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MINERVA BIOTEC 1997;9:85-92

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Cell movement is a critical step in normal embryonic development, tumour metastasis, inflammatory responses, and wound healing. Cell migration has been described as continuous cycles of specific and subsequent morphological changes of the cell body, due to cytoskeletal modifications and formation of adhesive contacts. There is increasing evidence that growth factor receptors can modulate cell motility, interfering either with the assembly/disassembly of the focal adhesion sites and with the mechanism of polymerisation/depolymerisation of the actin filaments. One particular subfamily of growth factors has been characterised by its ability to induce developmental, as well as growth, cell dissociation, and motility stimuli: Hepatocyte Growth Factor/Scatter Factor (HGF/SF) and Macrophage Stimulating Protein (MSP). HGF/SF protein has the property of dispersing or scattering epithelial cell colonies into single isolated cells, with enhanced random, non-polarised motility. The biological activity of the receptor, Met tyrosine kinase, depends on the presence of two phosphotyrosine residues in the carboxy-terminal tail, acting as a multifunctional docking site for SH2-containing effectors. Macrophage Stimulating Protein (MSP), originally discovered by its effect on macrophages, is also active on several epithelial cells. Its receptor, the Ron tyrosine kinase, can be constitutively activated in the absence of the ligand. In these conditions the signalling evoked by active tyrosine kinase induces a strong motile and invasive phenotype. The fact that HGF/SF and MSP elicit motile-invasive responses indicates the important role played by these factors and their receptors in cell physiology and pathology.

KEY WORDS: Cell movement - Growth substances - Signal transduction HGF/SF - Macrophage stimulating protein (MSP) - Hepatocyte growth factor (HGF/SF).

Cell migration plays a crucial role in a wide spectrum of biological events not only for simple organisms but also for animals. The aptitude of several cells to migrate is an important process during embryogenesis, ranging from gastrulation to development of the nervous system. Moreover it lasts also during the adult life, remaining prominent during physiological processes (e.g. inflammatory response, wound healing, and tissue regeneration) as well as in disease, during the metastatic process.[1] Not every cell type is specialised for locomotion, but in given circumstances some of them (e.g. neutrophils, fibroblasts, neurons) are able to move. In many tissues cell motility is normally repressed but it is activated only by certain physiological and pathological conditions (e.g. oncogenic transformation, inflammatory process, tissue regeneration).

Cell locomotion has been described as continuous cycles of specific and subsequent morphological changes of the cell body.[2] These major morphological changes can be summarised as fol-

This work has been supported by AIRC (Associazione Italiana per la Ricerca sul Cancro) and by CNR Project 95.00367.PF39.

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lows: (i) cytoskeletal-mediated extensions (*protrusion*), (ii) formation of adhesive contacts at the cell leading edge (*adhesion*), (iii) movement along the substrate (*traction*), (iv) breaking adhesive contacts (*deadhesion*), (v) cytoskeletal-dependent retraction of the trailing edge (*tail retraction*). Every step of these cycles needs a complex array of molecular events, that requires the physically co-ordinated involvement, both spatially and temporally, of cytoskeletal network, plasma-membrane compartment, and adhesion system.

Cell migration has been described as the formation of intimate and extensive adhesive contacts between cells and substratum. This results from a co-operation between the adhesive system and the actin cytoskeleton, followed by generation of forces across the cell.[3] Therefore, it's of paramount importance to investigate the mechanisms controlling: (i) the status of adhesive contacts with the matrix or with the substrate, determined by the assembly/disassembly of the focal adhesion sites; (ii) the cytoskeletal reorganisation inside the cell, visible as stress fibers and lamellipodia formation, based on polymerisation/de-polymerisation of the actin filaments.

Focal adhesion is the common type of adhesive contact made by cells with the Extracellular Matrix (ECM).[4] Focal adhesions are characterised by integrins as the major adhesion receptors [5,6] and by associated cytoplasmic plaque proteins, including vinculin, talin, paxillin and a number of protein tyrosine kinases, such as FAK (Focal Adhesion Kinase).[4,7] They are the major sites of actin filament attachment at the contact interface, their formation is associated with the process of cell spreading and are sites for co-ordination between cell adhesion and cell migration.[8]

The assembly of the focal adhesions is regulated by ECM ligand binding events. The combination of integrin receptor occupancy and clustering, induced by the ECM ligands triggers a synergistic response that includes re-organisation of the cytoskeleton, association of cytoplasmic plaque proteins, and activation of a local signalling pathway.[6,9] On the other hand, adhesive complexes assembly and disassembly can also be regulated by intracellular signals.[10,11] These signals are generated by biochemical modifications and production of soluble second messengers typical of the signal transduction pathways.

It has been postulated that one of the mechanisms of adhesive complexes formation is tyrosine phosphorylation of integrins, paxillin, tensin, and FAK by specific tyrosine kinases. The phosphorylation on tyrosine residues generates on the focal adhesion components, specific recognition sites for proteins containing SH2 (Src-homology 2) domains [12-14] In addition, FAK exhibits tyrosine kinase activity and phosphorylates cytoskeletal-associated substrates such as talin, Src and paxillin.[15,16] This might be necessary for recruiting additional structural and signalling components of the focal adhesion complex. Furthermore, the focal adhesion proteins can also bind to each other and to actin filaments, through SH3 (Src Homology-3)-binding domains or by LIM domains.[17] The molecular interconnections between the components of the focal adhesion complex and cytoskeletal actin can be enhanced and modified by these multiple biochemical interactions, in order to transmit mechanical forces for cell locomotion.

Focal adhesions and actin-membrane interactions are also regulated by the Rho subfamily of the GTP-binding proteins.[18] Cdc 42, Rac, and Rho stimulate the assembly of structures resembling focal adhesions in association with filopodia, lamellipodia, and actin stress fibers, respectively.[19] Interestingly, Rho can regulate actin polymerisation [20] through a pathway involving the increase in phosphatidylinositol 4,5 bisphosphate (PIP_2) levels.[21] PIP_2 promotes actin filament polymerisation by direct interaction with actin-binding proteins.[22] In addition, Rho and other related GTPases may also function in a local signalling pathway, coupling ligand binding of integrins to focal adhesion assembly.[9] Given the roles played by Cdc42, Rac and Rho in the regulation of the different actin-membrane interactions, these transducers could be sufficient to drive the entire process co-ordinating the cycles of cell extension, adhesion, and detachment that are implicated in cell motility. However, the mechanisms exerted by Cdc42, Rac and Rho in the processes relevant to cell locomotion are not well characterised yet. Many protein tyrosine kinases and tyrosine phosphatases as well as lipid enzymes, including the focal adhesion kinase (FAK), phosphatidylinositol (PI) 3-kinase (PI 3-kinase), phosphatidylinositol phosphate (PIP) 5-kinase (PIP 5-kinase), and the phospholipase C-(PLC-γ) have been identified as key mediators of

the signalling pathway induced by these small G proteins.[4] FAK probably initiates formation of adhesions, PIP-5 kinase generates PIP_2 implicated in the assembly of actin filaments, PI 3-kinase is involved in chemotactic responses and in the modulation of integrin affinity, PLC-γ mobilise actin-binding proteins *via* hydrolysis of PIP_2.[9]

The multitude of intracellular complex interactions, leading to cell motility are dependent upon several stimuli evoked by the extracellular environment, as growth and differentiation factors, cytokines, and chemoattractants. In particular, the rapid effects on cell adhesion and on cell motility, exerted by some growth factors and their receptors are mediated by some of the transducers and effectors mentioned above. In addition, growth factor receptors trigger signalling pathways very similar to the local signals of focal adhesion regulation. For example, Cdc42, Rac and Rho are activated by many growth factors,[23 24] and PI metabolism is linked to the growth factor receptor-mediated cell motility.[25-27] In this review, attention will be focused on the specific growth factors inducing cell migration.

Motogenic growth factors

In general, tissue growth factors are soluble proteins acting on the growth and on the proliferation of specific target cells. Nevertheless, many growth factors work as multifunctional agents, that induce not only cell proliferation, but also a variety of additional effects including cell movement.

Growth factors exert their effect through binding to the cell surface, where a specific transmembrane receptor with protein tyrosine kinase activity (PTK-R) is located. Binding of the growth factor to the extracellular domain of these receptors leads to the transient activation of the kinase and of the signal transduction cascade reactions.[28-31] Ligand binding induces dimerization of receptor molecules, which in turn leads to an increase in catalytic activity. This allows auto-transphosphorylation of the intracellular domain of the receptor on tyrosine residues embedded in specific amino acid sequences. These phosphotyrosines and their flanking sequences become docking sites for proteins containing conserved structural modules, known as SH2 domains. This module is a typical feature of sequences encoding molecules involved in signal transduction.[32]

As mentioned above, there is increasing evidence that growth factor receptors can modulate cell motility, interfering either with the assembly/disassembly of the focal adhesion sites and with the mechanism of polymerisation/depolymerisation of the actin filaments. It is worth nothing that signals elicited by growth factor receptors can activate the same specific intracellular signalling pathways induced by integrins and/or other components of the focal adhesion.[33] For example, PDGF, insulin, and IGF-1 are able to induce re-organisation of the actin cytoskeleton, as during formation of lamellipodia and membrane ruffles, following activation of PI 3-kinase.[34-36] Many data have shed light on the mechanisms by which PI 3-kinase and its lipid products can initiate cell movement. Some evidence suggest that the lipid products of the PI 3-kinase may be involved in mediating interactions with actin filaments and/or microtubular motors.[37 38] Recently, it has been demonstrated that one of the downstream effectors of PI 3-kinase is the small GTP-binding protein Rac, that can be directly responsible for the PDGF-induced chemotactic response by fibroblasts *in vitro*.[39 40]

There are several examples of involvement of growth factors in the regulation of focal adhesion and actin filaments assembly. It has been demonstrated that the platelet-derived growth factor receptor (PDGF-R), upon ligand activation is able to interfere with adhesion and cytoskeletal systems. Phosphorylated PDGF-R induced cytoskeletal reorganisation in the skeletal muscle cell, as well as the tyrosine phosphorylation of paxillin, FAK, and talin in Swiss 3T3 fibroblasts.[41 42] In adherent Swiss 3T3 cells the activated PDGF-R induced the association of PI 3-kinase with FAK.[26] PDGF binding to its receptor induced tyrosine phosphorylation of a 190 kDa protein that co-immunoprecipitated specifically with integrin $\alpha_v\beta_3$.[43] Furthermore, PDGF induces neurite outgrowth of PC12 cells in a PI 3-kinase and PLC-γ dependent manner.[44]

Another growth factor, the Epidermal Growth Factor (EGF) is directly involved in the cytoskeletal organisation of the cell, because its receptor (EGF-R) contains an F-actin binding domain in its intracellular region.[45]

Upon activation of their respective receptors, both PDGF and EGF induce the formation of focal adhesion through the stimulation of the GTP-binding proteins of the Ras family. In addition, PDGF

and EGF receptors phosphorylated PLC-γ1,[46] a prerequisite for the dissociation of the profilin:PIP_2 [47] and gelsolin:PIP_2 complexes,[48] important steps in the regulation of actin polymerisation/depolymerisation. Moreover, stimulation with insulin has been found to induce the direct association between the phosphorylated specific Insulin Receptor Substrate-1 (IRS-1) and the integrin $\alpha_v\beta_3$. Similarly, the $\alpha_v\beta_3$ ligand enhanced several fold DNA synthesis induced by insulin, in cells plated on vitronectin.[49] This result reveals the existence of a direct cross-talk between these two classes of molecules: Integrins and Tyrosine Kinase Receptors.

On the other hand, there are synergistic effects among Growth Factors and integrins involved in the regulation of cell migration and cell adhesion. The migration of FG carcinoma cells on vitronectin matrices needs the activation of the signal transduction pathway by EGF-R, including PKC and PLC-activation.[25 50] In addition, Stem Cell Factor (SCF) enhanced mast cells adhesion to fibronectin, *via* PI 3-kinase activation upon binding to its receptor Kit.[51] This synergism is strictly required for a complete biological effect conveyed by a growth factor. The addition of PDGF to non-adherent fibroblasts, does not induce 4,5 PIP_2 hydrolysis (although PLC-γ becomes activated), neither Ca_2+ mobilisation and PKC activation, three well known events exerted by PDGF and involved in its proliferative response. The failure of PLC-γ to hydrolyse PIP_2 stems from the lack of substrate (4,5 PIP_2), whose synthesis depends on PIP 5-kinase activity, induced by integrin binding to ECM. The full responsiveness of fibroblasts to growth factor stimulation can be rescued, only after plating on fibronectin.[52 53] Adherence to the ECM, and subsequent integrin activation stimulated the small GTP-binding protein Rho, resulting in an increase of PIP 5-kinase activity and synthesis of 4,5-PIP_2, substrate of PLC-γ.[54]

According to this model, mitogenic stimuli are under the double control of growth factors and extracellular matrices: cell proliferation is induced by the growth factor, through the activation of a specific enzyme (PLC-γ), but is modulated by integrins, that influence the cellular responsiveness to growth stimuli, regulating the level of the substrate (PIP_2) for the effector enzyme.

One particular subfamily of growth factors has been characterised by its ability to induce a wide spectrum of biological activities. Among these are differentiative, as well as growth, cell dissociation, and motility stimuli, all conveyed by two heterodimeric proteins, known as Hepatocyte Growth Factor/Scatter Factor (HGF/SF) and Macrophage Stimulating Protein (MSP).

Hepatocyte Growth Factor/Scatter Factor (HGF/SF)

This protein has the property of dispersing or scattering epithelial cell colonies into single isolated cells, by rupture of intercellular junctions and desmosomes. The scattered cells have enhanced motility, showing random, non polarised movement.[55] The determination of its amino acid sequence showed that it is identical to a strong mitogen for hepatocytes and for a wide variety of other epithelial cell types.[56] Hepatocyte Growth Factor/Scatter Factor (HGF/SF), as it is now named, has been generally found to be secreted by mesenchymal cells (fibroblasts and smooth muscle cells) rather than by epithelial and endothelial cells, although its effects are mainly obvious on the latter two cell types.

HGF/SF is a heterodimer with a larger α chain of about 60 KDa and a smaller β chain of about 30 KDa, linked by a single interchain disulphide bond. The protein is glycosylated to a significant extent. It is translated from a single mRNA, and the active form is produced by cleavage of the biologically inert precursor chain. Analysis of the structure of the molecule revealed several features that are not shared with any other growth factor. The α chain contains four kringles, structural motifs that also occurs in plasminogen, tissue-type and urokinase-type plasminogen activators, factor XII and prothrombin. The β chain consists of a serine protease domain that is inactive, because of substitution of two critical residues in the catalytic site.[57]

The high affinity receptor of HGF/SF has been identified as the MET proto-oncogene product. Met is a transmembrane receptor tyrosine kinase, made of a 145 KDa β subunit and a 50 KDa α subunit, that is synthesised as a single chain precursor. The α chain and the N-terminal portion of the β chain are exposed on the cell surface, whereas the C-terminal portion of the β chain is located in the cytoplasm and contains the tyrosine kinase domain and

phosphorylation sites involved in the regulation of enzyme activity and signal transduction.[58 59]

The biological activity of the receptor, including cell motility, depends on the presence of two phosphotyrosine residues in the carboxy-terminal tail, which act as a multifunctional docking site for SH2-containing effectors and activate an array of transductional pathways.[60]

The molecular mechanisms responsible for HGF/SF and Met receptor mediated cytoskeletal rearrangement and consequent cell migration are not fully understood. The role played by the PI 3-kinase in HGF/SF-mediated motility and scattering seems well defined. The HGF/SF induced scattering on MDCK cells can be blocked using the selective PI 3-kinase inhibitor, Wortmannin.[61] Similarly, Wortmannin treatment abrogates HGF-induced chemotaxis and tubulogenesis in renal epithelial cells.[63] These results show that the activation of the PI 3-kinase is critical in HGF/Met-mediated cell dissociation and motility of epithelial cells.

An interesting point is the involvement of the small GTP-binding proteins Ras, Rac and Rho, in the regulation of the migratory responses induced by HGF/SF. Different data have been reported on the Ras involvement in Met-mediated cell migration. Expression of a dominant negative mutant Ras protein or the injection of a neutralising antibody for Ras in MDCK cells blocked HGF/SF-mediated cell dissociation and scatter. This demonstrates that the Ras pathway is essential to mediate the motility signal of HGF/SF-Met receptor to the cell-cell adhesion system and the cytoskeleton of MDCK epithelial cells.[63] In addition, microinjection of an activated form of Ras promoted constituent cell spreading.[64] On the other hand, MDCK cultured cells transfected with a Met mutant, where the Grb2 binding site was specifically abrogated, but all other effectors could bind, were still able to dissociate and migrate. This shows that a direct link with Grb2/Ras is required for transformation, but it is not essential to trigger the scatter response in MDCK cells.[65]

Moreover, microinjection of MDCK cells with a dominant negative mutant of Rac inhibits cell spreading and actin reorganisation induced by HGF/SF, showing that Rac plays a crucial role in mediating the HGF/SF induction of these events.[64] On the other hand, it has been shown that the HGF/SF-mediated cell motility of cultured mouse keratinocytes requires the involvement of Rho, but not of Rac and Ras.[66] However, PI 3-kinase has been shown to be involved in the selective activation of Rac and Rho,[67] therefore the possibility that Met involves Rac and Rho via activation of the PI 3-kinase cannot be ruled out.

Interestingly those, has been observed a redistribution of E-cadherin and desmoplakins I/II following HGF/SF stimulation of epithelial cells,[68] as well as the phosphorylation of β-catenin and plakoglobin,[69] demonstrating the existence of a control-effect of the Met tyrosine kinase receptor on the adhesive elements connecting cells and regulating cell locomotion. Moreover, it has been demonstrated that HGF stimulates motility in oral squamous carcinoma cells mediating the assembly/disassembly of focal adhesions by involvement of $p125^{Fak}$.[70]

Recently, an increasing number of signalling molecules with properties similar to HGF/SF have been characterised, both in physiological and in pathological cells. A Scatter Factor-like factor (SFL) has been identified as a paracrine effector molecule, produced by a metastatic variant of a carcinoma cell line.[71] Another newly discovered factor with scattering activity has been identified in a monocyte-conditioned medium as stimulator of tumor cell motility.[72] Many well characterised cytokines (e.g. aFGF, IL-6, EGF, and TNFα) have also been found to induce dispersion of epithelial cell colonies, migration, and invasion of human carcinoma cells.[69 73 74]

These scattering agents all share the property of scattering epithelial cell colonies, but have different cell type specificity: SFL and HGF/SF do scatter MDCK cells, whereas aFGF, TNFα, EGF and the Monocyte Factor do not. On the other hand, EGF induces MDCK cell proliferation. Thus, the specific effects elicited by a factor depends on the type of target cells as well as on the extracellular environment.

Macrophage Stimulating Factor (MSP)

Macrophage stimulating Protein (MSP) was originally discovered by its ability to make resident peritoneal macrophages responsive to the chemoattractant C5a of the complement.[75]

Macrophage stimulating Protein (MSP) is an 80

KDa disulphide-linked heterodimer which belongs to the HGF/SF family.[76] MSP is synthesized by liver cells, circulates in blood as a biologically inactive precursor and is cleaved by members of the kallikrein family, as well as by macrophage bound proteases.[77 78]

Functional studies have revealed that, in addition to macrophages, MSP acts also on other cell lineages. These include growth stimulation of certain epithelial cell lines, suppression of colony formation of human bone marrow cells induced by steel factor plus GM-CSF, and induction of IL-6 production in primary human marrow megakaryocytes. The receptor for MSP was identified as the RON gene product, a transmembrane receptor protein tyrosine kinase, cloned from a human keratinocytes cell line.[79] The Ron gene encodes a 185 KDa heterodimeric protein composed of a 35 KDa extracellular α-chain and a 150 KDa transmembrane β-chain with intrinsic tyrosine kinase activity.[80 81] Ron belongs to a subfamily of receptor tyrosine kinase that includes Sea and the proto-oncogene Met.

As the HGF/SF prototype of the family, MSP is equally able to induce cell proliferation as well as cell motility on epithelial cells [82] and on murine keratinocytes.[83]

The MSP/Ron receptor can be constitutively activated in the absence of the ligand either as a naturally occurring splicing variant (Δ-Ron) or by expressing molecular chimaeras with constitutively dimerized kinase domains (Tpr-Ron). In these conditions the signalling evoked by the active tyrosine kinase induces a strong motile and invasive phenotype.[84]

Recently it has been demonstrated that the activation of PI 3-kinase is an absolute requirement for MSP-induced cell migration in keratinocytes and epithelial cells.[85] Motility induced by Ron is independent of the threshold of MAP-kinase level of activation, suggesting that the Ras pathway is not a critical step for MSP-induced cell migration. The Ras threshold required for the scattering response is far lower that the necessary for growth and transformation.[86] Ron fulfils the requirements for activating cell dissociation and matrix invasion and provides a naturally occurring example of dissociation between the two arms of the biological responses triggered by tyrosine kinase receptors.

Cell movement is a critical step in normal embryonic development, tumor metastasis, inflammatory responses, and wound healing. The fact that HGF/SF and MSP elicit motile-invasive responses indicates the important role played by these factors and their receptors in cell physiology and pathology. In particular, a possible role for Met and Ron can be envisaged in tumor progression toward metastasis.

References

1. Erickson CA. Cell migration in the embryo and adult organism. Curr Opin Cell Biol 1990;2:67-74.
2. Mitchison TJ, Cramer LP. Actin-based cell motility and cell locomotion. Cell 1996; 84: 371-9.
3. Cramer LP, Mitchison TJ, Theriot JA. Actin-dependent motile forces and cell motility. Curr Opin Cell Biol 1994;6:82-6.
4. Jockusch BM, Bubeck P, Giel K, Kroemker M, Moschner J, Rothkegel M, et al. The molecular architecture of focal adhesions. Ann Rev Cell Dev Biol 1995;11:379-416.
5. Turner CE, Burridge K. Transmembrane molecular assemblies in cell-extracellular matrix interactions. Curr Opin Cell Biol 1991;3:849-53.
6. Hynes RO. Integrins: versatility, modulation, and signalling in focal adhesion. Cell 1992;69:11-25.
7. Gumbiner BM. Proteins associated with the cytoplasmic surface of adhesion molecules. Neuron 1993; 11: 551-564.
8. Hynes RO, Lander AD. Contact and adhesive specificity in the association, migrations, and targeting of cell and axons. Cell 1992;68:303-22.
9. Schwartz MA, Schaller MD, Ginsberg MH. Integrins: emerging paradigms of signal transduction. Ann Rev Cell Dev Biol 1995;11:549-99.
10. Juliano RL, Haskill S. Signal transduction from the extracellular matrix. J Cell Biol 1993;120:577-85.
11. Clark EA, Brugge JS. Integrins and signal transduction pathways: the road taken. Science 1995;268:233-9.
12. Burridge K, Turner CE, Romer LH. Tyrosine phosphorylation of paxillin and pp125FAK accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. J Cell Biol 1992; 119:893-903.
13. Turner CE. Paxillin: a cytoskeletal target for tyrosine kinases. Bioassays 1994;16:47-52.
14. Lo SH, Weinsberg E, Chen LB. Tensin: a potential link between the cytoskeleton and signal transduction. Bioassays 1994;16: 817-23.
15. Chen HC, Appeddu PA, Parsons JT, Hildebrand JD, Schaller MD, Guan JL. Interaction of focal adhesion kinase with cytoskeletal protein talin. J Biol Chem 1995;270:16995-9.
16. Schaller M, Parsons JT. Focal adhesion kinase and associated proteins. Curr Opin Cell Biol 1994;6:705-10.
17. Turner CE, Miller JT. Primary sequence of paxillin contains putative SH2 and SH3 domain binding motifs and multiple LIM domains: identification of a vinculin and pp125 fak-binding region. J Cell Sci 1994;107:1583-91.
18. Hall A. Small GTP-binding proteins and the regulation of the actin cytoskeleton. Ann Rev Cell Dev Biol 1994;10:31-54.
19. Nobes CD, Hall A. Rho, Rac and Cdc 42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell 1995;81:53-62.
20. Hall A. Ras related GTPases and the cytoskeleton. Mol Cell Biol 1992;3:475-9.
21. Chong LD, Traynor-Kaplan A, Bokoch GM, Schwartz MA. The small GTP-binding protein Rho regulates a phosphatidylinositol 4-phosphate 5 kinase in mammalian cells. Cell 1994;79:507-13.

22. Stossel TP. On the crawling of animal cells. Science 1993;260: 1086-94.
23. Ridley AJ, Hall A. The small GTP-binding protein Rho regulate the assembly of focal adhesions and actin stress fibers in response to growth factors. Cell 1992;70:389-99.
24. Ridley AJ, Paterson AF, Johnston CL, Diekmann D, Hall A. The small GTP-binding protein Rac regulates growth factor-induced membrane ruffling. Cell 1992;70:401-10.
25. Chen P, Xie H, Sekar MC, Gupta K, Welis A. EGF receptor-mediated cell motility: phospholipase C activity is required, but mitogen-activated protein kinase activity is not sufficient for induced cell movement. J Cell Biol 1994;127:847-57.
26. Chen HC, Guan JL. Stimulation of PI-3 kinase association with focal adhesion kinase by platelet derived growth factor. J Biol Chem 1994;269:31229-33.
27. Wennstrom S, Siegbahn A, Yokote K, Arvidsson AK, Heldin CH, Mori S *et al.* Membrane ruffling and chemotaxis transduce by the PDGF receptor required the binding sites for PI-3 kinase. Oncogene 1994;9:651-60.
28. Yarden Y, Ullrich A. Growth factor receptor tyrosine kinase. Ann Rev Biochem 1988;57:443-78.
29. Schlessinger J, Ullrich A. Growth factor signalling by receptor tyrosine kinases. Neuron 1992;9:383-91.
30. Fantl WJ, Johnson DE, Williams LT. Signalling by receptor tyrosine kinases. Ann Rev Biochem 1993;62:453-81.
31. Heldin C-H. Dimerization of cell surface receptor in signal transduction. Cell 1995;80:213-23.
32. Pawson T., Protein modules and signalling networks. Nature 1995;373:573-80.
33. Ridley AJ. Membrane ruffling and signal transduction. BioEssays 1994;16:321-7.
34. Kotani K, Yonezawa K, Hara K, Ueda H, Kitamura Y, Sakaue H, *et al.* Involvement of phosphoinositide 3-kinase in insulin-induced or IGF-1-induced membrane ruffling. EMBO J 1994;13: 2313-21.
35. Wennstrom S, Hawkins PT, Cooke F, Hara K, Yonezawa K, Kasuga M *et al.* Activation of phosphoinositide 3-kinase is required for PDGF-stimulated membrane ruffling. Curr Biol 1994;4:385-93.
36. Wymann N, Arcaro A. Platelet-derived growth factor-induced phosphatidylinositol 3-kinase activation mediates actin rearrangement in fibroblasts. Biochem J 1994;298:517-20.
37. Booker GW, Gout I, Downing AK, Driscoll PC, Boyd J, Waterfield MD *et al.* Solution structure and ligand-binding site of the SH3 domain of the p85 α -subunit of phosphatidylinositol 3-kinase. Cell 1993;73:813-22.
38. Kapeller R, Chakrabarti R, Cantley L, Fay F, Corvera S. Internalisation of activated PDGF receptor-phosphatidylinositol-3 kinase complexes: potential interactions with the microtubule cytoskeleton. Mol Cell Biol 1993;13:6052-63.
39. Hawkins PT, Eguinoua A, Qiu R-G, Stokoe D, Cooke FT, Walters R *et al.* PDGF stimulates an increase in GTP-Rac *via* activation of phosphoinositide 3-kinase. Curr Biol 1995;5: 393-403.
40. Parker PJ. PI 3-kinase puts GTP on the Rac. Curr Biol 1995;5: 577-79.
41. Tidball JG, Spencer MJ. PDGF stimulation induces phosphorylation of talin and cytoskeletal reorganisation in skeletal muscle. J Cell Biol 1993;123:627-35.
42. Rankin S, Rozengurt E. PDGF modulation of focal adhesion kinase and paxillin tyrosine phosphorylation in Swiss 3T3 cells. J Biol Chem 1994;269:704-10.
43. Bartfeld NS, Pasquale EB, Geltosky JE, Languino LR. The αVβ3 integrin associates with a 190 kDa protein that is phosphorylated on tyrosine in response to PDGF. J Biol Chem 1993;268: 17270-6.
44. Vetter ML, Bishop JM. BPDGF receptor mutants defective for mitogenesis promote neurite outgrowth in PC12 cells. Curr Biol 1995;5:168-78
45. den Hartigh JC, van Bergen en Henegouwen PM, Verkleij AJ, Boonstra AJ. The EGF receptor is an actin-binding protein. J Cell Biol 1992;119:349-55.
46. Morrison DK, Kaplan DR, Rhee SG, Williams LT. Platelet derived growth factor (PDGF)-dependent association of phospholipase C-V with the PDGF receptor signalling complex. Mol Cell Biol 1990;10:2359-66.
47. Goldschmidt-Clermont PJ, Machesky LM, Baldassarre JJ, Pollard TD. The actin binding protein profilin binds to PIP2 and inhibits its hydrolysis by phospholipase C. Science 1990;247:1575-8.
48. Heldman AW, Goldschmidt-Clermont PJ. Cell signalling and motility activity. Symp Soc Exp Biol 1993;47:317-24.
49. Vuori K, Ruoslathi E. Association of insulin receptor substarte-1 with integrins. Science 1994;266:1576-8.
50. Klemke RL, Yebra M, Bayna EM, Cheresh DA. Receptor tyrosine kinase signalling required for integrin alpha v beta 5-directed cell motility but not adhesion on vitronectin. J Cell Biol 1994;127:859-66.
51. Serve H *et al.* Differential roles of PI3-kinase and Kit tyrosine 821 in Kit receptor-mediated proliferation, survival and cell adhesion in mast cells. EMBO J 1995;14:473-83.
52. McNamee HM, Ingber DE, Schwartz MA. Adhesion to fibronectin stimulates inositol lipid synthesis and enhances PDGF-induced inositol lipid breakdown. J Cell Biol 1992;121:673-78.
53. Schwartz MA, Ingber DE. Integrating with integrins. Mol Cell Biol 1994;5:389-93.
54. Chong LD, Traynor-Kaplan A, Bokoch GM, Schwartz MA. The small GTP-binding protein Rho regulates a phosphatidylinositol 4-phosphate 5-kinase in mammalian cells. Cell 1994;79:507-13.
55. Warn R, A scattering of factors. Curr Biol 1994;4:1043-5.
56. Naldini L. Weidner KM, Vigna E, Gaudino G, Bardelli A, Ponzetto C *et al.* Scatter Factor and Hepatocyte Growth Factor are indistinguishable ligands for the Met receptor. EMBO J 1991;10:2867-78.
57. Nakamura T, Nishizawa T, Hagiya, Seki T, Shimonishi M, Sugimura A *et al.* Molecular cloning and expression of human hepatocyte growth factor. Nature 1989;342:440-3.
58. Giordano S, Ponzetto C, Di Renzo MF, Cooper CS, Comoglio PM. Tyrosine kinase receptor indistinguishable from the c-Met protein. Nature 1989;339:155-6.
59. Naldini L, Vigna E, Ferracini R, Longati P, Gandino L, Prat M et al., Tyrosine kinase encoded by the MET proto-oncogene is activated by autophosphorylation. Mol Cell Biol 1991;11:1793-803.
60. Ponzetto C, Bardelli A, Zhen Z, Maina F, Dalla Zonca P, Giordano S *et al.* A multifunctional docking site mediates signalling and transformation by the Hepatocyte Growth Factor/Scatter Factor receptor family. Cell 1994;77:261-71.
61. Royal I, Park M. Hepatocyte growth factor-induced Scatter of MDCK cells requires phosphatidylinositol 3-kinase. J Biol Chem 1995;270:27780-7.
62. Derman MP, Cunha MJ, Barros E, Nigam S, Cantley LG. HGF-mediated chemotaxis and tubulogenesis require activation of the phosphatidylinositol 3-kinase. Am J Physiol 1995;268: 1211-7.
63. Hartmann G, Weidner KM, Schwartz H, Birchmeyer W. The motility signal of scatter factor/hepatocyte growth factor mediated through the receptor tyrosine kinase Met requires intracellular action of Ras. J Biol Chem 1994;269:21936-9.
64. Ridley AJ, Comoglio PM, Hall A. Regulation of scatter factor/hepatocyte growth factor responses by Ras, Rac, and Rho in MDCK cells. Mol Cell Biol 1995;15:1110-22.
65. Ponzetto C, Zhen Z, Audero E, Maina F, Bardelli A, Basile ML, *et al.* Specific uncoupling of Grb2 from the Met receptor: differential effect on transformation and motility. J Biol Chem 1996;271:14119-23.
66. Takaishi K, Sasaki T, Kato M, Yamochi W, Kuroda S, Nakamura T *et al.* Involvement of Rho p21 small GTP-binding protein and its regulator in the HGF-induced cell motility. Oncogene 1994; 9:273-9.

67. Reif K, Nobes CD, Thomas G, Hall A, Cantrell DA. Phosphatidylinositol 3-kinase signals activate a selective subset of Rac/RHO-dependent effectors pathways. Curr Biol 1996;6:1445-5.
68. Royal I, Park M. Hepatocyte growth factor-induced Scatter of MDCK cells requires phosphatidylinositol 3-kinase. J Biol Chem 1995;270:27780-7.
69. Shibamoto S, Hayakawa M, Takeuchi K, Hori T, Oku N, Miyazawa K *et al.* Tyrosine phosphorylation of beta-catenin and plakoglobin enhanced by hepatocyte growth factor and epidermal growth factor in human carcinoma cells. Cell Adh Comm 1994;1:295-305.
70. Matsumoto K, Nakamura T, Kramer RH. Hepatocyte Growth Factor/Scatter Factor induces tyrosine phosphorylation of focal adhesion kinase ($p125^{Fak}$) and promotes migration and invasion by oral squamous cell carcinoma cells. J Biol Chem 1994;269:31807-12.
71. Bellusci S, Moens G, Thiery JP, Jouanneau J. A scatter factor-like factor is produced by a metastatic variant of a rat bladder carcinoma line. J Cell Sci 1994;107:1277-87.
72. Jiang WG, Puntis MCA, Hallett M. Monocyte-conditioned media possess a novel factor which increased motility of cancer cells. Int J Cancer 1993;53:426-31.
73. Valles AM, Boyer B, Badet J, Tucker GC, Barritault D, Theiry JP. Acidic fibroblasts growth factor is a modulator of epithelial plasticity in a rat bladder carcinoma line. Proc Natl Acac Sci USA 1990;87:1124-8.
74. Tamm I, Cardinale I, Krueger J, Murphy JS, May LT, Sehgal PB. Interleukin-6 decreases cell-cell association and increases motility of ductal breast carcinoma cells. J Exp Med 1989;170: 1649-69.
75. Leonard Ij and Skeel AH. Enhancement of spreading, phagocytosis and chemotaxis by macrophage stimulating protein (MSP) Adv Exp Med Biol 1979;121B:181-94.
76. Yoshimura T, Yuhki N, Wang MH, Skeel A, Leonard IJ. Cloning, sequencing and expression of the human Macrophage Stimulating Protein (MSP) confirms as a kringle protein and locates the gene on chromosome 3. J Biol Chem 1993;268: 15461-7.
77. Wang MH, Yoshimura T. Skeel A, Leonard IJ. Proteolytic conversion of single chain precursor MSP to a biologically active heterodimeric by contact enzymes of the coagulation cascade. J Biol Chem 1994;269:3436-40.
78. Wang MH, Skeel A, Leonard IJ. Proteolytic cleavage and activation of pro-MSP by resident peritoneal macrophage membrane proteases. J Clin Invest 1996;97:720-26.
79. Ronsin C, Muscatelli F, Mattei MG, Breatnach R. A novel putative receptor protein tyrosine kinase of the Met family. Oncogene 1993;8:1195-202.
80. Gaudino G, Follenzi A, Naldini L, Collesi C, Santoro M, Gallo KA *et al.* Ron is an heterodimeric tyrosine kinase receptor activated by the HGF homolog MSP. EMBO J 1994;13:3524-32.
81. Wang MH, Ronsin C, Gesnel MC, Coupey L, Skeel A, Leonard EJ, et al. Identification of the Ron gene product as the receptor of human MSP. Science 1994;266:117-9.
82. Medico E, Mongiovì A, Huff J, Jelinek MA, Follenzi A, Gaudino G *et al.* The tyrosine kinase receptor Ron and Sea control "scattering" and morphogenesis of liver progenitor cells *in vitro.* Mol Biol Cell 1996;7:495-504.
83. Wang MH, Drucos AA, Sun Y, Suda T, Skeel AA, Leonard IJ. MSP induces proliferation and migration of human keratinocytes. Exp Cell Res 1996;226:39-46.
84. Collesi C, Santoro MM, Gaudino G, Comoglio PM, A splice variant of the Ron transcript induces constitutive tyrosine kinase activity and an invasive phenotype. Mol Cell Biol 1996;16: 5518-26.
85. Wang MH, Montero-Julian FA, Dauny I, Leonard IJ. Requirement of Phosphatidylinositol-3 kinase for epithelial cell migration activate by human MSP. Oncogene 1996;13: 2167-75.
86. Santoro MM, Collesi C, Grisendi S, Gaudino G, Comoglio PM Constitutive activation of the Ron gene promotes invasive growth but not transformation. Mol Cell Biol 1996;16:7072-83.