SF, thus leading to tumour cell migration and invasion.

The fact that HGF/SF plays such a key role in cancer metastasis, suggests that the serine protease HGFA and its inhibitors HAI-1 and HAI-2 are important factors influencing tumour cell progression. The balance between proteases and their inhibitors has been implicated as mediating the progression of tumour cells (Declerk and Imren, 1994). Furthermore, the balance between proteolytic activation and inhibition on a cells surface is reported as being disrupted in cancer cells (Takada et al., 1995).

It has been shown that HAI-1 expression is down regulated in adenocarcinoma tissues of the colorectum (Kataoka et al., 1998), whereas, overexpression of HGFA was observed in breast cancer (Hiscox et al., 1998), these situations results in enhanced tumour metastasis.

Contrary to this, the novel kunitz-type protease inhibitor known as KOP was overexpressed in pancreatic cancer cells. KOP has been recognised as a gene homolog to bikunin, which was suggested to be identical to HAI-2. However, it is uncertain what is KOP’s target protease for inhibition, and whether KOP has the ability to inhibit HGFA (Müller-Pillasch et al., 1998).

HAI-1 and HAI-2 could act to inhibit HGFA action concomittantly, or they may be needed to function in different situations and be expressed in different tissues. Shimomura et al., (1999), revealed that the new soluble forms of HAI-1 have different inhibitor action against HGFA. Although, the underlying mechanisms behind
HGF/SF inhibitors in cancer

this are not known at the present. Further examination of HAI-1, HAI-2 and HGFA interaction in the various tissues is required to discover if the down-regulation of HAI-1/2 and/or over expression of HGFA is a possible prognostic factor in cancer metastasis. Also, the specific proteolytic cleavage required to release a form of HAI-1 should be further investigated, as this processing by a specific protease plays a key role in the control of HAI-1 activity.

At the present time the role of HAI-1 and HAI-2 in tumours is uncertain. However, it appears that the cell expression and localisation of HAI-1 mediates tissue injury and regeneration, through interaction with HGFA.

Other roles for HAI-1 and HAI-2

Kunitz domain 1 in HAI-1 and 2 is responsible for the inhibition of HGFA action, however the role of the kunitz domain 2 is unknown, maybe HAI-1/2 play another inhibitory role for another serine protease such as trypsin, plasmin or kallikrein as HAI-2 has been reported as being identical to KOP and Bikunin. Although Kawaguchi et al., (1997), did report that the proteolytic processing of HAI-2 appeared to occur in the kunitz domain 2. Therefore, the proteolytic cleavage of HAI-2 will have to be further characterised to understand the function of the kunitz domain 2.

HGF/SF antagonists

Mature HGF/SF, after conversion by HGFA, is composed of an α- and β-chain, which are disulphide-linked. The mature form is processed from a single chain pro-HGF/SF (Fig. 3) (Nakamura et al., 1989). The α-chain of mature HGF/SF contains some of the most interesting features of this cytokine, as the N-terminus is composed of a hair-pin domain and four homologous kringle domains, while the C-terminus of the α-chain is linked to the β-chain. The hair-pin and the first two kringles are known to be essential for receptor binding.

Fig. 4. The effect of NK4 on HGF/SF induced angiogenesis (Jiang et al., 1999). NK4 was able to antagonise in vitro tubule formation induced by HGF/SF, from human vascular endothelial cells.
Interaction between the receptor and the binding domain stimulates the kinase domain of cMET, which further activates downstream events.

The striking effect of HGF/SF on cancer cells, the established relationship between the cytokine and its receptor, and disease progression and prognosis of breast cancer bearing patients, has prompted attempts to minimise the effect of HGF/SF in vitro and in vivo. The possibility of using part of HGF/SF as antagonists to HGF/SF through competitive binding to cMET has been postulated. The extensively tested strategy involved using the receptor-binding domain of the α-chain. Alternative spliced variants of the α-chain, which contain different parts of the subunit have been discovered previously. The variant that contains the hair-pin and the first kring domain (K1), is known as HGF/NK1 and is naturally occurring. The one that contains the hair-pin and the first and the second kring domains (K2), is known as HGF/NK2. Both variants are able to bind to the receptor and have been shown to act as partial agonists and partial antagonists (Chan et al., 1991; Hartman et al., 1992; Lokker and Godowski 1993; Ciocci et al., 1996; Schwall et al., 1996). For example, NK2 has no mitogenic effect on hepatocytes, but retains motogenic and pro-metastasis effect (Otsuka et al., 1991; Hartman et al., 1992; Lokker and Godowski 1993; Ciocci et al., 1996; Schwall et al., 1996). For example, NK4 was able to inhibit the in vitro angiogenesis without eliciting an agonistic activity on the process (Fig. 4) (Jiang et al., 1999b). NK4 also inhibited the migration, and in vitro invasion of breast, colon and bladder cancer cells (Fig. 5) (Hiscox et al., 2000; Parr et al., 2000). Interestingly, delivery of NK4 transcript by means of adenovirus has also been shown to exert strong anti-cancer effects (Maekaedo et al., 2000). Thus, this specific HGF/SF antagonist may offer a unique and strong opportunities for the treatment of cancer, associated with abnormal expression of HGF/SF and/or its receptor.

**Fig. 5.** Effects of NK4 on HGF/SF induced cell motility (left) and its receptor (MET) activation (right) (Parr et al 2000). **Left:** A: colon cancer cells without HGF/SF; B: cells with human HGF/SF; C: a combination of HGF/SF and NK4; and D: cells with NK4 alone. **Right:** The effects of NK4 on the HGF/SF receptor. HT115 human colon cancer cells were treated as indicated. Phosphorylated HGF/SF receptor was detected with immunoprecipitation and Western blotting. NK4 suppressed HGF/SF-induced receptor activation.
Non-specific inhibitors to HGF/SF

Interestingly EGF inhibits HGF-induced migration in the gastric cancer cell line, MKN7, possibly by regulating the phosphorylation status of c-met in these cells (Takeuchi, 1996).

Polyunsaturated fatty acids

Gamma linolenic acid (GLA) is an n-6 essential fatty acid which has been shown to cytotoxic towards tumours both in vitro and in vivo (Begin et al., 1986; Horrobin, 1990; Jiang et al., 1996c, 1998). We have shown that GLA exerts a potent inhibitory effect on HGF/SF-stimulated tumour cell motility, invasion, and membrane ruffling at concentrations that are non-toxic to the cells (Jiang et al., 1995a, 1996b), possibly by the regulation of the cell-surface adhesion molecule, E-cadherin (Jiang et al., 1995b,c). Interestingly, GLA also inhibits the adhesion of tumour cells to extracellular matrix via inhibition of FAK and paxillin phosphorylation (Jiang et al., 1996d). A link between such an effect and peroxisome proliferator activated receptor (PPAR) has been recently proposed (Jiang et al., 2000). This may represent a further mechanism by which GLA inhibits HGF/SF-induced cell motility.

IL-4 and IL-12

IL-4 has been found to be able to inhibit HGF/SF-induced motility and invasion of colorectal cancer cells (Uchiyama et al., 1996). IL-12 is a monocyte/macrophage derived cytokine which has a strong effect on NK cells and cytotoxic T lymphocytes and may thus represent an important anti-cancer cytokine (Hiscox and Jiang, 1997c). We have also shown that this immunoregulatory cytokine, inhibits tumour cell motility and basement membrane invasion stimulated by HGF/SF (Hiscox et al., 1995). These effects are thought to be mediated by alterations in cell-surface adhesion molecule levels.

Invasion inhibitory factor-2

Invasion inhibiting factors are small proteins extracted from the liver which exhibit anti-metastatic properties in melanoma and lung cancer cells in vivo (Isoai et al., 1992, 1993). Invasion inhibitory factor-2 (IIF-2) (Isoai et al., 1992, 1993, 1994), has been shown to inhibit HGF/SF-stimulated motility and invasion of tumour cells in vitro (Jiang et al., 1995d; Han et al., 1996) however the mechanism by which this occurs is not clear.

Retinoic acid

Retinoic acid, which belongs to a group of vitamin A metabolites, has been shown to exert opposite effects on the same cell (Koj et al., 1995), including regulation of cytokine production and acute phase responses at a transcription level. Retinoic acid has been widely reported to be involved in the regulation of other motility factor receptor functions, cell-matrix interactions and proteolytic enzymes (Edward et al., 1989; Hendrix et al., 1990; Lotan et al., 1992).

Cell adhesion molecule-mediated inhibition of the effects of HGF/SF

It has been reported that restoring the function of or raising the level of E-cadherin inhibits HGF/SF-induced cancer cell invasion (Jiang et al., 1995c; Jiang, 1996). Restoring E-cadherin mediated cell-cell adhesion by Tiam1 or RacV12 gene transfection also reduces HGF/SF induced cell scattering (Hordijk et al., 1997). HGF/SF also enhances the phosphorylational status of β-catenin, a possible mechanism surrounding the HGF/SF-induced scattering of epithelial cells. Clearly, further investigations concerning the interplay between HGF/SF and cell-cell adhesion complexes such as the E-cadherin/catenin complex may present further ways of inhibiting the metastatic-promoting effects of HGF/SF.

Modulation of intracellular Ca2+

HGF/SF-induced tumour cell membrane ruffling and motility have been shown to be inhibited by the addition of cytosolic calcium regulating agents such as ATP (Jiang et al., 1995a). Transient elevation of intracellular Ca2+ levels is thought play a role in the HGF/SF-inhibitory mechanism of ATP as inhibition of this Ca2+ raise by BAPTA abolishes the inhibitory effects of ATP. Elevation of cytosolic free Ca2+ with other agents including ionomycin and ADP also results in HGF/SF inhibition.

Recent new approaches

c-MET inhibitors

Although c-MET is a prime target for intervention, specific inhibitors to c-MET has not been described until recently. Lipson and colleagues have recently reported to have discovered selective pyrroleindolinone c-MET kinase inhibitors (Lipson et al., 2000). In a brief report, it revealed that these inhibitors can inhibit HGF/SF induced c-MET phosphorylation and cellular response, including cell migration and invasion. This may offer a new direction in the inhibition of HGF/SF activity. However, in vivo testing are required for this in order to evaluate the effectiveness and toxicity of these compounds.

Antisense-hammerhead ribozyme approach to target HGF/SF and/or c-MET

The antisense approach to suppress the expression of specific mRNA has been in testing for some time, with
limited success. However, a recent study using a small hammerhead ribozyme (a small RNA) to target HGF/SF and c-MET has yield some very interesting results. Abounader and Laterra (1999), have constructed short antisense sequences of HGF/SF and c-MET to a hammerhead ribozyme. The recognition of the specific sequence by the ribozyme result in the generation of anti-sense oligonucleotides. These ribozyme constructs were subsequently cloned into a bacteria vector. The authors use these plasmids to generate a stable tumour transfectant, which carry these construct. The authors elegantly showed that cells carrying these hammerhead construct constantly produce specific antisense oligos to either HGF/SF or c-MET mRNA and result in the destruction of these molecules. These transfectants exhibited far lower tumourigenecity and low capacity in tumour progression, compared with the wild type.

Anisamycin antibiotics

Recently, it has been reported that certain members of the geldanamycin family of anisamycin antibiotics are potent inhibitors of HGF/SF-mediated plasmin activation, displaying inhibitory properties at femtomolar concentrations and nine orders of magnitude below their growth inhibitory concentrations. These antibiotics were initially discovered as inhibitors of the src family of tyrosine kinases (Uehara et al., 1988; Whitesell et al., 1994; Roe et al., 1999). At nanomolar concentrations, the geldanamycins down-regulate c-Met protein expression, inhibit HGF/SF-mediated cell motility and invasion, and also revert the phenotype of both autocrine HGF/SF-c-Met transformed cells as well as those transformed by c-Met proteins with activating mutations (Webb et al., 2000).

Conclusions

The past decade has seen the rapid expansion of the knowledge on the role of HGF/SF and its receptor in cancer. The factor is now known to widely involved in cellular migration, matrix adhesion and degradation, and invasiveness of cancer cells. It is also a potent angiogenic factor. Clinical studies have demonstrated a clear inverse relationship between the factor/receptor and progression and prognosis of patients with cancer. Recent efforts to identify the activation pathways of the factor and importantly, inhibitors and antagonists, have presented some new opportunities in combating this factor in settings, such as cancer. Although most of the inhibitors are still at a development stage, further progress will undoubtedly lead to applying some of these technologies to the therapy of cancer, particularly those associated with a high HGF/SF and/or high c-MET conditions.

References


HGF/SF inhibitors in cancer


147, 386-396.


W., Daikuhara Y., Tsubouchi H., and expression of cultured epithelial cells by hepatocyte growth factor scatter factor is facilitated metastasis.


HGF/SF inhibitors in cancer

Accepted July 27, 2000