Chapter 10

HEPATOCYTE GROWTH FACTOR/SCATTER FACTOR AND PROSTATE CANCER METASTASIS

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Abstract: Metastasis is a life-threatening event in tumor bearing patients resulting in total organ failure and to subsequent death. Understanding the fundamental processes involved in how cancer cells detach from the primary tumor in order to enter the metastatic cascade is a key factor in delivering therapeutic starategies to prevent metatasis from occuring. Hepatocyte Growth Factor/Scatter Factor (HGF/SF) is an unique growth factor capable of inducing a number of biological responses in a wide variety of normal and neoplastic cells, including invasion, cell spreading, scattering, motility and shedding of cell-cell adhesion molecules. This review is intended to highlight some of the more recent discoveries made in scientific and clinical research, with particular emphasis on the effect of HGF/SF on protsate cancer metastasis and invasion.

Key words: prostate cancer, HGF/SF, invasion and metastasis

1. PREFACE TO TUMOR PROGRESSION AND METASTASIS IN THE DEVELOPMENT OF PROSTATE CANCER

Metastasis is perhaps the single, most important rate-limiting factor affecting the survival outcome of patients with cancer. In particular, prostate cancer remains one of the leading male cancers diagnosed in Western countries (1). The incidence and mortality rate for prostate cancer has increased dramatically during the last decade. It was estimated that the

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incidence alone for prostate cancer increased to 126% in the early 1990's, compared that that observed in the 1970's. During the same time period, the mortality rate for prostate cancer was also estimated to have increased at a rate of more than 1% per year. As a consequence, this has given rise to more than 15.6 deaths per 100,000 per year in the UK alone. Tumor metastasis to distant organs is dependent on the detachment of cancer cells from their primary location and their subsequent invasion to the surrounding tissues. There are a number of key factors known to play definitive roles in tumor metastasis including hepatocyte growth factor/scatter factor (HGF/SF), and this motogen has been shown to influence the development of prostate cancer. The mechanisms involving HGF/SF mediated responses in prostate cancer progression will be explained in greater detail at a later stage in this review. Finally, there remains very little knowledge concerning the underlying influences that contribute to the incidence of prostate cancer, such as tumor biology, the mechanisms of disease progression and its prognosis. Therefore, this article will attempt to address some of the more recent discoveries made in these areas.

2. HISTORICAL BACKGROUND CHRONICALING THE DISCOVERY OF HGF/SF

HGF was originally identified as a powerful stimulant for hepatocyte growth *in vitro* (2–4). However, subsequent cloning and sequencing of this molecule revealed that it was homologous to both hepatopoietin A and a tumor toxic factor (5–8). Previously, a fibroblast-derived protein was reported to scatter tightly-packed colonies of epithelial cells and this was subsequently termed scatter factor (SF) (9). In addition, partial amino acid sequencing of SF revealed that it was over 90% homologous to both rat and human variants of HGF (10–13). Therefore, it was generally accepted that the term HGF/SF should be used to describe this multi-functional cytokine (14–15).

2.1 Biological Activity and Structure of HGF/SF

HGF/SF is synthesised and secreted as a single peptide chain of 728 amino acid residues, containing a signal sequence of 29 amino acid residues and a pro-sequence of 25 amino acid residues (16–17). Furthermore, the single chain pro-form of HGF/SF needs to be converted to its mature active

heterodimeric form, by extracellular cleavage of a pro-sequence Arg^{494}-Val^{495} bond via an unique serine protease (6, 16–19). HGF/SF in its mature form is composed of one heavy peptide α-chain (69 kDa), and one light peptide β-chain (34 kDa), which are linked together by a disulfide bridge forming a heterodimer (16–17). The α-subunit of HGF/SF is composed of four triple-disulphide structures called kringle domains and a putative N-terminal hairpin loop domain (20), homologous to plasminogen preactivation peptide (PAP), and responsible for both heparin and *c-Met* receptor binding (21–23). The first and second of these kringle domains have been reported to be necessary for the correct biological functioning of HGF/SF (24). These kringle domains are also known to be involved in protein-protein binding and as such, mediate receptor-ligand binding on plasminogen activator (25–26). The β-subunit has a domain similar to that of a serine protease, although no protease activity occurs due to amino acid substitution within the catalytic site (27). The complete lack of protease activity within the β-subunit of HGF/SF results in the absence of highly conserved triad residues present within active serine proteases (5, 16).

HGF-converting enzyme has been implicated in the conversion of pro-HGF/SF into its active heterodimeric form via proteolytic cleavge of the single chain precursor form of HGF/SF (23). Similarly, urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) have also been shown (*in vitro*) to convert pro-HGF/SF into a mature active form of HGF/SF (19, 28). Another mechanism of activation exists which occurs in response to tissue injury (Figure 1), where a precursor of an HGF/SF activator (HGFA) is produced by epithelial cells and is then activated by thrombin (a component of the blood coagulation cascade), which in turn goes on to process pro-HGF/SF into its bioactive form (29). HGF/SF is regulated by an injurin like factor (humoral type factor) produced in non-injured distant organs following hepatic or renal injury and where it has been found to induce the expression of HGF/SF mRNA in rat lungs (30–32). Injurin is a small non-protein factor (8–15kDa) that is located in a number of tissue extracts (liver, kidney, brain and lung) and has been shown to translationally enhance HGF/SF production (33). A possible role for injurin in the activation of pro-HGF/SF is shown in Figure 1.

2.2 Structure and Function of the HGF/SF Receptor C-Met

There are various biological responses following stimulation by HGF/SF which are mediated by its mature proto-oncogene receptor *c-Met* (34–35). The HGF/SF receptor was first identified as an activated oncogene in an

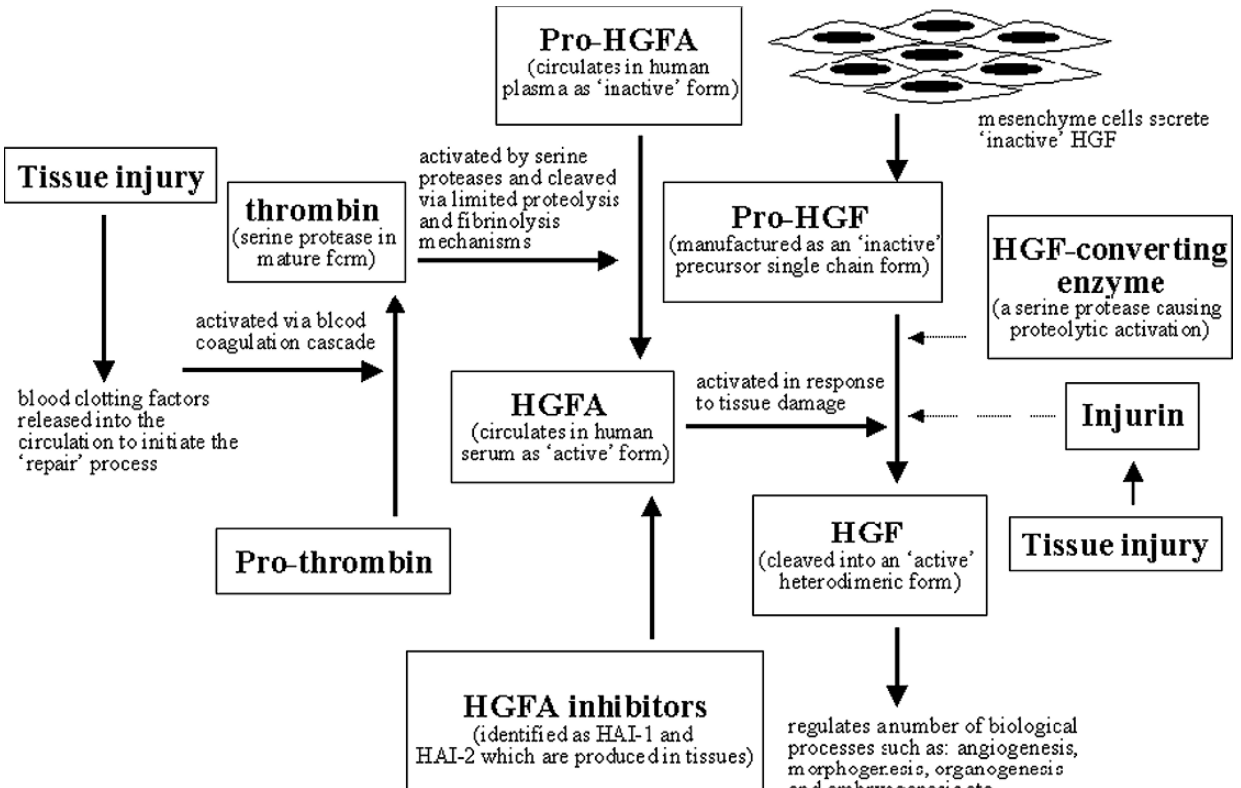

Figure 1. A pathway showing the secretion, activation and regulation of HGF/SF. The broken arrows indicate unknown mechanisms of HGF/SF activation involving injurin.

N-methyl-N'-nitrosoguanidine-treated human osteogenic sarcoma cell Line (MNNG-HOS), by its ability to transform NIH 3T3 mouse fibroblasts (36–37). The receptor encoded by the *c-Met* proto-oncogene is a two chain protein composed of an α-subunit (50 kDa), linked to a membrane-spanning β-subunit (145 kDa) joined together (producing an αβ complex of 190 kDa) by disulphide bonding (34–35, 38). The intracellular portion of the *c-Met* β-chain possesses a tyrosine kinase domain that identifies the HGF/SF receptor as a member of the receptor tyrosine kinase (RTK) family of cell surface molecules (34–35, 38–39).

HGF/SF binds to its receptor and induces tyrosine phosphorylation in the intracellular domain located on the β-chain of mature *c-Met*, causing ligand-dependent receptor homodimerisation and cross-phosphorylation within the tyrosine kinase domain (40). Such events are thought to promote binding of intracellular signalling proteins containing *src* homology (SH) regions, such as: phospholipse-C-γ (PLC-γ), Ras, GTPase-activating protein (GAP), phosphatidylinositol 3-kinase (PI3K), $pp^{60c-src}$ and the Grb2-SOS complex, to the activated HGF/SF receptor (41–44). A schematic representation of intracellular signal transducers that are associated with tyrosine phosphorylation of *c-Met*, have been described elsewhere (45). HGF/SF has also been shown to stimulate the *ras*-guanine nucleotide exchanger, thus promoting the GTP-bound active state of the Ras protein (46). This association implicates the Ras pathway in the mediation of HGF/SF-induced cell motility, via its interaction with components of the cell's cytoskeleton (47).

3. BIOLOGICAL EFFECTS OF HGF/SF ON TUMOR INVASION AND METASTASIS

The most significant factor affecting the survival outcome in cancer patients is metastatic spread. The process is referred to as the 'metastatic cascade' and it is controlled by a number of essential rate-limiting steps including: (i) the loss of cell-cell contact from the primary tumor site; (ii) enzymatic degradation of both basement and extracellular matrix components, resulting in adhesion to the matrix and to subsequent invasion through these components; (iii) intravasation; (iv) survival within the circulation; (v) adhesion to the endothelium followed by extravasation, and (vi) establishment of a secondary tumor and angiogenesis (48–49). HGF/SF has been shown to cause a number of biological responses, such as increased cell motility (50), cell growth (51–52), invasion (53), angiogenesis (54–55), morphogenesis (56) and embryogenesis (57). The profound stimulatory effects induced by HGF/SF upon tumor cell function are central to the process of metastasis *in vitro*, and as such this motogen has now been

implicated as a mediator of metastatic spread *in vivo* (14). An increase in HGF/SF-induced cell motility and cellular scattering been demonstrated in a variety of cell types including tumor-derived cells (58–61). Furthermore, HGF/SF promotes the growth of hepatopoietic and hematopietic cells in culture (51–52), since the removal of this growth factor induces apoptosis (62–63). Exposure to HGF/SF has also been shown to reverse the effects of TGF-β1 (transforming growth factor-β1) induced growth arrest in epithelial and endothelial cells (64). Therefore, regulation of cell growth by HGF/SF may represent part of a co-ordinated cell growth control state (14).

HGF/SF causes the disassembly of cadherin/catenin complexes in a number of epithelial derived cells, resulting in a more invasive phenotype through the dissociation of β-catenin from E-cadherin (65–66). This has been demonstrated in our laboratory using co-precipitation experiments on prostate cancer cells LNCaPFGC (67). Stimulation with HGF/SF over a 2h incubation period induced the dissociation of co-precipitated E-cadherin/β-catenin using a lysis buffer containing the detergent Triton X-100 (also known as the Triton soluble fraction). Previous studies by our group have also indicated that HGF/SF promotes the invasion of a number of cell types into collagen gels and artificial basement membranes (59, 68–69). Such studies have revealed quite conclusively that HGF/SF affects the functioning of cellular adhesion molecules such as E-cadherin, promoting the *in vitro* invasiveness of both bladder and prostate cancer cells through Matrigel, via the neutralisation of E-cadherin with an anti-E-cadherin antibody (66, 70). Cellular-matrix adhesion (mediated through cell-matrix receptors called integrins) of cancer cells is known to play a key role in the process of metastasis and is necessary for both tumor cell matrix degradation, and for subsequent invasion through components of the ECM. A study by Parr *et al.* (71) demonstrates quite clearly that HGF/SF enhances cell-matrix adhesion, membrane ruffling, cellular spreading and invasiveness in prostate cancer cells, thus implicating HGF/SF as an inducer of cancer spread *in vitro* (14).

3.1 Induction of Motogen Induced Cell-Cell Dissociation and Migration

HGF/SF inhibits cadherin function by altering the phosphorylation status of cadherin-associated catenin proteins, thus affecting their binding to the intracellular portion of E-cadherin (65–66). In addition, HGF/SF induces tyrosine phosphorylation of β-catenin causing down-regulation of cadherin-mediated cell-cell adhesion (66, 72–73). HGF/SF has also been shown to

promote dissociation in epithelial cells through the dismantling of cell-cell adhesion complexes, resulting in an increase in cell motility/migration (14, 66). Cell motility may be initiated through a number of steps including: (i) the assembly and disassembly of focal adhesion; (ii) as integrin attachment to, and detachment from, the extracellular matrix, and (iii) through actomyosin cytoskeleton reorganisation (74). We have recently shown that HGF/SF influences both cell motility and migration in prostate cancer (DU-145) cells *in vitro*, events necessary for metastatic spread *in vivo*. The biological effects of HGF/SF and its antagonist NK4, on cell motility/migration in prostate cancer were assessed using a computerised motion analysis technique (71). Addition of HGF/SF was found to increase both cell motility (Figure 2A) and migration (Figure 2B) in DU-145 cells, but its effect was inhibited by incubation with NK4, thus indicating HGF/SF's ability to enhance the metastatic potential of DU-145 cells *in vitro*.

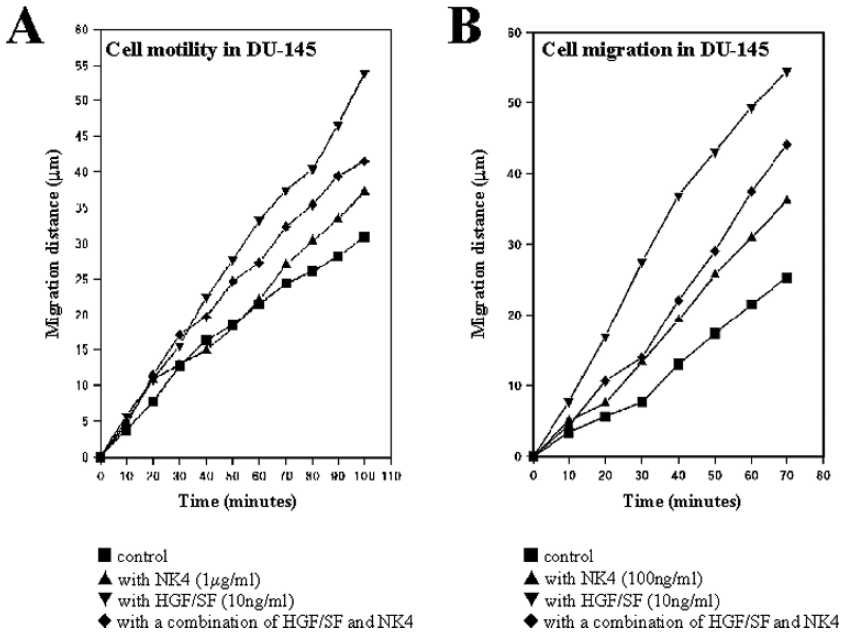

Figure 2. (A). Effect of NK4 on HGF/SF-induced cell motility in DU-145 cells using a motion analysis software package. The results indicate that HGF/SF (10ng/ml) induced tumor cell migration. However, NK4 (1 μg/ml) was able to antagonise the influence of HGF/SF. (B). NK4 suppressed HGF/SF action during migration of DU-145 cells in wound closure, using a wounding assay and motion analysis technique. HGF/SF dramatically increased migration of the cell fronts to close the wound; however, the addition of NK4 significantly ($p < 0.05$) reduced the biological influence of HGF/SF elicited in DU-145 cells (ref 71).

An enhanced metastatic phenotype after stimulation by HGF/SF has been demonstrated by Rong *et al.* (75), after transfecting *c-Met* cDNA into a cell line lacking the HGF/SF receptor. The *c-Met* receptor has been reported to be primarily located at intracellular junctions together with E-cadherin in normal epithelium. However, it remains unclear whether their peripheral localisation facilitates their physical interactions, or allows the regulation of cell adhesion (76, 77). Presumably the latter holds true, since we have shown that HGF/SF increases the co-precipitation level between the E-cadherin/catenin complex and the HGF/SF receptor *c-Met*, thereby regulating intercellular adhesion in prostate cancer cells following stimulation by this motogen (78). Miura *et al.* (79) have shown that exposure to HGF/SF demonstrates an induced cellular scattering in DU-145 cells, by decreasing the expression of E-cadherin and causing its subsequent translocation into the cytoplasm. Similarly, we also report that HGF/SF promotes the loss of cell-cell contact in LNCaPFGC cells due to a reduction in the level of co-localised peripheral staining (using immunofluoresecence) between E-cadherin and *c-Met*, and to an increase in the level of cytoplasmic staining (78). Cell scattering in prostate cancer via HGF/SF stimulation is thought to occur as a result of cellular regulation through the endocytosis and degradation of E-cadherin (79). Furthermore, the translocation of E-cadherin into the cytoplasm may help to either regulate, or redistribute protein levels within the cell's microenvironment. The most likely mode of mediation is via proteolytic degradation of endocytosed E-cadherin within lysosomes, or by the transportation of E-cadherin in recycling vesicles to the plasma membrane for future reuse (80–81). Therefore, it would appear that HGF/SF mediates the recycling of E-cadherin levels in prostate cancer cells, thus providing a potential mechanism for regulating cadherin dynamics within the cell's microenvironment.

4. ENHANCEMENT OF CELL MOTILITY VIA THE DEGRADATION OF EXTRACELLULAR MATRIX PROTEINS

Components of the extracellular matrix (ECM) including basement membranes, are critical entities in providing both structure, and maintenance to tissue architecture (82–84). The loss of tissue integrity involves both the degradation of ECM proteins, and the coordinated synthesis of newly processed matrix components, essential metabolic steps governing the cell's microenvironment (85–86). Enzymatic digestion of components of the ECM occurs during inflammatory responses, cell migration, tumor invasion,

cell motility and metastasis (86). Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes (comprising of more than 20 family members) implicated in the degradation of connective tissue and basement membranes, thereby playing pivotal roles during both tumor development and progression (85–88). HGF/SF can bind to a variety of ECM components (such as thrombospondin-1, fibronectin and heparan sulfate proteoglycan), thereby creating a pool of HGF/SF located within the ECM serving to localise the release of matrix-degrading proteolytic enzymes, which in turn enhances the metastatic potential and motile behaviour of cancer cells, thus allowing invasion to occur through the ECM (15).

4.1 The Release of Soluble Forms of Cell Adhesion Molecules by Matrix-Degrading Enzymes

Activated forms of MMPs are believed to be responsible for both tumor invasion and metastasis because of their ability to degrade extracellular matrix, and basement membrane components respectively (87). HGF/SF has been implicated in the upregulation of MMP-1, MMP-9 and MMP-14 production in mesothelioma cells in a dose-dependent manner (89). Similarly, Dunsmore *et al.* (90) has shown that HGF/SF stimulates the production of MMP-1 and MMP-3 in keratinocytes, in a dose-dependent and matrix-dependent manner. In another study by Bennett *et al.* (91), HGF/SF was shown to regulate the production of MMPs by oral carcinoma cells. We have shown in Figure 3 the release of matrilysin (otherwise known as MMP-7; the smallest known family member of MMPs) mediated by HGF/SF, causing cleavage to the ectodomain of E-cadherin (producing a soluble 80kDa fragment into the cell supernatant fraction) which in turn, causes an increase in both cellular scattering (Figure 4A) and invasion (Figure 4B) in prostate cancer (67). Furthermore, we have also demonstrated how we may effectively block this mechanism with an antisense-oligonucleotide specifically directed towards matrilysin.

Activation of MMPs are subject to regulation by their association with TIMPs (tissue inhibitors of metalloproteinases) and once activated, may be inactivated by the presence of TIMPs. However, other modes of metalloproteinase activation involving the shedding of cell-cell adhesion molecules are thought to exist. One such mechanism may include the involvement of HGF/SF in the activation of urokinase-type plasminogen, thereby converting the pro-active form (28kDa) of matrilysin into its active (19kD) form, in order to release a soluble 80kDa fragment of E-cadherin within the cell's supernatant fraction (67). A schematic representation of this possible mode

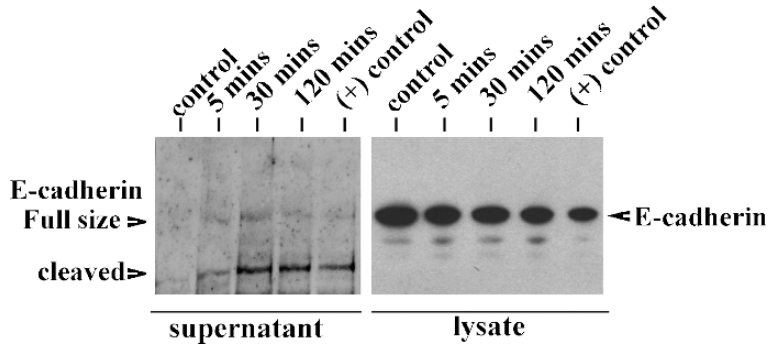

Figure 3. HGF/SF induced a loss of E-cadherin from LNCaPFGC cells. Cells were stimulated with HGF/SF (40ng/ml) for the times indicated. The supernatant fractions were collected and the cells lysed. The level of E-cadherin in the cell lysate fraction was reduced after HGF/SF stimulation (right). Consequently, a small fragment of approximately 80kDa appeared in the supernatant fractions (left), after challenging with HGF/SF. This fragment was recognised upon probing with an anti-E-cadherin antibody (ref 67).

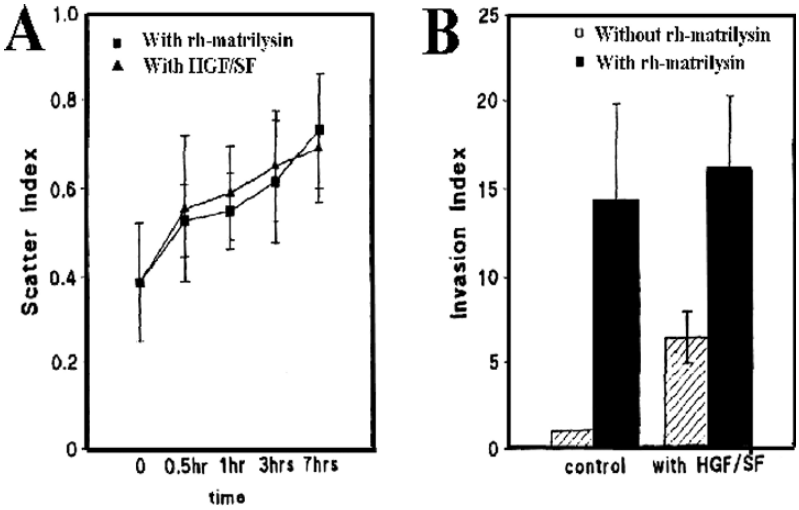

Figure 4. (A). The effect of rh-matrilysin (100units/ml), or HGF/SF (60ng/ml) on colony scattering in LNCaPFGC cells after incubation at 37oC for upto 7 hours. There was a significant difference in scattering index as determined by area morphometry after 30 mins exposure to either rh-matrilysin ($p = 0.008$), or HGF/SF ($p = 0.021$) respectively, using the mean + sem from 10 colonies per time point. (B). The invasive capacity of LNCaPFGC cells through basement membrane matrigel was significantly enhanced by inclusion of either; 100Units/ml of rh-matrilysin ($p = 0.0037$), or 40ng/insert of HGF/SF ($p = 0.0004$) respectively, after 96 hours of culture at 37oC (67).

of activation is demonstrated in Figure 5. MMPs-3 and -7 have also been reported to cleave the extracellular portion of E-cadherin from both MCF-7 and MDCKts.*src*Cl2 cells, releasing a circulating (80kDa) fragment into the culture medium of cells after stimulation with phorbol-12-myristate-13-acetate (92). This effect was inhibited by over-expression of TIMP-2, suggesting that MMPs were indeed responsible for E-cadherin cleavage in these cell types (92). Furthermore, forced expression of an activated form of MMP-3 has been reported to cleave and degrade E-cadherin levels, thus contributing to an invasive mesenchymal transformation in mammary epithelial cells (85). MMPs have also been implicated in the proteolytic cleavage of VE-cadherin producing a soluble 90kDa fragment after growth factor deprivation-induced apoptosis (93). It would appear that MMPs play definitive roles in enhancing the metastatic potential of cancer cells by firstly, degrading components of the ECM and secondly, disrupting cell-cell adhesion mechanisms giving rise to a more invasive phenotype.

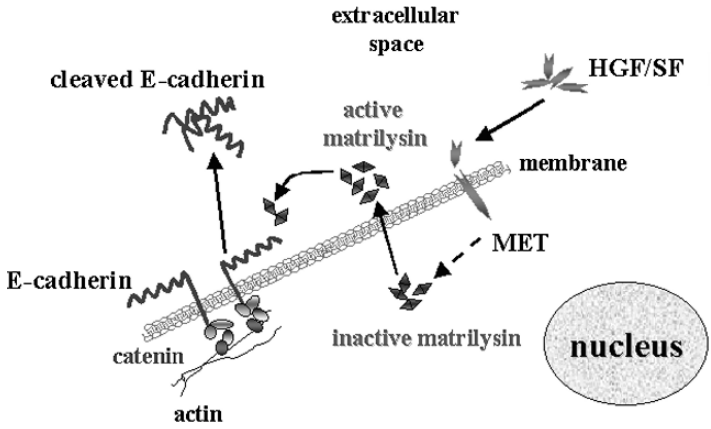

Figure 5. A schematic representation of the possible pathway involved in HGF/SF activation of its receptor c-Met, which in turn activates 'pro-active' matrilysin into its 'active' form (i.e. the pathway for this mechanism is not known as indicated by the broken arrow head). The subsequent release of activated matrilysin into cell supernatant fraction results in the cleavage of the extracellular domain of E-cadherin, producing an 80 kDa soluble fragment. Full length E-cadherin (120 kDa) associates with a group of cytoplasmic proteins called catenins (i.e. α-, β- and γ-catenins) which aid in homophilic interactions in the presence of extracellular calcium, forming tight cell-cell adhesion complexes.

5. HGF/SF, ITS RECEPTOR AND CLINICAL ASPECTS IN PROSTATE CANCER

A correlation between the level of HGF/SF and disease progression in prostate cancer has recently become established. For instance, immunoreactive HGF/SF in the serum of patients with prostate cancer has been detected. Naughton *et al*. (94) have shown a stepwise increase in the level of HGF/SF in serum from patients with adenocarcinoma of the prostate (i.e., 700 pg/ml in normal controls; 974 pg/ml in patients with localised prostate cancer; and 2117 pg/ml in patients with metastatic disease). It is interesting to note that in this study when the level of prostate-specific antigen (PSA) and tumor grade were known, the level of HGF/SF was the most significant predictor for the presence of metastatic disease. Interestingly, a similar pattern of increase for HGF/SF receptor staining has been observed. Furthermore, staining for the HGF/SF receptor was detected in 18% of benign prostate lesions; 84% of primary prostate cancers; and 100% of metastatic bone marrow, and lymph node lesions respectively (95). In addition, the same study showed a significant correlation between the intensity of staining for the HGF/SF receptor and grade of prostate cancer (i.e., 20% in Grade I; 88% in Grade II; 93% Grade III; and 100% in Grade IV). In an independent study conducted by Watanabe *et al*. (96), a stepwise increase in the expression of the HGF/SF receptor; prostatic intraepithelial neoplasia (PIN) (36%); latent prostatic cancer (33%); clinical prostate cancer (71%); and metastatic prostate cancer (100%) has been demonstrated. These clinical observations are supported by laboratory studies showing that the HGF/SF receptor is expressed in prostate cancer cell lines (78, 97); that HGF/SF is expressed; and that bioactive HGF/SF is produced by both prostate stromal fibroblasts, and myofibroblastic cells (97–99). *In vitro*, HGF/SF has been shown to increase the migration, invasiveness, and growth of prostate cancer cells (56, 78, 97, 99). Interestingly, HGF/SF has also been shown to activate the androgen receptor, and may thus be involved in androgen-dependent, and independent growth of prostate cancer cells (98, 100, 101).

The role of HGF/SF and its receptor in the interplay between prostate cancer cells and stromal cells has been well documented. Stromal fibroblasts from prostate tissues have been shown to express and produce bioactive HGF/SF. Furthermore, the amount of bioactive HGF/SF produced by prostate stromal fibroblasts was found to be significantly higher than the amount secreted from skin and bone marrow fibroblasts respectively (98–99). In addition, prostate fibroblasts have been shown to significantly increase the growth of prostate cancer cells *in vitro* and

in vivo (98, 102–104). The importance of HGF/SF in the development of prostate cancer is further reinforced by a more recent study showing that normal prostate epithelial cells respond differently to prostate cancer cells (99). In this study, Gmyrek *et al*. (99) demonstrated that both normal prostate epithelial, and prostate cancer cells express the HGF/SF receptor and respond to HGF/SF stimulation with increased migration. However, prostate cancer cells were found to exhibit a significant increase in proliferation to HGF/SF stimulation, while prostate epithelial cells had a reduced proliferation response to HGF/SF. Therefore, it would appear that the induction of invasiveness in response to HGF/SF is most likely to occur as a result of an increase in the amount of bioactive HGF/SF secreted by stromal cells, and by the presence of the HGF/SF receptor on cancer cells (105).

Another report implicating the level of HGF/SF and its receptor on the behaviour of prostate cancer has been clearly demonstrated in a study comparing the incidence of prostate cancer between African American and white Americans (i.e., the incidence of prostate cancer was found to be 2–3 times higher in African Americans than white Americans). In addition, this study has shown that African Americans have a 4-fold increase in the level of HGF/SF receptor expression present in prostate cancer tissues, compared with samples taken from matched Caucasian populations (106).

6. THE THERAPEUTIC ASPECT OF HGF/SF IN PROSTATE CANCER, STUDIES ON THE HGF/SF ANTAGONIST, NK4

Given the pivotal roles of HGF/SF and its receptor in cancer, considerable effort has been made to identify possible therapeutic targets to inhibit the actions of HGF/SF and its receptor in a variety of cancer cell types, including prostate cancer. A number of agents have now been shown to suppress the functions of HGF/SF in cancer cells, including retinoic acid and its derivatives (15); inhibitory factors to invasiveness (107); and gamma linolenic acid (69). Although the action of these products are not specific to HGF/SF; a more recently identified antagonist known as NK4, has been reported to specifically inhibit HGF/SF mediated responses.

6.1 Biological Structure of the HGF/SF Antagonist NK4

There have been a number of studies performed to date, examining the biological effects induced by HGF/SF and their consequences on cancer

development and progression. This has, therefore, prompted attempts to minimise the pleiotrophic actions of HGF/SF both *in vitro* and *in vivo*. The idea of using part of the HGF/SF molecule as a specific antagonist to this motogen through competitive binding to *c-Met* has been postulated at great length (71, 108–110). Date *et al*. (108) constructed a protein called NK4 (50kDa) through enzymatic (elastase-digested HGF/SF) cleavage of mature HGF/SF, which retained the N-terminal hairpin loop (i.e., *c-Met* receptor binding domain) plus all four kringle domains; but was completely devoid of the HGF/SF β-chain and amino acid residues responsible for dimerisation on the C-terminus of the α-chain, thus allowing NK4 to completely inhibit the biological responses driven by HGF/SF through *c-Met* receptor coupling; by competing for binding to the *c-Met*-HGF/SF receptor tyrosine kinase, but without causing the activation of *c-Met* (108, 111–112).

6.2 Inhibitory Effects of NK4 and Clinical Perspectives

NK4 has been shown to display complete antagonism towards HGF/SF mediated responses in hepatocytes and MDCK cells (108, 112) respectively. Furthermore, NK4 has been shown to inhibit tumor invasion through the basement membrane using a co-culture system mimicking tumor-stroma interactions (111, 113). NK4 has also been shown to inhibit the effects of HGF/SF on *in vitro* angiogenesis in human vascular endothelial cells (114). The HGF/SF antagonist NK4, also inhibits cell migration and *in vitro* invasion in a variety of cancer cell types including prostate, colon and breast cancer cells (71, 109, 115). Date *et al*. (111) also reported that local administration of NK4 into mice inhibited tumor growth, invasion and metastasis. Similarly, in a more recent study conducted by Kuba *et al*. (116), NK4 was reported to suppress both tumor growth and metastasis in nude mice by inhibiting HGF/SF mediated angiogenesis. Furthermore, in a separate study carried out by Kuba *et al*. (117) the four kringle domains of HGF/SF known as K1-4 (63kDa) have also been shown to have an anti-angiogenic effect on cultured endothelial cells, however, this variant was found to lack antagonistic activity to HGF/SF-induced responses. Therefore, NK4, reputed to be a specific antagonist to HGF/SF, may offer both unique and strong opportunities in the treatment of cancer, where abnormal expression of HGF/SF and/or its receptor are associated with the disease progression, and prognosis in tumor bearing patients (15, 110).

7. CONCLUSIONS

There is now abundant evidence demonstrating the diverse biological effects displayed by HGF/SF on cells *in vitro* and *in vivo*. HGF/SF has been implicated in the regulation of mitogenesis, motogenesis and morhogenesis; playing pivotal roles in a number of regulatory responses including physiological and pathological processes. In addition, HGF/SF is now known to be widely involved in cellular migration, scattering, motility, matrix adhesion and its degradation, shedding of cell surface adhesion molecules and increased invasiveness of cancer cells. Furthermore, HGF/SF is a known potent factor of angiogenesis in both normal and neoplastic cells. This motogen has also been shown to play a key regulatory role in cancer development. Thus, identifying mechanisms of HGF/SF activation, and more importantly inhibitors and antagonists to this motogen, may help to address the more important clinical issues of cancer treatment such as the prevention of invasion, and metastasis in tumor bearing patients. Although most inhibitors to HGF/SF are still at a developmental stage, recent studies on cancer treatment using NK4 in experimental models look quite promising, and have demonstrated quite clearly the unique therapeutic potential of this HGF/SF antagonist (111, 116). However, the possibility that NK4 can be of therapeutic value to tumor bearing patients clearly warrants further investigation. Therefore, preventing the metastatic spread of cancer cells remains a long-term objective that may greatly benefit the overall management of cancer patients.

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