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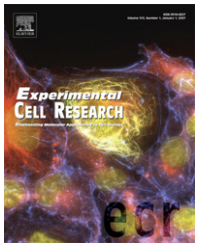

Research Article

c-*jun* amino-terminal kinase and mitogen activated protein kinase 1/2 mediate hepatocyte growth factor-induced migration of brain endothelial cells

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ABSTRACT

Hepatocyte growth factor (HGF) influences several components of the angiogenic response, including endothelial cell migration. While recent studies indicate a crucial role of HGF in brain angiogenesis, the signaling pathways that regulate brain endothelial cell migration by HGF remain uncharacterized. Herein, we report that HGF stimulated human brain microvascular endothelial cell (HBMEC) migration in a dose- and time-dependent manner. Challenge of HBMECs with HGF activated the c-*jun* amino-terminal kinase (JNK), increased phosphorylation of the proline-rich tyrosine kinase 2 (Pyk-2) at Tyr^{402} and activated c-Src. Inhibition of JNK by SP600125 or expression of a dominant negative JNK1 construct abrogated the migratory response of HBMECs to HGF. Treatment of HBMECs with the Src inhibitor PP2 markedly decreased HGF-stimulated JNK activation and migration to HGF. Moreover, expression of a mutant Pyk-2 construct prevented HGF-induced Pyk-2 phosphorylation at Tyr^{402} and stimulation of HBMEC migration. Next, we examined activation of the extracellular signal regulated kinase (ERK) pathway. Stimulation of HBMECs by HGF led to rapid activation of ERK1/2, phosphorylation of Raf-1 at Ser^{338} and $Tyr^{340/341}$ and MEK1/2 at Ser^{222}. Moreover, inhibition of ERK activation by UO126 and PD98059 markedly decreased HGF-stimulated HBMEC migration. HGF also activated AKT, while inhibition of AKT by LY294002 induced a modest decrease of HGF-induced HBMEC migration. These results highlight a model whereby JNK and ERK play a critical role in regulation of brain endothelial cell migration by HGF.

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Introduction

Endothelial cell migration is a complex process that plays a critical role during angiogenesis [1]. This process is regulated by highly orchestrated molecular events that allow endothelial cells to abandon the vascular bed when challenged by extracellular stimuli, infiltrate the membrane basement and reach the interstitial space where they proceed to differentiate into mature blood vessels [1]. The ability of endothelial cells to readily migrate in response to external stimuli is a prerequisite for successful angiogenesis during physiological as well as pathological conditions [2]. On the other hand, inappropriate migration of endothelial cells could compromise the formation of new blood vessels as well as vascular stability and lead, as a result, to an increase of vascular permeability and tissue damage [2,3]. Therefore, elucidation of the intracellular events orchestrating the migratory response of endothelial cells in response to extracellular signals is critical to modulate the angiogenic response for therapeutic purposes.

Hepatocyte growth factor, also known as scatter factor, is a multifunctional growth factor that potently stimulates migration of a variety of cell types, including vascular endothelial cells [4–6]. HGF also regulates other angiogenic cell responses, including proliferation and differentiation [7,8]. Moreover, accumulating evidence indicates that HGF stimulates angiogenesis during pathological conditions such as inflammation, ischemia and tumor growth [9–12]. HGF can act directly on the endothelial cells to induce specific responses that are initiated by binding of the growth factor to c-Met, a tyrosine kinase receptor composed of a 50-kDa extracellular α subunit and a 145-kDa transmembrane β subunit containing several tyrosine phosphorylation binding sites that link c-Met to several downstream signaling pathways [13,14]. Thus, activation of the phosphatidylinositol-3-kinase pathway has been shown to regulate the migratory response of human umbilical vein endothelial cells to HGF [15]. Moreover, HGF induces cytoskeletal-localized events, including phosphorylation of the focal adhesion kinase and its association with paxillin, which results in decreased permeability of pulmonary vascular endothelial cells [16]. Recent studies suggest a critical role of HGF in regulation of angiogenesis in the brain [17,18]. However, the mechanisms by which HGF influences angiogenic responses of brain endothelial cells remain largely uncharacterized.

Members of the mitogen-activated kinase (MAPK) family, including the c-*jun* amino-terminal kinases (JNKs) and the extracellular signal regulated kinase (ERK), are involved in regulation of a wide array of cellular functions, including cell motility [19–22]. Recent work indicates that activation of JNK is involved in regulation of neuronal cell migration [23]. Moreover, inhibition of JNK activity decreased tumor-associated angiogenesis in vivo, implying an important role of this kinase in angiogenesis [24]. The role of JNK in mediating the migratory response of brain endothelial cells has not been established. In this report, we have dissected the signaling pathways involved in regulation of brain endothelial cell migration by HGF. We demonstrate that challenge of brain endothelial cells with HGF leads to activation of JNK and ERK in addition to AKT. Moreover, we show that inhibition of JNK and ERK markedly decreases the ability of HGF to stimulate brain endothelial cell migration. Taken together, our results indicate a critical role for JNK and ERK in regulation of brain endothelial cell migration by HGF.

Materials and methods

Reagents

Endothelial cell medium, attachment factor and endothelial growth factor supplements were obtained from Cell Systems (Kirkland, WA). Hepatocyte growth factor was purchased from R&D Systems (Minneapolis, MN). Fetal bovine serum was purchased from Hyclone (Logan, UT). M199 medium was purchased from Gibco (Grand Island, NY). Myelin basic protein (MBP), enolase, fibronectin and β-actin antibodies were purchased from Sigma (St. Louis, MO). Phospho-ERK (Thr^{202}/Tyr^{204}) antibodies, Flag antibodies and the enhanced chemiluminescence system were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Phospho-Raf-1 (Ser^{338}) and phospho-Raf-1 ($Tyr^{340/341}$) antibodies were purchased from Upstate Biotechnology (Lake Placid, NY). Phospho-MEK1/2 (Ser^{222}) and phospho-Pyk-2 (Tyr^{402}) antibodies were purchased from Biosource (Camarillo, CA). C-Met, phospho-c-Met ($Tyr^{1234/1235}$) and phospho-AKT (Ser^{473}) antibodies were purchased from Cell Signaling (Beverly, MA). γ-^{32}P-adenosine orthophosphate was purchased from Amersham Life Science (Arlington Heights, IL). SP600125, U0125, PP2, PD98059 and LY294002 were purchased from Calbiochem (La Jolla, CA). Protein G plus/protein A agarose beads and $pp60^{src}$ antibodies were obtained from Oncogene Science (Carpinteria, CA). Transwell chemotactic chambers were purchased from Costar/Corning (Corning, NY). Lipofectamine/Plus reagent, precast Tris–Glycine gels and See-Blue protein marker were from Invitrogen (Carlsbad, CA). X-ray films were purchased from Phenix Research Products (Hayward, CA).

Brain endothelial cells

Primary HBMECs were purchased from Cell Systems (Kirkland, WA). Cells are routinely maintained in attachment factor-coated tissue culture flasks and grown in an atmosphere of 5% CO_2 and 37°C in tissue culture medium containing 10% fetal bovine serum and endothelial growth factor supplement. Cells are used between passage 2 and 12.

Plasmids

The pcDNA3-FLAG-JNK1 (APF) vector, which contains a catalytically inactive dominant negative mutant of JNK1 [25], was kindly provided by Dr. R.J. David (Howard Hughes Medical Institute Research Laboratories, University of Massachusetts Medical Center, Worcester, MA). The pME18s-FLAG-Pyk-2 (Y402F) vector contains Pyk-2 in which the autophosphorylation site Tyr^{402} is mutated to alanine [26]. This construct was a kind gift of Dr. T. Katagiri (Division of Biochemistry and Cellular Biology, National Institute of Neuroscience, Kodaira, Tokyo, Japan).