PERGAMON

European Journal of Cancer 36 (2000) 1269–1274

European Journal of Cancer

www.ejconline.com

Rho family proteins in cell adhesion and cell migration

E.E. Evers, G.C.M. Zondag, A. Malliri, L.S. Price, J.-P. ten Klooster, R.A. van der Kammen, J.G. Collard *

The Netherlands Cancer Institute, Division of Cell Biology, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Received 24 March 2000; accepted 3 April 2000

Abstract

Cell migration and the regulation of cadherin-mediated homotypic cell–cell interactions are critical events during development, morphogenesis and wound healing. Aberrations in signalling pathways involved in the regulation of cell migration and cadherin-mediated cell–cell adhesion contribute to tumour invasion and metastasis. The rho family proteins, including cdc42, rac1 and rhoA, regulate signalling pathways that mediate the distinct actin cytoskeleton changes required for both cellular motility and cell–cell adhesion. Recent studies indicate that rac directly influences rho activity at the GTPase level and that the reciprocal balance between rac and rho activity can determine epithelial or mesenchymal cell morphology and migratory behaviour of epithelial (tumour) cells.

Keywords: Rac; Rho; Adherens junctions; E-cadherin; Cell migration; Tiam1

1. Cadherin-mediated cell–cell adhesion of epithelial cells

The proper formation of adherens junctions is critical in development, morphogenesis and the maintenance of epithelial differentiation. Adherens junctions are regions of the plasma membrane where E-cadherin molecules of adjacent epithelial cells contact each other. Destabilisation of these junctions contributes to invasion and metastasis of epithelial tumour cells (see [1,2] and Fig. 1). Cadherins comprise a superfamily of transmembrane glycoproteins with important roles in calcium-dependent intercellular adhesion and signalling pathways. For adhesion, it is essential that the cytoplasmic domain of E-cadherin forms complexes with catenins, i.e. β-catenin, γ-catenin/plakoglobin and α-catenin (Fig. 1). α-Catenin is related to vinculin and is thought to associate with cytoskeleton components by direct interactions [3] or indirectly via α-actinin [4]. β-Catenin is the vertebrate homologue of the *Drosophila* segment polarity gene *Armadillo*, which links cadherin to the cytoskeleton through α-catenin [3,4], and participates in the wnt/wingless signalling pathway [5,6]. γ-Catenin/plakoglobin not only associates with E-cadherin but is also found in desmosomal junctions. In addition, a large number of other proteins are involved in E-cadherin mediated adhesion among those $p60^{Src}$, the Src substrate $p120^{Cas}$ also termed $p120^{Ctn}$, IQGAP a downstream effector of rac and cdc42, and the tumour suppressor protein adenomatous polypolis coli (APC), which might compete with E-cadherin for binding to β-catenin.

2. Functional cadherin-mediated cell–cell adhesions suppress invasiveness

A change in the expression or structure of the cadherins or catenins leads to adherens junction-disassembly and, as a consequence, to migratory and invasive cells. E-cadherin deletion mutants which lack the catenin binding region are unable to form functional adhesions, whereas expression of mutants which lack the extracellular domain act in a dominant-negative fashion and disturb epithelial cell–cell adhesions [7–9]. Apparently, the dominant-negative mutant competes with endogenous E-cadherin in binding catenins, thus preventing functional complex formation. Similarly, inactivation of E-cadherin in MDCK cells by specific antibodies leads to a loss of intercellular adhesion, a fibroblastoid morphology and an invasive phenotype [10,11]. Biochemical modification of E-cadherin and associated proteins correlates with the disassembly of functional E-cadherin complexes. MDCK cells expressing oncogenic ras or the

* Corresponding author. Tel.: +31-20-512-1932; fax: +31-20-512-1944.

E-mail address: jcoll@nki.nl (J.G. Collard).

0959-8049/00/$ - see front matter
PII: S0959-8049(00)00091-5

v-Src oncogene rapidly lose cell–cell contacts and acquire a fibroblastoid morphology and invasive capacity. V-Src mediated tyrosine phosphorylation of cytoskeletal components such as β-catenin, *zonula occludens* (ZO)-1, ZO-2, ezrin, radixin and moesin may play a role in destabilisation of the cell–cell adhesions. Phosphorylation of the E-cadherin/catenin complex and associated cytoskeletal proteins may thus counteract junctional assembly and thereby promote invasiveness and de-differentiation of epithelial cells.

3. Cadherin-mediated cell–cell adhesions in human tumours

Invasive carcinomas show reduced epithelial differentiation, particularly at the invasive fronts where the cells infiltrate into the surrounding stromal cells. The loosening of intercellular adhesiveness is due to the functional disturbance of E-cadherin-mediated cell–cell contacts. Several mechanisms for the inactivation of cadherin-mediated cell–cell adhesion have been proposed in human cancer [1,2]. These include: down-regulation of cadherin expression and mutations within the gene; downregulation, mutation or deletion of catenins; biochemical modification by phosphorylation of components in the cadherin complex; competitive protein interactions of components involved in the formation of stable adhesions, and more recently the activation state of the small GTPases rac1 and rhoA.

The majority of epithelial tumours show reduced E-cadherin expression in a heterogeneous manner and a correlation between reduced E-cadherin expression, loss of tumour differentiation and increased invasiveness has been found, reviewed in [1,2]. These findings are consistent with the hypothesis that the inhibition of E-cadherin function enhances the release of cancer cells from the primary site. Invasiveness of several carcinoma cell lines was suppressed upon transfection of mouse E-cadherin cDNA [11]. Moreover, in 50% of diffuse type human gastric carcinomas, mutations in E-cadherin were found which affect the Ca^{2+}-binding region [12]. Similar mutations of the E-cadherin gene have also been demonstrated in human ovarian carcinomas [13], and lobular breast cancer [14]. A normally expressed E-cadherin might thus not be functional because of specific mutations. Since catenins are essential in functional E-cadherin-mediated adhesions, histological studies have been extended to catenin expression levels in human tumours. For instance, in oesophageal and

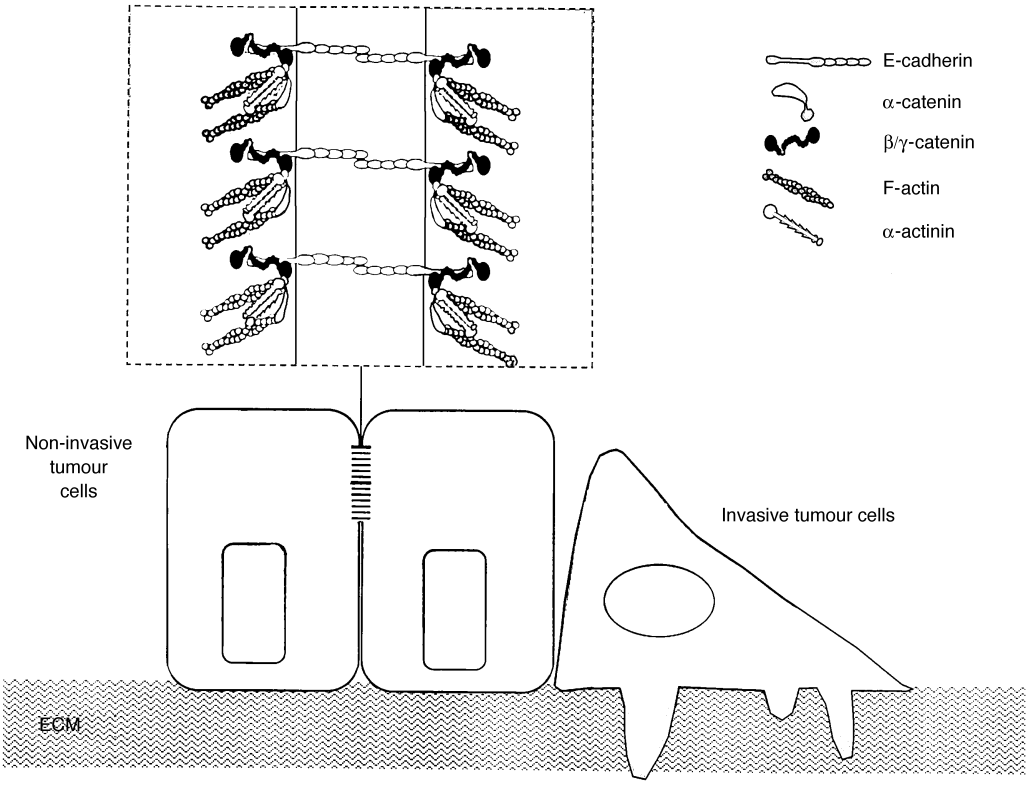

Fig. 1. Cadherins are transmembrane glycoproteins which play important roles in calcium-dependent intercellular adhesion of epithelial cells. For functional adhesion the cytoplasmic domain of E-cadherin forms complexes with catenins, i.e. β-catenin, γ-catenin/plakoglobin and α-catenin. α-Catenin is thought to associate with F-actin cytoskeleton components by direct interactions or indirectly via α-actinin. Loss of E-cadherin-mediated adhesions is associated with invasive capacity of epithelial tumour cells.

breast tumours the correlation of low α-catenin expression with de-differentiation and lymph-node metastasis is even stronger than that of E-cadherin. In bladder cancer, expression levels of α-, β- and γ-catenins and $p120^{Ctn}$ have similar prognostic values as found for E-cadherin [15]. The importance of catenins in functional complex formation is further illustrated by the findings that the adhesive properties of PC3 cells, which harbour a null mutation in α-catenin, could be restored by transfection with α-catenin cDNA [16].

4. Cadherins/catenins and cell signalling

Besides their roles in cell–cell adhesions, cadherins and catenins have also been implicated in cell signalling. Both E-cadherin and the colorectal cancer tumour suppressor gene product (APC) are able to bind to β-catenin/armadillo and regulate signalling cascades that determine tumour suppression, dorsal-ventral axis determination in *Xenopus* and segment polarity in *Drosophila* [17,18]. β-Catenin associates with the lymphoid enhancer binding factor (LEF1) and the *Xenopus* homologue XTcf-3 [19,20]. This complex translocates to the nucleus and leads to transcriptional activation. β-Catenin has been shown to play an essential role in the transformation of colonic epithelium as well as in melanoma development [21–23]. High levels of free β-catenin, as a result of either mutations in APC or in β-catenin itself, drive the formation of complexes with Tcf or Lef-1, which lead to gene transcription. β-Catenin thus has at least two functions: i.e. linking cadherin to the cytoskeleton and participation in the transcriptional activation of genes.

5. Rho-like GTPases

More recently, family members of the rho-like GTPases have been implicated in the establishment and maintenance of E-cadherin-mediated cell–cell adhesions as well as in invasion and migration of epithelial tumour cells. Like ras GTPases, rho-like GTPases cycle between the active GTP-bound state and the inactive GDP-bound state. Their activity is regulated by three classes of proteins, i.e. guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs) [24,25]. Upon receptor stimulation, GEFs stimulate the exchange of bound GDP for GTP leading to activation of the rho-like GTPases. Only the active forms of the GTPases bind downstream effectors thereby leading to the transmission of receptor-induced signals [25]. GAPs promote the inactivation of the GTPases by stimulating the intrinsic GTP hydrolysis rate of rho-like proteins. Association of rho-like proteins with GDIs can block the exchange of GDP for GTP as well as the hydrolysis of GTP and are thought to keep the GTPases in an inactive form.

The most well known rho-like proteins, cdc42, rac1 and rhoA, participate in the formation of distinct patterns of actin organisation and the assembly of integrin complexes [26]. In fibroblasts, cdc42 regulates the formation of focal complexes and actin-containing microspikes or filopodia. Rac regulates the formation of focal complexes, lamellipodia and membrane ruffles, for instance upon stimulation of PDGF- or EGF tyrosine kinase receptors. RhoA activation by the serum component lysophosphatidic acid (LPA) regulates the formation of actin filament bundles (stress fibres) and the assembly of focal contacts. In epithelial cells, rac and rho also play a role in the establishment and maintenance of E-cadherin-mediated cell–cell adhesions. In human keratinocytes and Madin Darby canine kidney (MDCK) cells, dominant negative N17Rac and C3 transferase, the latter inactivates rhoA, have been found to perturb the organisation of actin filaments at sites of cell–cell contact. The inactivation of rac or rho results in the dislocation of E-cadherin and its complex members from the adherens junctions leading to loss of cell–cell adhesions [27,28]. Consistent with these observations are the findings that expression of constitutively active V12Rac in MDCK cells leads to increased accumulation of members of the E-cadherin complex and F-actin at sites of cell–cell contact resulting in enhanced cell–cell adhesions [29,30]. The GEF Tiam1, a specific activator of rac [31,32], is localised to sites of cell–cell contact in epithelial MDCK cells [29]. Overexpression of Tiam1 inhibits the hepatocyte growth factor (HGF)-induced scatter response of MDCK cells, by increasing E-cadherin-mediated cell–cell adhesion [29]. Moreover, Tiam1-mediated rac activation reverts the oncogenic ras-induced fibroblastoid phenotype of MDCK cells into a non-invasive epithelioid phenotype by restoring E-cadherin-mediated adhesions in these cells [29]. Increased rac signalling thus seems to suppress invasion of epithelial cells by enhancing E-cadherin mediated cell–cell adhesions.

6. Matrix-dependent signalling of rho-like proteins

The composition of the extracellular matrix can influence the adhesive or migratory response of cells. For instance, epithelial bladder carcinoma cells show α2β1 integrin-mediated migration on collagens but not on fibronectin or laminin substrates [33,34]. The consequence of signalling by rho-like GTPases in T47D mammary carcinoma cells is also determined by the extracellular matrix. Expression of activated V12rac and V12cdc42 in T47D cells stimulates α2β1-mediated motility on collagen [35]. On a fibronectin or laminin

matrix, Tiam1-mediated rac activation restores E-cadherin-mediated adhesion and inhibits migration of V12ras-transformed MDCK cells, whereas on collagen Tiam1 stimulates motility of these cells [30]. Thus, specific integrin signals determine whether cells respond to rac activation by promoting either cell migration or cell–cell adhesions [30]. These matrix-dependent effects of rac signalling may explain the reported controversial results with respect to rac-induced suppression or promotion of migration [29,35]. Rac-mediated effects on invasive and motile behaviour of epithelial cells appear thus to depend on the balance between cell–cell and cell–substrate interactions, which either suppress or promote motility.

7. Cross-talk between rho-like proteins

The distinct rho-like GTPases act in concert to remodel the actin cytoskeleton in response to receptor stimulation (Fig. 2). In most studies, the activation state of cdc42, rac and rho, has been deduced from the appearance of specific cytoskeletal changes in cells such as the formation of filopodia, lamellipodia and stress fibres, respectively [36,37]. Recently, we have used biochemical pull-down assays to monitor the direct activation state of rac and cdc42, taking advantage of the specific binding of the downstream effector pak to GTP- but not GDP-loaded rac and cdc42 [30,38–40]. Similarly the activation state of rhoA can be determined by measuring the binding of the rhoA-specific downstream effector rhotekin [41] to GTP-bound rhoA [38,39,42]. Simultaneous analysis of rac and rho activities allows us to address the relationship between the activation state of both GTPases at the molecular level, and to get insight into the regulation of rac- and rho-dependent signalling pathways and phenotypes. Using these pull-down assays we found that signalling by constitutively active mutants of cdc42 and rac as well as platelet derived growth factor (PDGF)-mediated activation of rac leads to downregulation of endogenous rho activity in NIH3T3 fibroblasts [39] (see also Fig. 2). While rac activation leads to the inactivation of rho, receptor-mediated or sustained activation of rho did not affect rac activity, suggesting a unidirectional signalling from rac to rho [39]. Similar studies in COS7 cells and epithelial MDCK cells confirmed that the downregulation of rho activity by rac is a general phenomenon that occurs in different cell types. Rac and rho activation may, thus be regulated by receptor mediated-signalling as well as by signalling from rac to rho [39]. Moreover, a cross-talk between these GTPases has been described at the level of downstream pathways which regulate the cytoskeleton [40,43,44].

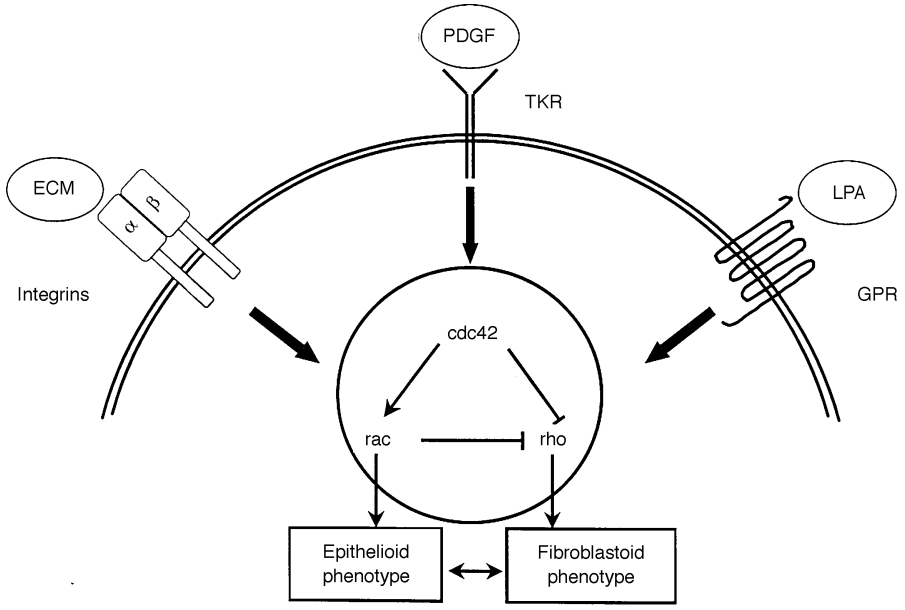

Fig. 2. Rho-like GTPases, which include cdc42, rac1 and rhoA, are differentially regulated in response to receptor stimulation. Signals transduced by integrin receptors, by tyrosine kinase receptors (TKR) such as the rac-linked receptor for platelet derived growth factor (PDGF), and by heterotrimeric G-protein receptors (GPR) such as the rho-linked receptor for lysophosphatidic acid (LPA), are able to activate small rho-like GTPases. The GTPases act in concert to remodel the cytoskeleton. Cross-talk exists between these small GTPases through downstream signalling in which cdc42 and rac can downmodulate rho activity. The epithelioid and fibroblastoid phenotype is determined by a balance between rac and rho activities, which are thought to be regulated locally at distinct sites at the cell membrane. Increased rac activity leads to decreased rho activity and a non-invasive, epithelioid phenotype caused by an increase in the formation of cadherin-based functional cell–cell adhesions. In contrast, increased rho activity is associated with a fibroblastoid phenotype characterised by disassembly of cadherin-based cell–cell adhesions and acquired invasive capacity.

8. The balance between rac and rho activity determines cellular morphology and migratory behaviour

Epithelial MDCK cells break up their cell–cell junctions and scatter in response to hepatocyte growth factor (HGF), which is the ligand of the c-Met proto-oncogene transmembrane tyrosine kinase receptor [45]. HGF activates the small GTPase ras and both HGF- and V12ras-induced membrane ruffling and scattering require rac activity [46–49]. However, activated V12rac is not sufficient to induce cell scattering, in agreement with the established role of rac in promoting cell–cell adhesion. The failure of activated rac to induce cell motility in MDCK cells indicates that HGF, in addition to rac, stimulates other signalling pathways to induce scattering which includes the MAP-kinase pathway [49]. Using pull-down assays we found that HGF/cMet receptor stimulation leads to a transient activation of rac but also to a prolonged activation of rhoA (data not shown). This suggests that the differential activation of rac and rho by HGF/cMet receptor signalling may play an important role in the induction of the migratory response. Biochemical analyses of the actual activities of the rho-like GTPases in NIH3T3 fibroblasts support a model that rac and rho signalling antagonise each other to control the cellular phenotype and migratory behaviour (Fig. 2). The activation of rac can lead to downregulation of rho activity. Increased rac signalling by Tiam1 or V12Rac results in inhibition of fibroblast migration and induces an epithelial-like morphology characterised by increased cadherin-based cell–cell adhesion [39]. Restoration of rho activity in these Tiam1-expressing cells by expression of constitutively active V14rho results in a fibroblastoid phenotype associated with migratory behaviour [39].

Taken together, in addition to changes in expression or mutations in E-cadherin and molecules involved in cadherin-based adhesions, recent data indicate that the reciprocal balance between rac and rho activity may also determine the cellular phenotype and migratory behaviour in epithelial MDCK cells and NIH3T3 fibroblasts. Oncogene- and growth factor-induced signalling as well as integrin-mediated cell interactions with different matrix components may influence the activity state of rac and rho proteins locally and thereby regulate epithelial-mesenchymal transition and migratory behaviour of epithelial (tumour) cells.

Acknowledgements

The work of J.G. Collard is supported by grants from the Dutch Cancer Society. E.E. Evers is supported by a fellowship from the Deutsche Forschungsgemeinschaft, L.S. Price by The Netherlands Organisation for Scientific Research, and A. Malliri by a fellowship from the European Commission.

References

1. Birchmeier W, Behrens J. Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. *Biochim Biophys Acta* 1994, **1198**, 11–26.
2. Bracke ME, Van Roy FM, Mareel MM. The E-cadherin/catenin complex in invasion and metastasis. *Curr Top Microbiol Immunol* 1996, **213**, 123–161.
3. Nagafuchi A, Takeichi M, Tsukita S. The 102 kd cadherin-associated protein: similarity to vinculin and post-transcriptional regulation of expression. *Cell* 1991, **65**, 849–857.
4. Knudsen KA, Soler AP, Johnson KR, Wheelock MJ. Interaction of alpha-actinin with the cadherin/catenin cell–cell adhesion complex via alpha-catenin. *J Cell Biol* 1995, **130**, 67–77.
5. McCrea PD, Turck CW, Gumbiner B. A homolog of the Armadillo protein in *Drosophila* (Plakoglobin) associated with E-Cadherin. *Science* 1991, **254**, 1359–1361.
6. Hinck L, Nelson WJ, Papkoff J. Wnt-1 modulates cell–cell adhesion in mammalian cells by stabilizing beta-catenin binding to the cell adhesion protein cadherin. *J Cell Biol* 1994, **124**, 729–741.
7. Nagafuchi A, Takeichi M. Cell binding function of E-cadherin is regulated by the cytoplasmic domain. *EMBO J* 1988, **7**, 3679–3684.
8. Ozawa M, Ringwald M, Kemler R. Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. *Proc Natl Acad Sci USA* 1990, **87**, 4246–4250.
9. Kintner C. Regulation of embryonic cell adhesion by the cadherin cytoplasmic domain. *Cell* 1992, **69**, 225–236.
10. Behrens J, Mareel MM, Van Roy FM, Birchmeier W. Dissecting tumor cell invasion: epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell–cell adhesion. *J Cell Biol* 1989, **108**, 2435–2447.
11. Vleminckx K, Vakaet L Jr, Mareel M, Fiers W, Van Roy F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell* 1991, **66**, 107–119.
12. Becker KF, Atkinson MJ, Reich U, Becker I, Nekarda H, Siewert JR, Hofler H. E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. *Cancer Res* 1994, **54**, 3845–3852.
13. Risinger JI, Berchuck A, Kohler MF, Boyd J. Mutations of the E-cadherin gene in human gynecologic cancers. *Nat Genet* 1994, **7**, 98–102.
14. Berx G, Cleton Jansen AM, Nollet F, *et al*. E-cadherin is a tumour invasion suppressor gene mutated in human lobular breast cancers. *EMBO J* 1995, **14**, 6107–6115.
15. Shimazui T, Schalken JA, Giroldi LA, *et al*. Prognostic value of cadherin-associated molecules (alpha-, beta-, and gamma-catenins and p120(cas)) in bladder tumors. *Cancer Res* 1996, **56**, 4154–4158.
16. Hirano S, Kimoto N, Shimoyama Y, Hirohashi S, Takeichi M. Identification of a neural alpha-catenin as a key regulator of cadherin function and multicellular organization. *Cell* 1992, **70**, 293–301.
17. Rubinfeld B, Souza B, Albert I, *et al*. Association of the apc gene product with beta-catenin. *Science* 1993, **262**, 1731–1734.
18. Hulsken J, Birchmeier W, Behrens J. E-cadherin and APC compete for the interaction with beta-catenin and the cytoskeleton. *J Cell Biol* 1994, **127**, 2061–2069.
19. Behrens J, Vonkries JP, Kuhl M, *et al*. Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* 1996, **382**, 638–642.

20. Molenaar M, Vandewetering M, Oosterwegel M, *et al*. XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos. *Cell* 1996, **86**, 391–399.
21. Korinek V, Barker N, Morin PJ, *et al*. Constitutive transcriptional activation by a beta-catenin–Tcf complex in APC-/-colon carcinoma. *Science* 1997, **275**, 1784–1787.
22. Morin PC, Sparks AB, Kronek V, *et al*. Activation of beta-catenin-Tcf signalling in colon cancer by mutations in beta-catenin or APC. *Science* 1997, **275**, 1787–1790.
23. Rubinfeld B, Robbins P, El-Gamil M, Albert I, Porfiri E, Polakis P. Stabilization of beta-catenin by genetic Defects in melanoma cell lines. *Science* 1997, **275**, 1790–1793.
24. Boguski MS, McCormick F. Proteins regulating ras and its relatives. *Nature* 1993, **366**, 643–654.
25. Aelst L, D'Souza-Schorey C. Rho GTPases and signalling networks. *Genes Dev* 1997, **11**, 2295–2322.
26. Hall A. Rho GTPases and the actin cytoskeleton. *Science* 1998, **279**, 509–514.
27. Braga VMM, Machesky LM, Hall A, Hotchin NA. The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell–cell contacts. *J Cell Biol* 1997, **137**, 1421–1431.
28. Takaishi K, Sasaki T, Kotani H, Nishioka H, Takai Y. Regulation of cell–cell adhesion by Rac and Rho small G proteins in MDCK cells. *J Cell Biol* 1997, **139**, 1047–1059.
29. Hordijk PL, ten Klooster J-P, van der Kammen RA, Michiels F, Oomen LCJM, Collard JG. Inhibition of invasion of epithelial cells by Tiam1-Rac signaling. *Science* 1997, **278**, 1464–1466.
30. Sander EE, van Delft S, ten Klooster J-P, *et al*. Matrix-dependent Tiam1/Rac signaling in epithelial cells promotes either cell–cell adhesion or cell migration and is regulated by phosphatidylinositol 3-kinase. *J Cell Biol* 1998, **143**, 1385–1398.
31. Habets GGM, Scholtes EHM, Zuydgeest D, *et al*. Identification of an invasion-inducing gene, Tiam-1, that encodes a protein with homology to GDP-GTP exchangers for Rho-like proteins. *Cell* 1994, **77**, 537–549.
32. Michiels F, Habets GGM, Stam JC, van der Kammen RA, Collard JG. A role for Rac in Tiam1-induced membrane ruffling and invasion. *Nature* 1995, **375**, 338–340.
33. Tucker GC, Boyer B, Gavriovic J, Emonard H, Thiery JP. Collagen-mediated dispersion of NBT-II rat bladder carcinoma cells. *Cancer Res* 1990, **50**, 129–137.
34. Vallés AM, Boyer B, Tarone G, Thiery JP. $\alpha_2\beta_1$ integrin is required for the collagen and FGF-1 induced cell dispersion in a rat bladder carcinoma cell line. *Cell Adhes Commun* 1996, **4**, 187–199.
35. Keely PJ, Westwick JK, Whitehead IP, Der CJ, Parise LV. Cdc42 and Rac1 induce integrin-mediated cell motility and invasiveness through PI(3)K. *Nature* 1997, **390**, 632–635.
36. Ridley AJ, Hall A. The small GTP-binding protein Rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* 1992, **70**, 389–399.
37. Nobes CD, Hall A. Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* 1995, **81**, 53–62.
38. Reid T, Bathoorn A, Ahmadian MR, Collard JG. Identification and characterization of hPEM-2, a guanine nucleotide exchange factor specific for Cdc42. *J Biol Chem* 1999, **274**, 33587–33593.
39. Sander EE, ten Klooster J-P, van Delft S, van der Kammen RA, Collard JG. Rac downregulates Rho activity: reciprocal balance between both GTPases determines cellular morphology and migratory behavior. *J Cell Biol* 1999, **147**, 1009–1022.
40. van Leeuwen FN, van Delft S, Kain HE, van der Kammen RA, Collard JG. Rac regulates phosphorylation of the myosin-II heavy chain, actinomyosin disassembly and cell spreading. *Nature Cell Biol* 1999, **1**, 242–248.
41. Reid T, Furuyashiki T, Ishizaki T, *et al*. Rhotekin, a new putative target for Rho bearing homology to a serine/threonine kinase, PKN, and rhophilin in the rho-binding domain. *J Biol Chem* 1996, **271**, 13556–13560.
42. Ren X-D, Kiosses WB, Schwartz MA. Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. *EMBO J* 1999, **18**, 578–585.
43. Sanders LC, Matsamura F, Bokoch GM, de Lanerolle P. Inhibition of myosin light chain kinase by p21-activated kinase. *Science* 1999, **283**, 2083–2085.
44. Sells MA, Boyd JT, Chernoff J. P21-activated kinase 1 (Pak1) regulates cell motility in mammalian fibroblasts. *J Cell Biol* 1999, **145**, 837–849.
45. Naldini L, Vigna E, Narsimhan R, *et al*. Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene c-MET. *Oncogene* 1991, **6**, 501–504.
46. 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**, 1110–1122.
47. Rodriguez-Viciana P, Warne PH, Khwaja A, *et al*. Role of phosphoinositide 3-OH kinase in cell transformation and control of the actin cytoskeleton by ras. *Cell* 1997, **89**, 457–467.
48. Scita G, Nordstrom J, Carbone R, *et al*. EPS8 and E3B1 transduce signals from ras to Rac. *Nature* 1999, **401**, 290–293.
49. Potempa S, Ridley AJ. Activation of both MAP kinase and phosphatidylinositide 3-kinase by ras is required for hepatocyte growth factor/scatter factor-induced adherens junction disassembly. *Mol Biol Cell* 1998, **9**, 2185–2200.