Mini Review

Novel mechanism of hepatocyte growth factor against prevention of inflammation and oxidative stress

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Hepatocyte growth factor (HGF) was originally identified as a potent mitogen stimulating hepatocyte growth. It also has mesenchyme-derived pleiotropic effects of regulating growth, motility, and morphogenesis of various types of cells, and is thus considered a humoral mediator of morphogenic tissue interactions. The protective effects of HGF against oxidative stress are important in the early stage of inflammation. Many studies suggest that epidermal growth factor receptor (EGFR) plays an important role in the production of oxidative stress. Some substances such as lipopolysaccharide (LPS) increase EGFR expression. LPS triggers sepsis shock and the systemic inflammatory response syndrome, which results in multiple organ failure. Recent reports demonstrated that HGF attenuated LPS- or angiotensin II (Ang II)-induced oxidative stress via EGFR degradation, and protected against vascular damage. Similarly, HGF inhibited the increase in expression of vascular cell adhesion molecule-1 in pathological conditions attributed LPS or Ang II. The protective effects of HGF are associated with the HGF/c-Met system. Since the HGF/c-Met system inhibits the translocation of SHIP2 to EGFR in pathological conditions, EGFR degradation is triggered via EGFR ubiquitination through SHIP2 binding to its own receptor, c-Met. This newly described mechanism of HGF to regulate SHIP2 recruitment to EGFR and inhibit LPS-induced inflammation via EGFR degradation may be useful for the development of anti-inflammatory agents.

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Introduction

Hepatocyte growth factor (HGF) was originally identified as a potent mitogen stimulating hepatocyte growth. It also has mesenchyme-derived pleiotropic effects of regulating growth, motility, and morphogenesis of various types of cells, and is thus considered a humoral mediator of morphogenic tissue interactions. The presence of its specific receptor, c-Met, is observed in not only hepatocytes but also cardiomyocytes, vascular cells, kidney cells, neurons, skeletal muscle cells and fibroblasts. HGF has recently been classed as a new member of growth factors with anti-apoptotic activity. Since HGF also has angiogenic activity, many studies on it have been conducted in clinical disciplines like cancer research and cardiovascular research. The protective effect of HGF against oxidative stress production is important in the early stage of inflammation. Yoshikage et al. suggested that the anti-apoptotic action of HGF could abrogate the decrease in DNA synthesis in epithelial cells¹. The mechanisms by which HGF prevents apoptosis are still unclear. However, we found that HGF up-regulated an anti-apoptotic factor, bcl-2, in human endothelial cells². It is known that HGF stimulates phosphatidylinositol-3'-kinase (PI3K), protein tyrosine phosphatase 2, phospholipase C- α , pp60^{c-src} and grb2/hSos^{3, 4}. Moreover, HGF also stimulated the Rho- and Ras-mediated signal transduction pathways, resulting in an increase in actin fibers⁵. The activation of these signal transduction pathways suggests that HGF will act to prevent cell death such as apoptosis. Especially, phosphorylation of Akt and ERK plays an essential role in the mitogenic and anti-apoptotic actions of HGF in vascular cells^{6, 7}. It is interesting that the HGF signal transduction pathway contains re-phosphorylation of ERK. Indeed, addition of neutralizing anti-HGF antibody after HGF stimulation attenuated re-phosphorylation of ERK⁷. These data indicate that re-phosphorylation of ERK was due to stimulation of endogenous HGF production. Overall, HGF signal transduction is strongly involved in the anti-apoptotic action of HGF.

Stimulation of LPS affects EGFR expression and signal transduction

Epidermal growth factor receptor (EGFR) is identified as the receptor of EGF observed in many kinds of cells. The ligands are members of the EGF family and TGF family. Some biochemicals are reported to affect EGFR signaling. A recent report suggested that EGFR also facilitated pro-

duction of reactive oxygen species (ROS)⁸. It is known that EGFR is down-regulated by both proteasome and endocytosis, and EGFR signal transduction is controlled by EGFR phosphorylation. Some ligands trigger EGFR signaling through EGFR activation with some phosphorylated sites. EGFR has many sites of phosphorylation, and Tyr845 and Tyr 992 are especially reported to play important roles in anti-apoptosis⁹⁻¹¹.

Recently, it was found that lipopolysaccharide (LPS) and angiotensin II (Ang II) stimulation increase EGFR signaling as well as its expression¹². Ang II has multiple effects on many organs; it induces high blood pressure and inflammation of veins, while fibrosis occurs in the kidney. On the other hand, LPS triggers sepsis shock and systemic inflammatory response syndrome (SIRS), which results in multiple organ failure. LPS, a fat-polysaccharide complex in the outer cell membrane of Gram-negative bacteria¹³, causes clinical symptoms of fever, low blood pressure, inflammation, shock and angioedema during bacterial infection¹⁴. Since current therapy is limited to blood transfusion, antibiotic administration and plasma-apheresis, novel therapeutic approaches are needed. Toll like receptor (TLR) 4, a LPS receptor, recognizes lipid A in LPS through myeloid differentiation factor 2 (MD-2)¹⁵⁻¹⁸. MD-2 is an important link between TLR4 and the LPS signaling pathway^{19, 20}. Administration of LPS triggers the release of many cytokines such as VEGF, TNF- α , IL-1, IL-6, and IL-8²¹⁻²³.

Although the pathogenesis of LPS-induced diseases is complex, inflammation induced by LPS is known to be largely mediated by the EGFR pathway. Both LPS and Ang II induced the expression of vascular cell adhesion molecule-1 (VCAM-1), which is one of the markers of systemic inflammation, via the EGFR/Akt pathway^{12, 24, 25}. In addition, since LPS induced ROS production in several organs^{26, 27}, we determined ROS production in the kidney, liver and lung using erlotinib, an EGFR tyrosine kinase inhibitor. LPS-induced vascular damage through ROS production in endothelial cells triggers lung injury^{28, 29}. Lin et al. showed that LPS induces VCAM-1 expression via the Src/EGFR/PI3-K/Akt pathway²⁵, consistent with our study. They also reported that LPS induced VCAM-1 expression through MAPK and NF- κ B in human tracheal smooth muscle cells²⁸. At the same time, Koff et al. showed that LPS induced EGFR activation via the TLR4-TACE pathway^{30, 31}. These reports suggest that LPS induces inflam-

mation via EGFR activation. These recent data indicate that EGFR plays an important role in LPS-induced inflammation. Our experiment under EGFR siRNA treatment to determine the effect of EGFR on LPS-induced inflammation suggested a direct relationship between EGFR and LPS- or Ang II-induced inflammation^{25, 32}.

Relationship of HGF/c-Met to EGFR

Recently, it was reported that EGFR ubiquitination via casitas B-lineage lymphoma (c-Cbl) is an important step in the mechanism of EGFR expression, and EGFR was mainly expressed in the plasma membrane, whereas EGFR was moved into the cytoplasm by endocytosis when EGFR bound to c-Cbl via ubiquitination. Under pathological conditions, a reasonable amount of Src homology domain 2 (SH2)-containing inositol 5'-phosphatase 2 (SHIP2) might not be recruited to EGFR, which would maintain the binding of c-Cbl with EGFR³³⁻³⁵. Down-regulation of conjugation between SHIP2 and EGFR occurs during c-Cbl binding to EGFR³⁴. The EGFR-c-Cbl complex is broken down by proteasomal endocytosis^{35, 36}. In these studies, SHIP2 was identified as key in ROS production. SHIP2 is known to be expressed ubiquitously and possesses 5'-phosphatase activity and an SH2 domain³⁷. Recently, SHIP2 was reported to interact with c-Cbl and c-Cbl-associated protein in COS-7 cells³⁸. Prasad et al. showed that SHIP2 sequesters c-Cbl from EGFR, thereby regulating the kinetics of the EGFR-c-Cbl association and subsequent degradation of EGFR³⁹. They also reported a role of SHIP2 in EGFR endocytic uptake. More recently, some reports have shown that CIN85 is a multiple domain adaptor protein involved in c-Cbl-mediated downregulation of EGFR⁴⁰. CIN85 src homology 3 domains specifically bind to a proline-arginine motif in c-Cbl, and this association seems to be important for EGFR endocytosis⁴¹. SHIP is one of the CIN85 effectors with PxxxPR motifs. Acting as a molecular scaffold, CIN85 clusters its effectors and recruits them to their site of action⁴². These reports suggest that the SHIP2-CIN85 complex might be recruited to EGFR and sequester Cbl from EGFR.

Novel mechanism of anti-inflammation and anti-oxidative stress

The direct relationship between EGFR and LPS- or Ang II-induced inflammation was made clear by our experiment under EGFR siRNA treatment. It also showed that EGFR

Fig.1 Possible mechanism of inhibition of EGFR signaling transduction by HGF/c-Met system in VSMC

Under basal conditions, SHIP2 prevents excess ubiquitination by inhibiting c-Cbl binding to EGFR after stimulation. The HGF/c-Met system starts to inhibit SHIP2 translocation to EGFR under EGFR activation through binding of SHIP2 to its own receptor, c-Met. Under pathological conditions, c-Cbl binds to EGFR significantly, which might degrade EGFR and act to inhibit EGFR signal transduction.

plays an important role in inflammation and ROS production. Moreover, our data showed that HGF treatment significantly stimulated EGFR degradation under LPS or Ang II stimulation, while MG-132, a proteasome inhibitor, significantly inhibited EGFR degradation induced by HGF treatment^{25, 32}. These data mean that HGF may inhibit inflammation induced by stimulation of EGFR degradation, leading to inhibition of VCAM expression⁴³. As SHIP2 is an important molecule involved in EGFR expression³³, we should focus on SHIP2 under HGF treatment. Because the HGF/c-Met system inhibits the translocation of SHIP2 to EGFR after LPS or Ang II stimulation, through SHIP2 binding to c-Met, EGFR degradation is triggered via EGFR ubiquitination⁴⁴. Under these conditions, a reasonable amount of SHIP2 might not be recruited to EGFR, which would maintain the binding of c-Cbl with EGFR. Here we showed that c-Met regulates LPS- or Ang II-induced inflammation and vascular damage through EGFR down-regulation via the ubiquitin-proteasome system (Fig.1). HGF does not affect EGFR expression in basal conditions. Under the basal conditions, SHIP2 prevents excess the ubiquitination by inhibiting c-Cbl binding to EGFR after stimulation. The HGF/c-Met system starts to inhibit SHIP2 translocation to EGFR under EGFR activation through binding of SHIP2 to its own receptor, c-Met. Under pathological conditions, c-Cbl binds to EGFR significantly, which

might degrade EGFR and act to inhibit EGFR signal transduction. Since there is a sufficient amount of intracellular SHIP2 under basal conditions, SHIP2 can bind to EGFR to preserve EGFR expression. However, a reasonable amount of SHIP2 might not be recruited to EGFR because of the increase in EGFR expression under LPS or Ang II stimulation.

In summary, HGF decreases EGFR activity by EGFR degradation through the ubiquitin system under pathological conditions, but doesn't decrease it under physiological conditions.

Conclusion

Overall, HGF has several protective effects against inflammation such as anti-apoptotic actions, anti-oxidative stress, and acceleration of regeneration of endothelial cells. Many reports demonstrated that HGF prevented LPS-induced multiple organ injuries⁴⁵⁻⁴⁸. The most important finding of recent studies is the protective effects of HGF against multiple organ damage induced by LPS through the inhibition of inflammation and ROS production, which is achieved via EGFR degradation by an E3 ubiquitin protein ligase, the c-Cbl-mediated ubiquitin-proteasome system as well as Ang II stimulation. Under pathological conditions, the HGF/c-Met system starts to have an anti-inflammatory effect on vascular cells. Moreover, HGF/c-Met could interact with EGFR to maintain homeostasis of many organs in the immune system. HGF has been identified as a novel candidate for therapy for SIRS and other vascular diseases, based upon the HGF/c-Met system via EGFR ubiquitination. Considering these unique properties of the HGF/c-Met system, HGF should be developed as a potential anti-inflammatory agent in the future.

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ogy, of the Japanese Government.

Conflict of interest

Ryuichi Morishita is the founder and a board member of AnGes MG, which developed the HGF gene therapy drug.

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