Hypothesis

Mode of receptor binding and activation by plasminogen-related growth factors

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Abstract Hepatocyte growth factor/scatter factor (HGF/SF) and macrophage stimulating protein (MSP) are plasminogen-related kringle proteins that lost serine protease domain enzymatic activity and became ligands for cell surface tyrosine kinase receptors. They are activated by cleavage to disulfide-linked αβ chains. Surprisingly, despite structural similarities, the high affinity receptor binding regions of the two proteins are different: α chain for HGF, and β chain for MSP. We propose that after cleavage exposes a β chain binding site (high affinity for MSP, low affinity for HGF), monomeric ligand induces receptor dimerization and activation via α and β chain binding sites of different affinity.

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1. Introduction

Met [1] and Ron [2,3] genes encode cell membrane receptor protein tyrosine kinases, that mediate cell proliferation or motility in several tissues. Their activating ligands, hepatocyte growth factor/scatter factor (HGF/SF) and macrophage stimulating growth factor (MSP) respectively, comprise a distinct family of growth factors that evolved from plasminogen [4]. HGF promotes growth, differentiation and migration of a wide variety of cells, including epithelia of numerous organs, vascular endothelium, neurons, myogenic precursor cells, osteoblasts and osteoclasts (for a review see [5]). Activating mutations of Met have recently been shown to occur in selected cases of renal papillary carcinoma [6], and there is evidence for autocrine HGF-Met signaling in various human carcinomas and sarcomas. Uncontrolled activation of Met is associated with tumor invasion and metastasis in several experimental models (for a review, see [7]). There is great interest in the possible therapeutic potential of HGF for tissue regeneration [5] and of HGF-Met antagonists for a broad spectrum of human malignancies. Targets for MSP include keratinocytes [8,9], subpopulations of macrophages [10], and selected cells of the hematopoietic system [11,12]. Although it is closely related to HGF-Met in structure and function, MSP-Ron has not yet been shown to have a role in tumorigenesis.

HGF and MSP have 45% sequence homology to each other, and 40% to plasminogen. They have the same domain organization: N-terminal domain (N domain) containing a hairpin loop motif, four kringles, a cleavage site for activating the protein, and a serine protease-like (sp) domain. They are secreted as single chain precursors, which are converted to mature proteins by cleavage at a single site to make a disulfide-linked αβ chain heterodimer [13,14]. The α chain contains the N domain and the kringles; the β chain comprises the serine protease (sp) domain, which is devoid of enzymatic activity due to catalytic triad mutations.

The receptor subfamily comprises Met, Ron and Sea [15]. Each receptor is composed of two disulfide linked subunits: an extracellular α chain and a β chain containing extracellular, transmembrane and intracellular regions. The domain with tyrosine kinase activity is located within the intracellular part of the β chain. Other unique structural features of this family include similar location of cysteine residues in the extracellular domain and two conserved tyrosines in the carboxy-terminal tail.

2. How do HGF and MSP activate their specific receptors?

2.1. The primary receptor binding site of HGF is located within the N-terminal region NK1

Studies of HGF receptor binding and biological responses [16,17] have established the following: (i) single-chain pro-HGF binds to Met, though affinity is lower than that of cleaved, biologically active αβ chain HGF; (ii) a fragment comprising the N-terminal 272 residues of HGF binds to Met with a K_d 4-fold higher than that of HGF; an HGF variant lacking the N-domain does not bind to Met; (iii) only full length, unmodified HGF can induce an optimal downstream biological response.

Studies of two HGF variants provided additional data on binding to Met. One variant, called HGF-NK2, is derived from an alternative transcript, and comprises the N-terminus and the first two kringles [18]. HGF-NK2 binds to Met with high affinity, and inhibits binding and activity of wild-type HGF. The role of the N domain in binding was studied with another HGF isoform, NK1. The hairpin loop is characterized by a high density of positively charged residues. Substitution of two positively charged residues in the loop with alanine markedly reduced biological potency [19,20].

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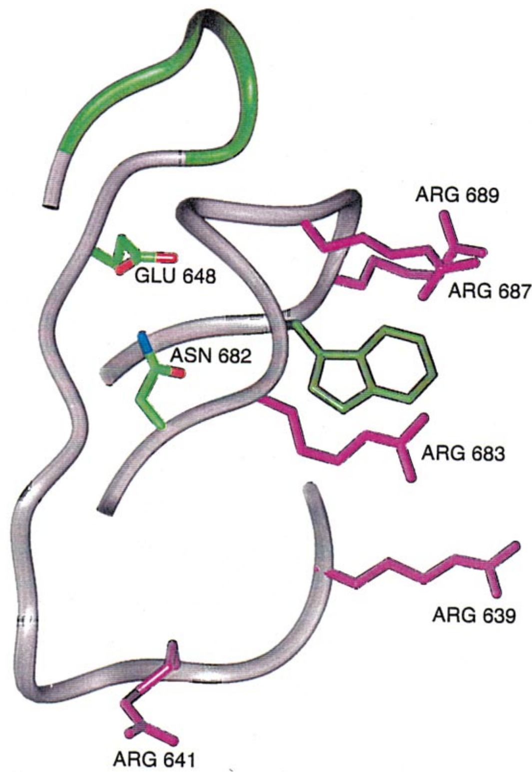

Fig. 1. Distinct features in the MSP β chain, which are not present in the corresponding region of HGF, that may account for receptor binding (based on comparison of 3D models [4]). (1) Cluster of arginines. (2) Conformation of Arg-683, 687 and 689 is stabilized by hydrophobic interactions between alkyl portions of their side chains and the indole ring of Trp-690. (3) Glu-648 and Asn-682 are buried in the region corresponding to the substrate binding pocket in trypsin. (4) Backbone tracing of the exposed hydrophobic loop (green) comprises sequence Leu-650, Leu-651, Ala-652, Pro-653 and Val-654.

2.2. The primary receptor binding site of MSP is located in the β-chain

A structure-activity study using pro-MSP, MSP, α chain, β chain, and an MSP-NK2 variant, revealed that only MSP was biologically active; the β chain was a weak MSP antagonist [21]. In contrast to HGF, free MSP β chain binds with high affinity to its receptor (K_d 1.4 nM for β chain, K_d 0.6–0.8 nM for MSP), whereas a binding site on the α chain is undetectable, as shown by no binding of pro-MSP or MSP-NK2. Absence of pro-MSP binding to Ron shows that the β chain binding site is exposed only after proteolytic cleavage to the MSP heterodimer. Modeling studies (based on an HGF β chain three-dimensional model [4]), revealed that Arg-639, 641, 683, 687, and 689 form a cluster of positively charged residues on the MSP sp domain surface (Fig. 1), which may account for its high affinity binding to Ron.

2.3. Hypothetical mechanism of receptor dimerization and activation

It has been generally accepted [22] that ligand-induced receptor activation involves dimerization of receptors followed by autophosphorylation occurring in *trans* (between two molecules) of specific tyrosines in the cytoplasmic portion of the β chain. Phosphorylation of conserved tyrosine residues within the active site of the tyrosine kinase domain upregulates the kinase activity of the receptor; the phosphorylated carboxy-terminus is a docking site for several SH2 containing proteins [23]. How do MSP and HGF induce dimerization of their receptors? Two different experimentally verified models of receptor dimerization by growth factors are relevant. In the case of the binding of stem cell factor (SCF) to its receptor, Kit, SCF dimers bind to pairs of Kit receptors, the stoichiometry being 2:2 [24,25]. In the human growth hormone (HGH) model, the stoichiometry is 1:2: one region of an HGH monomer binds with high affinity to its receptor (R1), after which another region of HGH binds to a second receptor (R2). Although the second site binding affinity is lower, the complex is stabilized by an R1/R2 interaction as the receptors are brought into proximity by HGH [26,27]. Several observations lead us to favor the HGH model for ligand-induced dimerization of Met and Ron (Fig. 2A).

(1) Sequence and domain structure of MSP/Ron and HGF/Met signaling systems are very similar. These include the N domain, which is homologous to the plasminogen preactivation peptide, and is retained in the mature form of both ligands. The evolution of MSP and HGF from plasminogen suggests that the same domains would be utilized by both growth factors for interaction with their receptors. (2) Although the ligand-receptor interface is usually complex and extensive, only a few residues account for most of the binding free energy [28]. It is likely that positively charged residues are critical for binding to Met or Ron, clusters of which are located in the α chain of HGF [4] and in the β chain of MSP (Fig. 1). Thus, despite their structural similarity, the regions of MSP and HGF that bind with high affinity to their receptors are different. For HGF, it is the α chain (specifically, the N domain of the α chain); for MSP, it is the β chain. (3) Interaction of ligand with receptor via the high affinity binding site is insufficient to activate the receptor; a second region of the ligand is required. For HGF, this second site is cryptic, and becomes exposed after specific cleavage of pro-HGF to the disulfide-linked αβ chain heterodimer. Thus, non-cleavable pro-HGF mutants bind to soluble Met, but have no biological activity on hepatocyte target cells [16]. Since HGF and MSP evolved from zymogens, the serine protease β chains of which become active after αβ chain cleavage, it is likely that proteolytic cleavage of pro-HGF to biologically active HGF results in exposure of a second binding site that is located in the β chain. This idea is supported by the fact that HGF mutants without β chain bind but do not activate [16]. (4) MSP binding is the converse of HGF. The high affinity binding site is hidden in the β chain of pro-MSP,

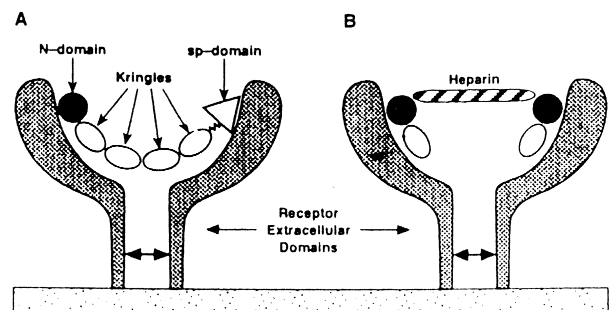

Fig. 2. Two models of receptor dimerization. A: Dimerization of Met or Ron with a ligand:receptor stoichiometry of 1:2. B: Dimerization of Met by a heparin-NK1 complex, with an NK1:Met stoichiometry of 2:2.

and becomes exposed by proteolytic cleavage to MSP. This accounts for the fact that – in contrast to pro-HGF, which has its high affinity binding site in the α chain – pro-MSP does not bind to receptor. Although free β chain binds with high affinity to Ron, it does not induce biological activity or receptor phosphorylation. Requirement for the whole molecule suggests that there is a second receptor binding site in the MSP α chain. This possibility is currently being evaluated. (5) The K_d values of HGF or MSP variants that bind to receptor (HGF-NK1, HGF-NK2, pro-HGF, MSP β chain) are 2–10-fold higher than the K_ds of the mature intact ligands. This is consistent with the suggestion that the mature ligands have two binding sites for their receptors.

Is the experimental evidence consistent with the alternative model of receptor dimerization induced by a ligand dimer? Neither MSP nor HGF are dimers in solution at physiological concentrations. Heparin-like oligosaccharides can induce dimerization of HGF [29], and therefore dimer formation might be induced by cell surface proteoglycan. HGF-NK1 did not bind to glycosaminoglycan-deficient CHO-745 cells, but could bind and cause Met tyrosine phosphorylation in the presence of added heparin [20]. In view of this proteoglycan-dependent agonist activity, it is possible to imagine a proteoglycan-linked NK1 dimer that binds to a pair of Met receptors via the NK1 high affinity sites (Fig. 2B and [30]). However, this model may have no relationship to how full-length HGF induces receptor dimerization, for which mature HGF β-chain is a requirement. Furthermore several lines of evidence show that sulfated polysaccharides are not required for HGF/Met interaction. HGF binds to soluble Met receptor in the absence of heparan sulfate, and it induces biological activity in cell line mutants that do not express heparan sulfate ([30], pp. 150–153). If the reason that both chains are required for receptor activation is formation of ligand dimers, this would involve binding of HGF to Met via α chains and interaction of a pair of HGF molecules via their β chains [30]. For MSP, binding to Ron would be via β chains, with dimer formation via α chain pairing. This requires postulating two different sets of intermolecular interaction sites (β chains for HGF and α chains for MSP), which cannot be ruled out, but are unknown at present. In contrast, the 1 ligand:2 receptor model involves ligand/receptor interaction regions that are already known, and a common mechanism for pro-MSP and pro-HGF activation by cleavage that exposes a β chain receptor binding site. In analogy to the HGH model, HGF α chain binds to Met receptor 1, after which HGF β chain can bind to Met receptor 2 (Fig. 2A). The converse is the case for MSP and Ron. It is assumed that the ligand/receptor interfaces for these two growth factors are similar, the differences in affinities of their corresponding chains being accounted for by a small number of surface residues. The complex is presumably stabilized by interactions between receptor pairs. When soluble recombinant receptor becomes available, it may be possible to determine ligand:receptor stoichiometry and also to test our hypothesis that HGF and MSP ligands are bivalent with respect to receptor binding.

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