Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 298 (2002) 80–86
BBRC
www.academicpress.com

Hepatocyte growth factor triggers signaling cascades mediating vascular smooth muscle cell migration

Taher E.I. Taher, Patrick W.B. Derksen, Onno J. de Boer, Marcel Spaargaren, Peter Teeling, Allard C. van der Wal, and Steven T. Pals*

Department of Pathology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Received 6 September 2002

Abstract

A key event in neointima formation and atherogenesis is the migration of vascular smooth muscle cells (VSMCs) into the intima. This is controlled by cytokines and extracellular matix (ECM) components within the microenvironment of the diseased vessel wall. At present, these signals have only been partially identified. In this study, we demonstrate that Met, the receptor tyrosine kinase for hepatocyte growth factor (HGF), is expressed on VSMCs isolated from the intima of atherosclerotic plaques of carotid arteries. Stimulation with HGF led to activation of Met as well as to activation of PI3-K, PKB/Akt, MEK, and the MAP kinases Erk1 and -2. Moreover, HGF induced lamellipodia formation, a characteristic feature of motile cells, and promoted VSMC migration across fibronectin-coated filters. The HGF-induced cell migration was mediated by β1 integrins and required PI3-K activation. Our results suggest a role for the HGF–Met signaling pathway in the pathogenesis of atherosclerosis and restenosis.

Keywords: Hepatocyte growth factor; Met; Vascular smooth muscle cell; Atherosclerosis; Migration

Atherosclerosis is the most common fatal disease in Western societies. Early atherosclerotic lesions consist of subendothelial cholesterol-engorged macrophages [1–4]. These 'fatty streak' lesions are the precursors of more advanced lesions characterized by a core consisting of lipid-rich necrotic debris and a fibrous cap consisting of vascular smooth muscle cells (VSMCs) and extracellular matrix (ECM) [1–4]. Migration of VSMCs from the arterial media to the intima is a fundamental aspect of atherosclerotic plaque formation [1–4]. Also, it is critical to restenosis after balloon angioplasty [1,2]. Environmental signals that can regulate VSMC migration include growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), and transforming growth factor (TGF)-β [5]. Furthermore, components of the ECM, including fibrinogen and fibrin, fibronectin, and hyaluronate [6,7], also contribute to the control of VSMC migration.

Recently, it has been shown that the receptor tyrosine kinase Met, the receptor for hepatocyte growth factor (HGF), is expressed on VSMCs after balloon denudation [8,9] and that HGF can promote migration of rabbit neointimal SMC in vitro [9]. HGF, also called scatter factor (SF), is a pleiotropic cytokine that induces complex responses, including growth, motility, and morphogenesis, in target cells, which include normal and malignant epithelia, endothelium, neurons, and B lymphocytes [10–12]. Knock-out mice deficient in either Met or HGF die in utero due to placental and liver defects and, in addition, show a severe defect in the migration of muscle cell precursors [13].

Upon HGF binding, Met is phosphorylated on tyrosine residues at the carboxy-terminus, which creates docking sites for a number of different cytoplasmic proteins. This may result in activation of several signaling cascades [12,14,15]: HGF stimulation triggers the Ras pathway through recruitment of the Grb2–Sos complex to Met, leading to activation of downstream effector molecules, including MAP kinases (MAPK) [12]. HGF-induced Ras activation has been implicated

* Corresponding author. Fax: +31-20-696-0389.
E-mail address: s.t.pals@amc.uva.nl (S.T. Pals).

0006-291X/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
PII: S0006-291X(02)02397-5

in a wide variety of cellular responses, including growth, cytoskeletal reorganization, and motility [16,17]. A second major signaling cascade that has been implicated in the HGF-induced motogenic effects is the PI3-K pathway [12]. Interaction of PI3-K with activated Met may enhance PI3-K activity and/or localize PI3-K in the proximity of its substrates. PI3-K activity has been implicated in the cytoskeletal reorganization required for cell motility and the formation of focal adhesions. These changes in actin organization in motile cells include the formation of a highly compact meshwork of actin filaments at the leading edge of cells, called lamellipodia and membrane ruffles, or the formation of short bundles of actin filaments protruding from the cell surface, called microspikes and filopodia [18]. Furthermore, PI3-K is involved in the regulation of integrin-mediated adhesion, a critical component of cell migration.

In this report, we have explored the role of HGF/Met signaling in VSMCs and show that the HGF–Met signaling pathway is functional in atherosclerotic plaque-derived VSMCs. We demonstrate that HGF stimulation of VSMCs induces lamellipodia formation, a characteristic feature of motile cells, and promotes cell migration. The HGF-induced VSMC migration is PI3-K dependent.

Materials and methods

Materials. Recombinant human HGF was purchased from R&D Systems (Abingdon, UK). Foreskin fibronectin was from Sigma Chemical (Sigma, Bornem, Belgium). Monoclonal antibodies used were: PY20, anti-phosphotyrosine (Affiniti, Nottingham, United Kingdom); 4B4, anti-integrin β1 (Coulter Hialeah, FL). The rabbit polyclonal antibodies used were: anti-hMet (anti-human Met), C-12 (Santa Cruz Biotechnology, Santa Cruz, CA); anti-phospho-p42/44 MAP kinase and anti-phospho-PKB/Akt (New England Biolabs, Beverly, MA). The pharmacological inhibitors used were: PD-98059, LY-294002, and Wortmannin (Biomol, Plymouth Meeting, PA).

Cell cultures. VSMCs were isolated from carotid atherosclerotic plaques by the "explant outgrowth technique." Carotid endarterectomy specimens were obtained from patients with symptomatic atherosclerotic disease. Fresh intimal plaque tissue was cut in small pieces ($1\,mm^3$) and placed in 24-well tissue culture plates that were coated with 1% gelatin. The explants were cultivated in DMEM (ICN Biomedicals, Aurora, OH) containing 10% fetal calf serum (Integro, Zaandam, The Netherlands), 10% pooled human serum (Biowhittaker, Walkersville, MD), 100 U/ml penicillin, 100 μg/ml streptomycin (both from Life Technologies, Breda, The Netherlands), epidermal growth factor (10 ng/ml), and basic fibroblast growth factor (bFGF, 10 ng/ml, Strathmann Biotechnology, Hannover, Germany) in a humidified atmosphere at 37 °C with 5% CO_2. Cells began to migrate from the explants within one to two weeks of culture and reached confluence within another two weeks. Cells were subcultured after trypsinization in $75\,cm^2$, gelatin-coated culture flasks. Growth factors (bFGF and EGF) were omitted from the culture medium in the final passage. The purity of the SMC cultures was determined using immunohistochemistry with anti-smooth muscle actin antibody (1A4, Dako, Glostrup, Denmark) specific for SMC, and anti-von Willebrand factor (vWF) antibody (Dako), to exclude contamination with endothelial cells. VSMC cells from four different patients were used in the experiments.

Immunoprecipitation and Western blot analysis. Briefly, 10×10^5 cells were lysed in lysis buffer containing 10 mM Tris–HCl (pH 8), 150 mM NaCl, 1% Nonidet P-40, 10% glycerol, 10 μg/ml aprotinin (Sigma), 10 μg/ml leupeptin (Sigma), 2 mM sodium orthovanadate, 5 mM EDTA, and 5 mM sodium fluoride. The lysates were spun at $10^4 g$ at 4 °C for 20 min and the immunocomplexes were collected by adding the anti-Met antibodies coupled to protein A–Sepharose for at least 2 h. The bound proteins were washed three times with lysis buffer, resolved by SDS–PAGE, and electrotransferred to nitrocellulose membranes. Detection of proteins by immunoblotting was performed using enhanced chemiluminescence lighting (ECL).

Immunofluorescence microscopy. To investigate the organization of the actin cytoskeleton (stress fibers, lamellipodia or filopodia formation) immunofluorescence microscopy studies were performed as described earlier [19].

Cell migration assay. Cell migration was assayed using a transwell chamber assay. Briefly, second or third passage VSMCs were harvested with trypsin (0.1 mg/ml trypsin), centrifuged, and resuspended in 0.3 % BSA DMEM. Cells (5×10^4) were plated on the upper surface of an uncoated or fibronectin-coated, polycarbonate membrane (8 μm pores) separating two chambers of a 6.5-mm transwell culture plate (Costar, Cambridge, MA). In the bottom chamber 20 ng/ml HGF was added. After the indicated time, cells on the upper face of the membrane were scraped off using a cotton swab. Cells that had migrated to the lower surface of the membrane were fixed with 10% formaldehyde and stained with Giemsa. The number of migrated cells on the lower surface of the membrane was counted in four fields at 100× magnification. Assays were performed in triplicate.

Results

Human carotid artery VSMCs express Met

To assess the expression of Met on cultured human VSMCs, cell lysates of second passage carotid artery VSMCs were immunoblotted with an anti-Met antibody. As shown in Fig. 1A, a band of approximately 145 kDa corresponding to the Met β-chain, as well as a 180 kDa band corresponding to the single chain Met precursor, was detected in the cell lysates. Immunoblots of the cell lysates of the B cell line Namalwa, non- or Met-transfected, are shown as positive and negative controls, respectively.

Stimulation of VSMC with HGF leads to activation of the PI3-K/PKB and RAS/MAPK pathways

To demonstrate the functionality of the HGF–Met signaling pathway in VSMC, we assessed the autophosphorylation of Met in response to HGF stimulation. As shown in Fig. 1B, stimulation of VSMC with HGF resulted in a strong tyrosine phosphorylation of Met. This finding prompted us to explore the activation of two major signaling cascades downstream of Met, i.e., the Ras/MAPK pathway and PI3-K/PKB pathway. These signaling routes have been implicated in the regulation of cell survival and proliferation, respectively [12]. Moreover, the Ras/MAPK pathway and PI3-K have been implicated in cell migration [16,17,20–22]. The MAPKs Erk1 and -2 are activated through phos-

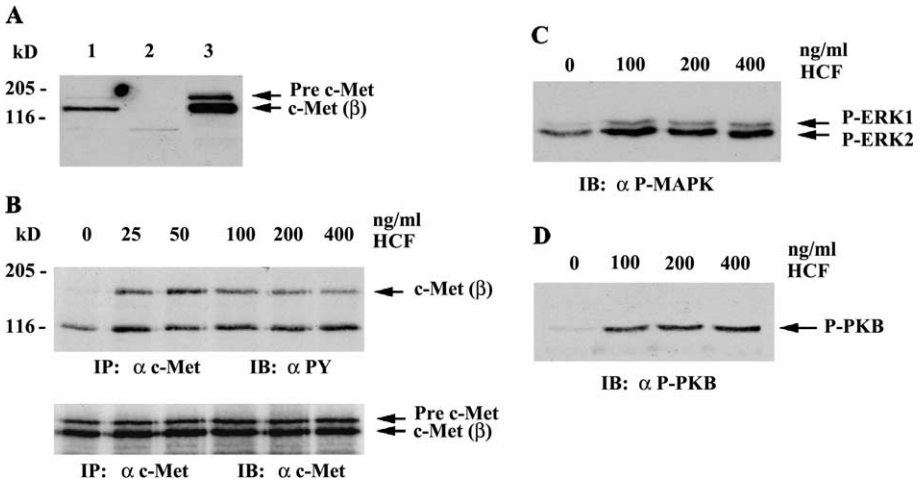

Fig. 1. Activation of Met on VSMCs induces MAP kinase and PKB/Akt activation. (A) Expression of Met on VSMCs. VSMCs (lane 1), Met negative (lane 2), and Met positive (lane 3) B cells were lysed and analyzed for the expression of Met by Western blotting analysis using anti-Met antibodies. The Met precursor and the Met β-chain are indicated (arrows). (B) Dose kinetics of the tyrosine phosphorylation of Met in VSMCs. VSMCs were incubated with increasing concentration of HGF for 5 min. The immunoprecipitates of c-Met were Western blotted, stained with anti-phosphotyrosine antibodies (upper panel), and then restained with anti-Met antibodies. (C) HGF induces activation of MAPK. Total cell lysates of VSMC were stimulated with increasing concentration of HGF and subjected to Western blotting using anti-phospo-MAPK. The phosphorylated ERK1 and -2 are indicated. (D) HGF induces activation of PKB/Akt. Anti-phospho-PKB staining of VSMCs lysates collected after stimulation with different concentrations of HGF. The phosphorylated PKB is indicated.

phorylation of threonine and tyrosine residues in their regulatory motif. Phosphorylation of this motif can be assessed with anti-phospho-MAPK antibodies and indicates MAPK activation. Similarly, phosphorylation of PKB/Akt on threonine/serine can be assessed with anti-phospho-PKB antibodies and reflects the level of PKB activation. As shown in Figs. 1C and D, HGF stimulation of VSMCs induces activation of Erk1 and -2 as well as PKB/Akt. Hence, HGF stimulation leads to the activation of pathways with a potential role in VSMC proliferation, survival, and migration.

HGF induces lamellipodia formation and promotes the integrin-mediated migration of VSMCs

We subsequently explored the role of HGF in VSMC migration, a process that plays a central role in the pathogenesis of both atherogenesis and restenosis [1–4]. Since the actin cytoskeleton maintains cellular shape and plays a pivotal role in cell motility, we initially investigated the effect of HGF stimulation on the reorganization of the cytoskeleton of VSMCs. Unstimulated VSMCs showed a well-organized cytoskeleton with abundant stress fibers organized into bundles (Fig. 2A). However, upon stimulation with HGF, the cells rapidly (within minutes) developed typical lamellipodia, which are characteristic of a motile and migratory phenotype (Figs. 2B and C).

For migration studies, VSMCs were plated on transwell filters, which were either uncoated or coated with fibronectin. HGF was added to the lower compartment of the system. Migration was quantified by determining the number of cells that migrated through the filter after 2, 4, and 8 h. As shown in Fig. 3A, VSMC did not migrate through uncoated filters, neither in the presence nor absence of HGF. In the presence of a fibronectin coating, VSMCs showed a baseline migration rate resulting in the accumulation of VSMC on the lower surface of the filter. However, in the presence of HGF this migration was enhanced 2–3-fold after 8 h (Fig. 3A). This increase is comparable to the migration induced by other established stimulants of VSMC motility [23,24]. The effect of HGF on VSMC migration was due to chemotaxis rather than chemokinesis, since addition of HGF to the upper compartment of the transwell system

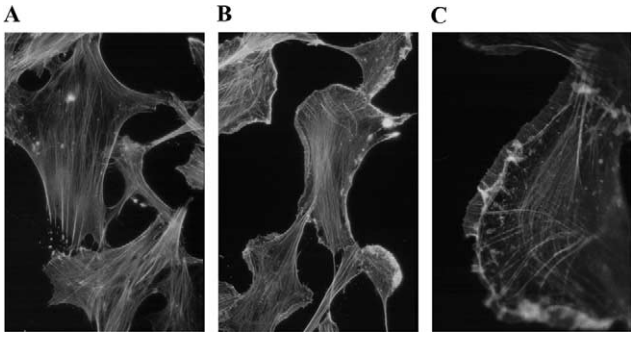

Fig. 2. HGF induces lamellipodia formation. Subconfluent (50–70%) cultures of VSMCs were stimulated with 100 ng/mL HGF for 10 min. Actin filaments were visualized using phalloidin–FITC. (A) Unstimulated cells show stress fibers but no lamellipodia. (B) Cells stimulated with HGF show lamellipodia formation and membrane ruffling. (C) Higher magnification of (B) focussing on lamellipodia and membrane ruffles.

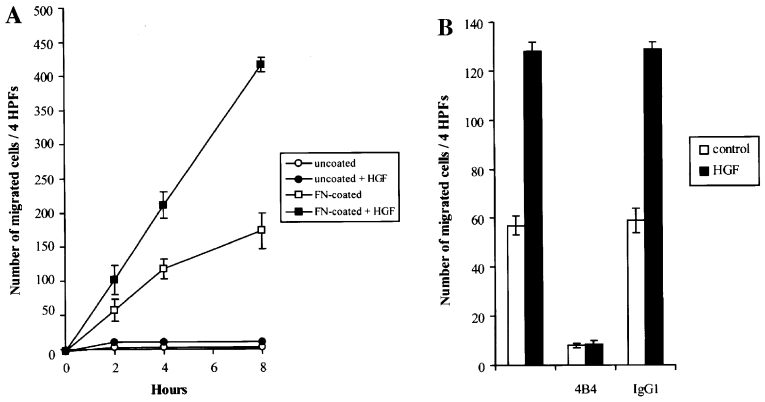

Fig. 3. HGF promotes β1 integrin-dependent migration of VSMCs. (A) HGF induces migration of VSMCs. Migration assays were performed in Transwell chambers either non- or fibronectin (FN)-coated. HGF was added to the lower compartment at a concentrattion of 100 ng/mL for the indicated time. The number of migrated VSMCs was determined at the indicated time points. (B) HGF-induced migration of VSMCs is β1 integrin dependent. To assess the role of β1 integrins in HGF-induced VSMC migration, migration was measured on fibronectin-coated membranes in the absence or presence of anti-β-integrin (4B4) or control antibodies (IgG1). After 8 h, the number of migrated VSMCs was determined. Data represent means ± SD of triplicates.

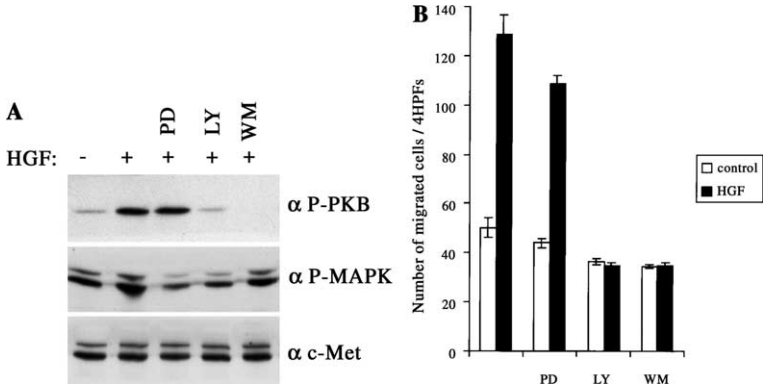

Fig. 4. HGF-induced VSMC migration is PI3-K dependent. (A) HGF-induced activation of PKB and Erk1 and -2 is blocked by using pharmacological inhibitors. Cells were deprived of serum for 3 h and incubated for 30 min at 37 °C with the PI3-K inhibitor Wortmannin (WM, 100 nM) and LY294002 (LY, 20 μM), the MEK inhibitor PD98059 (PD, 50 μM), or control media with DMSO only, prior to incubation with HGF. Activation of PKB and MAP kinases was determined in total cell lysates by immunoblotting with anti-phospho PKB (top) and anti-phospho Erk1 and -2 (α p-MAPK) (middle), respectively. Staining with anti-human Met represents loading controls (bottom panel). (B) HGF-induced VSMCs migration was measured in the absence or presence of WM (100 nM), LY (20 μM), or PD (50 μM). After 8 h, the number of migrated VSMCs was determined. Data represent means ± SD of triplicates.

did not significantly enhance migration (data not shown). Noteworthy, no effect of HGF on VSMC proliferation was observed (data not shown).

Since VSMCs express β1 integrins, the major receptors for fibronectin, we investigated their role in the HGF-mediated VSMC migration. Antibodies against β1 integrins completely inhibited the HGF-induced, as well as the spontaneous, VSMC migration through fibronectin-coated filters (Fig. 3B).

HGF-mediated VSMC migration is dependent on PI3-kinase activity

As demonstrated above, HGF stimulation of VSMC leads to activation of both the Ras/MAPK and the PI3-K/PKB pathways and enhances VSMC migration in a β1 integrin-dependent fashion. Since both PI3-K and Ras have been implicated in the control of integrin-mediated adhesion as well as cell migration, we studied the contribution of these pathways to VSMC migration in response to HGF. To this end, the effect of pharmacological inhibitors of either PI3-K, i.e., Wortmannin and LY294002, or MAPK, i.e., PD98059, on VSMC migration was studied. HGF-induced activation of PI3-K could be specifically blocked by Wortmannin and LY294002, as illustrated by the abolishment of PKB phosphorylation, whereas PD98059 prevented HGF-induced phosphorylation of the MAP kinases Erk-1 and -2 (Fig. 4A). Interestingly, both Wortmannin and LY completely inhibited the HGF-mediated migratory response (Fig. 4B). Also, the spontaneous (HGF independent) migration of VSMC was inhibited by

approximately 50%. By contrast, PD98059 had little or no effect on the HGF-induced and spontaneous VSMC migration (Fig. 4B). Hence, HGF-mediated VSMC migration is dependent on PI3-K, but not on MAPK, activity.

Discussion

Migration of VSMCs from the media to the intima of arteries is one of the major pathological vascular responses involved in atherosclerotic plaque formation as well as in the development of restenosis after percutaneous transluminal coronary angioplasty (PTCA). The present study demonstrates that the HGF receptor Met is involved in the control of the motility of human VSMCs. The HGF-induced cell motility is mediated by β1 integrins and depends on PI3-K activation. Our data suggest that the HGF/Met signaling pathway plays a role in the pathogenesis of atherosclerosis and restenosis.

We demonstrated that primary cultured VSMCs express a functional Met receptor protein (Figs. 1A and B). Stimulation of VSMCs with HGF resulted in enhanced tyrosine phosphorylation of Met (Fig. 1B) as well as in activation of the MAPKs Erk1 and -2, and PKB/Akt (Figs. 1C and D), components of the PI3-K/PKB and the RAS/MAPK pathways, two major signaling routes downstream of Met [12]. Activation of these pathways in cells other than VSMC has been implicated in the complex biological responses mediated through Met, including regulation of cell growth and motility [12]. We observed that Met can control VSMC motility. Stimulation with HGF resulted in a rapid reorganization of the actin cytoskeleton of VSMCs, leading to the formation of membrane ruffles and lamellipodia (Fig. 2), characteristics of a motile and migratory phenotype [18]. Moreover, Met activation by HGF enhanced migration of VSMCs through fibronectin-coated filters (Fig. 3). HGF has been reported to be present in the atherosclerotic plaques [25]. In the diseased arterial wall, macrophages and endothelial cells represent a potential paracrine source of HGF, since both cell types have been reported to produce HGF [9,26,27]. Activated macrophages are not only present in atherosclerotic plaques but have also been detected at the site of PTCA-induced injury [28]. Alternatively, (V)SMCs have been reported to produce HGF [8] and may thus represent an autocrine source of HGF.

Our studies indicate that at least two distinct mechanisms contribute to the HGF-controlled motility changes of VSMCs. First, HGF-induced reorganization of the actin cytoskeleton. These changes evidenced by the rapid induction of membrane ruffles and lamellipodia upon HGF stimulation (Fig. 2) create the cytoskeletal flexibility required for cell locomotion. Second, HGF-controlled regulation of integrin activities. Regulation of the affinity and/or avidity of adhesion molecules of the integrin family, which mediate interactions with ECM components, is fundamental to the control of cell migration in many systems [29]. As shown in our present study (Fig. 3B), integrins, viz., β1 integrins, are also critical for the HGF-stimulated VSMC migration. Recent studies from our own and other laboratories have shown that the HGF/Met signaling pathway is capable of regulating integrin activity in several cell types, including B cells, neutrophils, and epithelial cells [11,21,30,31]. In B cells, HGF-stimulation leads to activation of β1 integrins and promotes adhesion to VCAM-1 and fibronectin [11]. In VSMCs, β1 integrins are prominent players in adhesion to ECM components, including fibronectin, and VSMCs produce vast amounts of fibronectin in response to vascular injury [7,29].

Upon stimulation of the VSMCs with HGF, we observed activation of the Ras/MAPK, as well as PI3-K/PKB signal transduction pathways (Figs. 1C and D). Since a vast amount of evidence supports a role for Ras/ERK and PI3-K in promoting cell migration through receptor tyrosine kinases, we investigated the possible role of these cascades in HGF-induced VSMC migration. The pharmacological PI3-K inhibitors Wortmannin and LY294002 were found to inhibit both spontaneous and HGF-induced VSMC migration, whereas the MAPK inhibitor PD98059 had no effect (Fig. 4B). Previous studies revealed that several stimuli have the ability to control VSMC migration, including PDGF, IGF-I, IL-3, NGF, and TNF-α [2–5]. In the studies addressing the molecular aspects of the underlying signaling mechanisms there appears to be some discrepancy as to the requirement for activation of PI3-K and MAPK in the migratory response [27,32–37]. PI3-K activation has been implicated in the control of integrin β1 activity by a variety of stimuli in other cell types [38], which is in agreement with the critical role of integrin β1 and activation of PI3-K in HGF-induced VSMC migration (Fig. 4B). However, activation of Erk, which has been implicated in integin β1 activation as well [39,40], does not appear to be involved in the HGF-mediated stimulation of VSMC migration (Fig. 4B). In contrast, HGF-induced migration of fibroblasts was recently reported to occur through activation of both Erk and PI3-K [41].

By means of either specific pharmacological inhibitors such as Wortmannin and LY294002, or by expression of dominant negative or constitutively active mutants, the function of PI3-K in Met signaling has been extensively studied. These studies revealed a prominent regulatory role for PI3-K in Met-induced mitogenesis, motility, and morphogenesis [42–45]. PI3-K was one of the first molecules that was shown to become associated with Met upon HGF-stimulation [46,47]. This interaction of PI3-K with Met may enhance

PI3-K activity and/or localize PI3-K in the proximity of its substrate [47]. PI3-K is composed of a p85 adapter subunit, which contains the Met interacting SH2 domain, and a p110 catalytic subunit. PI3-K is able to phosphorylate PIP2 to produce PIP3. PIP3 in its turn can bind to the PH domain of target proteins, resulting in their translocation, membrane localization and, indirectly, in their activation. Besides the ability of PI3-K to directly interact with Met, two additional mechanisms may account for Met-induced PI3-K activation. First, the p85 subunit of PI3-K was also found to associate with the docking protein Gab1 [48]. Second, PI3-K has been identified as an effector molecule for Ras, as Ras has the ability to directly interact with the p110 catalytic subunit of PI3-K [49]. To what extent these three different mechanisms contribute to HGF-induced PI3-K activation and migration in VSMCs remains to be established.

In conclusion, our current results indicate that Met is expressed on VSMCs and that HGF/Met signaling through PI3-K controls β1 integrin-mediated VSMC migration. These data indicate that the HGF/Met signaling pathway may play a role in the pathogenesis of atherosclerosis and restenosis.

References

[1] A.J. Lusis, Atherosclerosis, Nature 407 (2000) 233–241.

[2] S.M. Schwartz, Smooth muscle migration in atherosclerosis and restenosis, J. Clin. Invest. 99 (1997) 2814–2816.

[3] R. Ross, The pathogenesis of atherosclerosis: a perspective for the 1990s, Nature 362 (1993) 801–809.

[4] R. Ross, Cell biology of atherosclerosis, Annu. Rev. Physiol. 57 (1995) 791–804.

[5] J. Waltenberger, Modulation of growth factor action: implications for the treatment of cardiovascular diseases, Circulation 96 (1997) 4083–4094.

[6] E.W. Raines, The extracellular matrix can regulate vascular cell migration, proliferation, and survival: relationships to vascular disease, Int. J. Exp. Pathol. 81 (2000) 173–182.

[7] N. Boudreau, E. Turley, M. Rabinovitch, Fibronectin, hyaluronan, and a hyaluronan binding protein contribute to increased ductus arteriosus smooth muscle cell migration, Dev. Biol. 143 (1991) 235–247.

[8] Y. Nakamura, R. Morishita, J. Higaki, I. Kida, M. Aoki, A. Moriguchi, K. Yamada, S. Hayashi, Y. Yo, H. Nakano, et al., Hepatocyte growth factor is a novel member of the endothelium-specific growth factors: additive stimulatory effect of hepatocyte growth factor with basic fibroblast growth factor but not with vascular endothelial growth factor, J. Hypertens. 14 (1996) 1067–1072.

[9] M. Aoyagi, S. Yamamoto, H. Azuma, M. Yamamoto, M. Tamaki, Y. Niimi, K. Hirakawa, K. Yamamoto, Localization and effects of hepatocyte growth factor on smooth muscle cells during neointimal formation after balloon denudation, Histochem. Cell Biol. 111 (1999) 419–428.

[10] D.S. Grant, H.K. Kleinman, I.D. Goldberg, M.M. Bhargava, B.J. Nickoloff, J.L. Kinsella, P. Polverini, E.M. Rosen, Scatter factor induces blood vessel formation in vivo, Proc. Natl. Acad. Sci. USA 90 (1993) 1937–1941.

[11] R. van der Voort, T.E. Taher, R.M. Keehnen, L. Smit, M. Groenink, S.T. Pals, Paracrine regulation of germinal center B cell adhesion through the c-met-hepatocyte growth factor/scatter factor pathway, J. Exp. Med. 185 (1997) 2121–2131.

[12] R. van der Voort, T.E. Taher, P.W. Derksen, M. Spaargaren, R. van der Neut, S.T. Pals, The hepatocyte growth factor/Met pathway in development, tumorigenesis, and B-cell differentiation, Adv. Cancer Res. 79 (2000) 39–90.

[13] F. Bladt, D. Riethmacher, S. Isenmann, A. Aguzzi, C. Birchmeier, Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud, Nature 376 (1995) 768–771.

[14] A. Bardelli, F. Maina, I. Gout, M.J. Fry, M.D. Waterfield, P.M. Comoglio, C. Ponzetto, Autophosphorylation promotes complex formation of recombinant hepatocyte growth factor receptor with cytoplasmic effectors containing SH2 domains, Oncogene 7 (1992) 1973–1978.

[15] L. Naldini, E. Vigna, R.P. Narsimhan, G. Gaudino, R. Zarnegar, G.K. Michalopoulos, P.M. Comoglio, Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene c-MET, Oncogene 6 (1991) 501–504.

[16] Y. Ueoka, K. Kato, Y. Kuriaki, S. Horiuchi, Y. Terao, J. Nishida, H. Ueno, N. Wake, Hepatocyte growth factor modulates motility and invasiveness of ovarian carcinomas via Ras-mediated pathway, Br. J. Cancer 82 (2000) 891–899.

[17] R. Terauchi, N. Kitamura, Requirement of regulated activation of Ras for response of MDCK cells to hepatocyte growth factor/scatter factor, Exp. Cell Res. 256 (2000) 411–422.

[18] R. Warn, D. Brown, P. Dowrick, A. Prescott, A. Warn, Cytoskeletal changes associated with cell motility, Symp. Soc. Exp. Biol. 47 (1993) 325–338.

[19] M. Spaargaren, J.L. Bos, Rab5 induces Rac-independent lamellipodia formation and cell migration, Mol. Biol. Cell 10 (1999) 3239–3250.

[20] K. Nakanishi, J. Fujimoto, T. Ueki, K. Kishimoto, T. Hashimoto-Tamaoki, J. Furuyama, T. Itoh, Y. Sasaki, E. Okamoto, Hepatocyte growth factor promotes migration of human hepatocellular carcinoma via phosphatidylinositol 3-kinase, Clin. Exp. Metastasis 17 (1999) 507–514.

[21] L. Trusolino, G. Serini, G. Cecchini, C. Besati, F.S. Ambesi-Impiombato, P.C. Marchisio, R. De Filippi, Growth factor-dependent activation of αvβ3 integrin in normal epithelial cells: implications for tumor invasion, J. Cell Biol. 142 (1998) 1145–1156.

[22] S. Kermorgant, T. Aparicio, V. Dessirier, M.J. Lewin, T. Lehy, Hepatocyte growth factor induces colonic cancer cell invasiveness via enhanced motility and protease overproduction. Evidence for PI3 kinase and PKC involvement, Carcinogenesis 22 (2001) 1035–1042.

[23] X. Wang, T.L. Yue, E.H. Ohlstein, C.P. Sung, G.Z. Feuerstein, Interferon-inducible protein-10 involves vascular smooth muscle cell migration, proliferation, and inflammatory response, J. Biol. Chem. 271 (1996) 24286–24293.

[24] M. Kohno, K. Yokokawa, K. Yasunari, M. Minami, H. Kano, T. Hanehira, J. Yoshikawa, Induction by lysophosphatidylcholine, a major phospholipid component of atherogenic lipoproteins, of human coronary artery smooth muscle cell migration, Circulation 98 (1998) 353–359.

[25] H. Ueda, M. Imazu, Y. Hayashi, K. Ono, W. Yasui, M. Yamakido, Immunohistochemical analysis of hepatocyte growth factor in human coronary atherectomy specimens: comparison with transforming growth factor β isoforms, Virchows Arch. 430 (1997) 407–415.

[26] F. Galimi, E. Cottone, E. Vigna, N. Arena, C. Boccaccio, S. Giordano, L. Naldini, P.M. Comoglio, Hepatocyte growth factor is a regulator of monocyte–macrophage function, J. Immunol. 166 (2001) 1241–1247.

[27] Q. Chen, M.C. DeFrances, R. Zarnegar, Induction of met proto-oncogene (hepatocyte growth factor receptor) expression during human monocyte–macrophage differentiation, Cell Growth Differ. 7 (1996) 821–832.
[28] A.C. van der Wal, A.E. Becker, Atherosclerotic plaque rupture—pathologic basis of plaque stability and instability, Cardiovasc. Res. 41 (1999) 334–344.
[29] J. Seki, N. Koyama, N.L. Kovach, T. Yednock, A.W. Clowes, J.M. Harlan, Regulation of β1-integrin function in cultured human vascular smooth muscle cells, Circ. Res. 78 (1996) 596–605.
[30] S. Mine, Y. Tanaka, M. Suematu, M. Aso, T. Fujisaki, S. Yamada, S. Eto, Hepatocyte growth factor is a potent trigger of neutrophil adhesion through rapid activation of lymphocyte function-associated antigen-1, Lab. Invest. 78 (1998) 1395–1404.
[31] L. Beviglia, R.H. Kramer, HGF induces FAK activation and integrin-mediated adhesion in MTLn3 breast carcinoma cells, Int. J. Cancer 83 (1999) 640–649.
[32] L. Pukac, J. Huangpu, M.J. Karnovsky, Platelet-derived growth factor-BB, insulin-like growth factor-I, and phorbol ester activate different signaling pathways for stimulation of vascular smooth muscle cell migration, Exp. Cell Res. 242 (1998) 548–560.
[33] R. Cospedal, H. Abedi, I. Zachary, Platelet-derived growth factor-BB (PDGF-BB) regulation of migration and focal adhesion kinase phosphorylation in rabbit aortic vascular smooth muscle cells: roles of phosphatidylinositol 3-kinase and mitogen-activated protein kinases, Cardiovasc. Res. 41 (1999) 708–721.
[34] R. Kraemer, H. Nguyen, K.L. March, B. Hempstead, NGF activates similar intracellular signaling pathways in vascular smooth muscle cells as PDGF-BB but elicits different biological responses, Arterioscler. Thromb. Vasc. Biol. 19 (1999) 1041–1050.
[35] M.S. Lundberg, K.A. Curto, C. Bilato, R.E. Monticone, M.T. Crow, Regulation of vascular smooth muscle migration by mitogen-activated protein kinase and calcium/calmodulin-dependent protein kinase II signaling pathways, J. Mol. Cell Cardiol. 30 (1998) 2377–2389.
[36] K. Graf, X.P. Xi, D. Yang, E. Fleck, W.A. Hsueh, R.E. Law, Mitogen-activated protein kinase activation is involved in platelet-derived growth factor-directed migration by vascular smooth muscle cells, Hypertension 29 (1997) 334–339.
[37] M. Higaki, H. Sakaue, W. Ogawa, M. Kasuga, K. Shimokado, Phosphatidylinositol 3-kinase-independent signal transduction pathway for platelet-derived growth factor-induced chemotaxis, J. Biol. Chem. 271 (1996) 29342–29346.
[38] W. Kolanus, W. Nagel, B. Schiller, L. Zeitlmann, S. Godar, H. Stockinger, B. Seed, αL β2 integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1, a cytoplasmic regulatory molecule, Cell 86 (1996) 233–242.
[39] P.E. Hughes, M.W. Renshaw, M. Pfaff, J. Forsyth, V.M. Keivens, M.A. Schwartz, M.H. Ginsberg, Suppression of integrin activation: a novel function of a Ras/Raf-initiated MAP kinase pathway, Cell 88 (1997) 521–530.
[40] P.E. Hughes, M. Pfaff, Integrin affinity modulation, Trends Cell Biol. 8 (1998) 359–364.
[41] M. Delehedde, N. Sergeant, M. Lyon, P.S. Rudland, D.G. Fernig, Hepatocyte growth factor/scatter factor stimulates migration of rat mammary fibroblasts through both mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt pathways, Eur. J. Biochem 268 (2001) 4423–4429.
[42] I. Royal, M. Park, Hepatocyte growth factor-induced scatter of Madin–Darby canine kidney cells requires phosphatidylinositol 3-kinase, J. Biol. Chem. 270 (1995) 27780–27787.
[43] I. Royal, T.M. Fournier, M. Park, Differential requirement of Grb2 and PI3-kinase in HGF/SF-induced cell motility and tubulogenesis, J. Cell Physiol. 173 (1997) 196–201.
[44] A. Khwaja, K. Lehmann, B.M. Marte, J. Downward, Phosphoinositide 3-kinase induces scattering and tubulogenesis in epithelial cells through a novel pathway, J. Biol. Chem. 273 (1998) 18793–18801.
[45] N. Rahimi, E. Tremblay, B. Elliott, Phosphatidylinositol 3-kinase activity is required for hepatocyte growth factor-induced mitogenic signals in epithelial cells, J. Biol. Chem. 271 (1996) 24850–24855.
[46] A. Graziani, D. Gramaglia, L.C. Cantley, P.M. Comoglio, The tyrosine-phosphorylated hepatocyte growth factor/scatter factor receptor associates with phosphatidylinositol 3-kinase, J. Biol. Chem. 266 (1991) 22087–22090.
[47] C. Ponzetto, A. Bardelli, F. Maina, P. Longati, G. Panayotou, R. Dhand, M.D. Waterfield, P.M. Comoglio, A novel recognition motif for phosphatidylinositol 3-kinase binding mediates its association with the hepatocyte growth factor/scatter factor receptor, Mol. Cell. Biol. 13 (1993) 4600–4608.
[48] M. Holgado-Madruga, D.R. Emlet, D.K. Moscatello, A.K. Godwin, A.J. Wong, A Grb2-associated docking protein in EGF- and insulin-receptor signalling, Nature 379 (1996) 560–564.
[49] P. Rodriguez-Viciana, P.H. Warne, R. Dhand, B. Vanhaesebroeck, I. Gout, M.J. Fry, M.D. Waterfield, J. Downward, Phosphatidylinositol-3-OH kinase as a direct target of Ras, Nature 370 (1994) 527–532.