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Case Report

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Macrophage Stimulating Protein (MSP): significance in cell biology, life science, and clinical medicine

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ABSTRACT

Macrophage stimulating protein (MSP) was discovered as a serum protein affecting macrophage motility, and found to be similar with a hepatocyte growth factor (HGF)-family molecule, HGF-liked protein (HLP). MSP/HLP has 45% sequence similarity with HGF. Inactive pro-MSP is synthesized by hepatocytes and released into the circulating blood, and its activation is regulated in disease pathologies such as in inflammation and cancer progression. Through binding to the RON receptor, MSP acts in innate-immune responses and anti- inflammation, and prevents the induction of iNOS, COX-2, and PGE_2 in response to endotoxin and interferon-□. Therefore, MSP is a crucial regulator of inflammation in multiple animal disease models of the liver, kidney, lung, gut and other organs. Also, MSP suppresses glucose production and negatively regulates the expression of gluconeogenic enzymes. HGF-Met and MSP-Ron signaling have analogous functions. In future, MSP/RON signaling becomes a new important drug target, especially for excess inflammation, and the inhibition of Ron inhibitors is expected for cancer therapy.

Profile of MSP and its receptor RON:

Macrophage stimulating protein (MSP) was discovered as a serum protein promoting mouse macrophage motility in 1976 [1], and was later purified to homogeneity from human plasma.On the other hand, while searching for hepatocyte growth factor (HGF)-family molecules, a unique protein was isolated with a domain structure similar to that of HGF, and the putative protein was named HGF-liked protein (HLP) in 1991 [2]. Based on the

amino acid sequence homology and the biological activity in macrophages, Shimamoto et al. found that HLP was identical to MSP [3]. MSP/HLP is a heterodimeric protein consisting of a disulfide-linked 53-kDa □-chain and 25-kDa □-chain, and has a 45% amino acid sequence similarity to HGF [2]. Similar to HGF, MSP is characterized by kringle domains (highly conserved triple disulfide loop structures) in the □-chain, and a serine protease domain in the □-chain, but is devoid of enzymatic activity because of amino acid substitutions in the catalytic triad. The activation mechanism by which biologically inactive pro-MSP is cleaved (at Arg483-Val484 bond) to make the active disulfide- linked heterodimer by serine protease also remains. This cleavage has been detected during blood coagulation, tissue injury, and local inflammation. A number of proteases within different tissues have been found to cleave pro-MSP, such as kallikrein, coagulation factor XII and XI, nerve growth factor-□ and epidermal growth factor-binding protein (EGF-BP), hepatocyte growth factor activator (HGFA), hepsin, human airway trypsin- like protease (HAT) and membrane serine protease 1(MT-SP1). The conversion to mature MSP may also be mediated by proteolytic enzymes associated with the cell membrane from resident or exudate peritoneal macrophage. Pro-MSP is synthesized by hepatocytes [4] and is released into the circulating blood, where concentrations in the low nanomolar range are found. Pro-MSP activation is not observed in freshly prepared human serum, thus the activation by serum convertases is an important physiological response to tissue injury.MSP mediates its biological activities through a receptor tyrosine kinase, RON/STK [5]. The *RON* (recepteurd'originenantais) gene was isolated from a human keratinocyte cell line, and the stk (stem cell-derived tyrosine kinase) gene, the mouse homolog, was isolated from mouse hematopoietic stem cells. RON/STK belongs to a family of receptor tyrosine kinases that includes Met, and MSP-induced dimerization leads to its autophosphorylation and kinase activation. Cross-interactions between HGF and Ron, as well as MSP and Met do not occur [5]. Several studies established that the beta chin of MSP (MSP□) binds to the RON sema domain with high affinity [7], and the alpha chain of MSP (MSP□) binds weakly to RON and does not bind in intact cells [7]. At first, MSP□ binds to Ron, and then MSP□ binds, which leads to activation of Ron signaling. MSP□ can be used as a specific RON inhibitor. Of interest, this is contrary to the affinity pattern in HGF. HGF□ can bind to Met occupied with HGF□ and the binding of HGF□ induces tyrosine phosphorylation of the receptor and subsequent cell functions. The N-terminal hairpin- and kringle- containing □-chain is a motif which specifies high-affinity binding to Met, while the□□-chain may play a role in optimal activation of the Met, which enables mitogenic, motogenic, and morphogenic actions of HGF. *RON*/*stk* genes generate two transcripts: 5.0 kb and 2.0 kb mRNAs in human [9]. The longer transcript encodes the full- size receptor, and the short- form (SF), including an alternative start site, has a partial extracellular domain followed by the transmembrane and intracellular sequences (also known as Ron□55). Short *Ron* mRNA is expressed in human lung, ovary, and gastrointestinal tract tissues and also in several human ovarian, breast, pancreas, and lung cancers, as well as leukemia. SF-Ron has nonredundant biological functions relative to full-length Ron in the progression of

immune responses *in vivo* and tumor progression.Ron activation leads to the trans-autophosphorylation of several intracellular C-terminal tyrosine residues. These phosphorylated tyrosine residues serve as high-affinity docking sites for effector proteins containing Src homology-2 and phosphotyrosine-binding domains, including PI3K, phospholipase C□ and growth factor receptor-bound 2 (Grb2). MSP-Ron signaling involves FAK, Src, Jun kinase and STAT3, and further regulation of the □-catenin and nuclear factor kappa B (NF-□B) signaling pathways. Thus, Ron activation leads to the activation of numerous signaling pathways that are capable of inducing pleiotrophic responses.

MSP functions:

MSP transcripts are mostly present in the liver. RON transcripts were detectable in mouse liver from early embryonal stages (day 12.5 p.c.) until adulthood. The adrenal gland, spinal ganglia, skin, lungs, and ossification centers of the developing mandible, clavicle and ribs were also positive at later stages (day 13.5-16.5). From day 17.5, RON was expressed in the gut epithelium and in a specific area of the central nervous system, corresponding to the nucleus of the hypoglossus [10]. In adult mice, RON transcripts are almost ubiquitous, except in the spleen and heart [10]. At first, MSP-RON signaling was found to directly act in the innate- immune responses by inducing phagocytosis of C3bi (complement component C3 fragment)-coated erythrocyte [11], function as an activator of chemotaxis [11], an essential step in the immune defense system, and effectively promote C5a (complement component C5 fragment)-mediated chemotaxis.Among mononuclear phagocytes, expression of RON is restricted to specific populations of terminally differentiated macrophages. Ron is expressed on resident peritoneal macrophages, but not on exudate peritoneal macrophages or mononuclear phagocytes from the bone marrow, peripheral blood, spleen, or alveoli [12]. The stimulatory activities of MSP include the induction of macrophage spreading, migration and phagocytosis. MSP plays important roles in anti- inflammatory activity, and for example, decreases LPS-induced cyclooxygenase-2 (COX-2) and its product prostaglandin E_2 (PGE_2). Notably, MSP prevents the increase of inducible nitric oxide synthase (iNOS) in macrophages in response to endotoxin and interferon- □□(IFN-□), and thus reduces the nitric oxide (NO) production, which is cytotoxic. $RON^{-/-}$ mice exhibited an elevated serum NO level and more severe inflammation after IFN-□ stimulation [13]. Treatment with MSP in murine peritoneal macrophages leads to the up-regulated expression of arginase I, an enzyme that promotes the production of polyamines and proline that promotes the healing process, and competes with iNOS for the substrate- L-arginine, thus yielding the inhibition of NO production [14]. ProinflammatoryM1 (classic) and anti- inflammatory M2 (alternative) macrophages are classified by the expression of iNOS and arginase I, respectively. The switch from the M1 phenotype to the M2 phenotype has prospective therapeutic implications. Thus, MSP-RON signaling regulates the transition between M1 and M2 macrophage activation. MSP-RON has the anti- inflammatory activity via suppression of NF-□B signaling [14]. MSP-RON signaling inhibits serine phosphorylation of NF-□B p65 after LPS stimulation. $RON^{-/-}$ mice exhibited an increased IL-12p40 level in response to LPS, accompanied with an elevated IFN-□ level. MSP

provides classical feedback regulation by TLR signaling and thus, protects animals from septic shock.MSP is a crucial regulator of inflammation in multiple animal disease models of the liver, kidney, lung, gut and other organs [15-18]. RON$^{-/-}$ mice with LPS-induced acute endotoxemia exhibited lowered anti- inflammatory cytokine IL-10 expression in the liver accompanied with decreased level of superoxide dismutase (SOD), an important antioxidant enzyme that works against oxidative stress in the liver. Mice with deletion of tyrosine kinase domain of RON (RON TK$^{-/-}$ mice) were more prone to severe inflammation, showing an increased TNF-□ level and a decreased serum IL-10 level, in response to LPS and galactosamine (GalN), a model of acute liver injury. RON TK$^{-/-}$Kupffer cells treated with LPS lead to a remarkable up-regulation of the serum TNF-□ level, whereas RON TK$^{-/-}$ hepatocytes exhibited an increased resistance to inflammatory factors and cell death [15]. Furthermore, MSP is involved in regulating hepatic glucose metabolism. MSP$^{-/-}$ mice were fed a normal diet, and grew to adulthood without any obvious abnormalities, but exhibited the accumulation of lipid-containing cytoplasmic vacuoles in hepatocytes. MSP dramatically suppressed the cAMP/dexamethasone (Dex)- mediated glucose production and negatively regulated the gene promoter activities and expression levels of key hepatic gluconeogenic enzyme genes, i.e. phosphoenolpyruvatecarboxykinase (PEPCK) and glucose-6-phospatase (Glc-6-Pase). Since AMPK (AMP-activated protein kinase is a main regulator of the cellular responses in both inflammation and metabolic deregulation, the participation of AMPK in MSP-RON signaling in hepatocytes strongly points toward the research value of MSP in liver and systemic metabolism. MSP expression is also correlated with the pathogenesis of inflammatory bowel disease (IBD). A non-synonymous variant in macrophage stimulating 1 (MST1) gene - the gene encoding MSP protein - was linked to IBD, which included both Crohn's disease and ulcerative colitis [17]. This variant in the MST1 gene leads to a mutant MSP that has a R689C substitution in its □ chain, and these mutants showed impaired affinity to RON and/or thermally less stable. Due to the decreased anti- inflammatory potential of the R689C mutant, MSP is required to inhibit the development of IBD. RON TK$^{-/-}$ mice developed more severe colitis following the administration of dextran sulfate sodium, a chemical used to induce chronic colitis. More serious symptoms were observed in these animals, with increased inflammation and pronounced histological changes in the colonic epithelium. During the development of the mouse metanephric kidney, RON has been observed in tubular epithelia. In adults, tubular and mesangial cells express RON, and tubules also express MSP. MSP-RON signaling plays various roles in renal physiology and inflammatory glomerular disorders, especially contrary roles that play either a protective or pathological role. Elevated MSP plasma levels were found both in critically ill patients with acute renal failure and in recipients of renal allografts before the first week following transplantation. MSP stimulated tubular epithelial cell (TEC) proliferation and conferred resistance to cisplatin- induced apoptosis by inhibiting caspase activation. Furthermore, MSP enhanced migration, scattering, branching morphogenesis, tubulogenesis, and mesenchymal dedifferentiation of surviving TECs. Also, RON is expressed in the developing neurons,

such as dorsal root ganglia (DRG) sensory neurons, the hypoglossal nucleus, and trigeminal ganglion, and MSP is recognized as a potent neurotrophic factor [10,19].As a neurotrophic factor for DRG sensory neuron, MSP promotes neurite extension and cellular migration in E15 chick DRG explants with low levels of NGF, and prevents motorne uron atrophy upon axotomy [19]. In addition to neurons, MSP has the potential to modulate the inflammatory actions of microglia. RON is expressed in the primary microglia, and MSP promots microglia migration without affecting cell survival and proliferation. Thus, MSP is one of the key players during neural degeneration and regeneration through the activation of cytokine production and cell migration in microglia. MSP-Ron signaling impacts human neuroinflammatory diseases [20]. MSP and RON present in human brain perivascular macrophages and microglia, but RON was diminished in both multiple sclerosis (MS) patients and MS animal models with experimental autoimmune encephalomyelitis (EAE). After induction of EAE, $RON^{-/-}$ animals exhibited significantly increased CNS pro-inflammatory gene expression. Thus, $RON^{-/-}$ mice show an exacerbation of symptoms during EAE with overall worsened disease severity, increased demyelination, axonal loss, and neuroinflammation. MSP also induces proliferation and migration of murine keratinocytes, and promotes hair growth and from the telogen to anagen stage of hair follicles. Incidentally, human immunodeficiency virus-1 (HIV) infects macrophages for long-term cellular reservoir for virus dissemination and alters cytokines and chemokines.HIV encodes several proteins, including Tat, to modulate the expression of receptors critical for innate immunity. A target of Tat is Ron, which negatively regulates inflammation and HIV transcription [21]. Tat specifically reduces cell surface RON expression in HIV-infected monocytes depending on protein degradation through the ubiquitin-proteasome pathway. By targeting MSP-RON signals, Tat may create a microenvironment for HIV replication and progression of AIDS-associated diseases in the CNS, lung, lymph nodes, and skin.In the future, MSP-RON signalingmayexhibit distinguished worthand become a new important drug target, especially the tissue/organ injuries related to excess inflammation.

Conflict of interest:

The author declares no conflict of interest.

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