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Minireview

Rho GTPases in cancer cell biology

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Abstract Rho GTPases contribute to multiple cellular processes that could affect cancer progression, including cytoskeletal dynamics, cell cycle progression, transcriptional regulation, cell survival and vesicle trafficking. In vitro several Rho GTPases have oncogenic activity and/or can promote cancer cell invasion, and this correlates with increased expression and activity in a variety of cancers. Conversely, other family members appear to act as tumour suppressors and are deleted, mutated or downregulated in some cancers. Genetic models are starting to provide new information on how Rho GTPases affect cancer development and progression. Here, we discuss how Rho GTPases could contribute to different steps of cancer progression, including proliferation, survival, invasion and metastasis.

Keywords: Rho GTPases; Cytoskeleton; Signaling; Migration; Invasion; Cancer

1. Introduction

Rho GTPases form a distinct family within the Ras-like protein superfamily, which also includes the Ras, Rab, Arf and Ran families (Fig. 1). Rho members differ from other Ras-like GTPases by the presence of a Rho-specific insert domain. Rho proteins are highly conserved from lower eukaryotes to plants and mammals [1]. In mammals the family comprises 20 members, divided into 8 different subfamilies (Figs. 1 and 2). Splice variants of Rac1 and Cdc42 have also been identified. RhoBTB3 and Miro1 and 2 are now considered as outside of the Rho family because they lack a proper Rho insert domain and are of distinct phylogenetic origin [1].

Most Rho family members act as molecular switches, cycling between a GTP-bound active form and a GDP-bound inactive form [2] (Fig. 3). Their activity is increased by guanine nucleotide exchange factors (GEFs), which promote the release of bound GDP and subsequent binding of the more abundant GTP, and downregulated by GTPase-activating proteins (GAPs), which stimulate the hydrolysis of GTP [3]. Rho proteins are also frequently post-translationally modified at the C-terminus with the addition of a lipidic group by prenylation (farnesylation or geranylgeranylation) or palmitoylation, thereby enhancing their interaction with membranes. GDI proteins (guanine–nucleotide-dissociation inhibitors) also regulate the activity of Rho GTPases by binding to the C-terminal prenyl group, preventing their membrane association and sequestering them in the cytoplasm, and thus frequently inhibiting their access to downstream targets [4]. RhoGDIs can bind to either the GTP- or the GDP-loaded forms. To date over 70 RhoGEFs, 60 RhoGAPs and 3 RhoGDIs have been identified in mammals, reflecting the complexity of the regulation of this class of proteins.

The Rho family members Rnd1, Rnd2, Rnd3/RhoE, RhoH, RhoBTB1 and RhoBTB2 have key amino acid substitutions that make them lack GTP hydrolysis activity and GDP binding and they are therefore permanently bound to GTP [5,6] (Fig 2). These proteins are likely to be regulated by expression level, phosphorylation or protein interactions through specific protein domains. Phosphorylation regulates the activity and localization of RhoA and Rnd3/RhoE, which are phosphorylated by cAMP-dependent protein kinase (PKA) and Rho-associated, coiled-coil containing protein kinase (ROCK), respectively [7,8].

Once activated, Rho GTPases bind different effector molecules and trigger a signalling cascade to direct cellular responses. Rho GTPases have been implicated in many cellular processes including actin and microtubule cytoskeleton organization, cell division, motility, cell adhesion, vesicular trafficking, phagocytosis and transcriptional regulation [2]. Considerable insight into their function has come from the study of model organisms. *Dictyostelium discoideum* has more than 15 genes in the family including members of the Rac and RhoBTB subfamilies but not the other human subfamilies. Several Rac isoforms affect migration and cytokinesis [9]. The nematode *Caenorhabditis elegans* has six members, including Rho, Rac and Cdc42. They are required for cytoskeleton-based processes including cell migration, cell polarity, phagocytosis, spindle formation, axon guidance, locomotion and embryonic development [10]. *Drosophila melanogaster* has seven members including Rho, 3 Rac isoforms, Cdc42 and RhoBTB, which have been shown to affect cell shape, morphology, polarity, cell division, and the maintenance of epithelial architecture.

As well as contributing to physiological processes, Rho GTPases have been found to contribute to pathological processes including cancer cell migration, invasion, and metastasis, inflammation, and wound repair [2,5]. In this review, we

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Abbreviations: ECM, extracellular matrix; JNK, Jun N-terminal kinase; MLC, myosin light chain; MMP, matrix metalloproteases; PAK, p21-activated kinase; PKA, cAMP-dependent protein kinase; ROCK, Rho-associated, coiled-coil containing protein kinase

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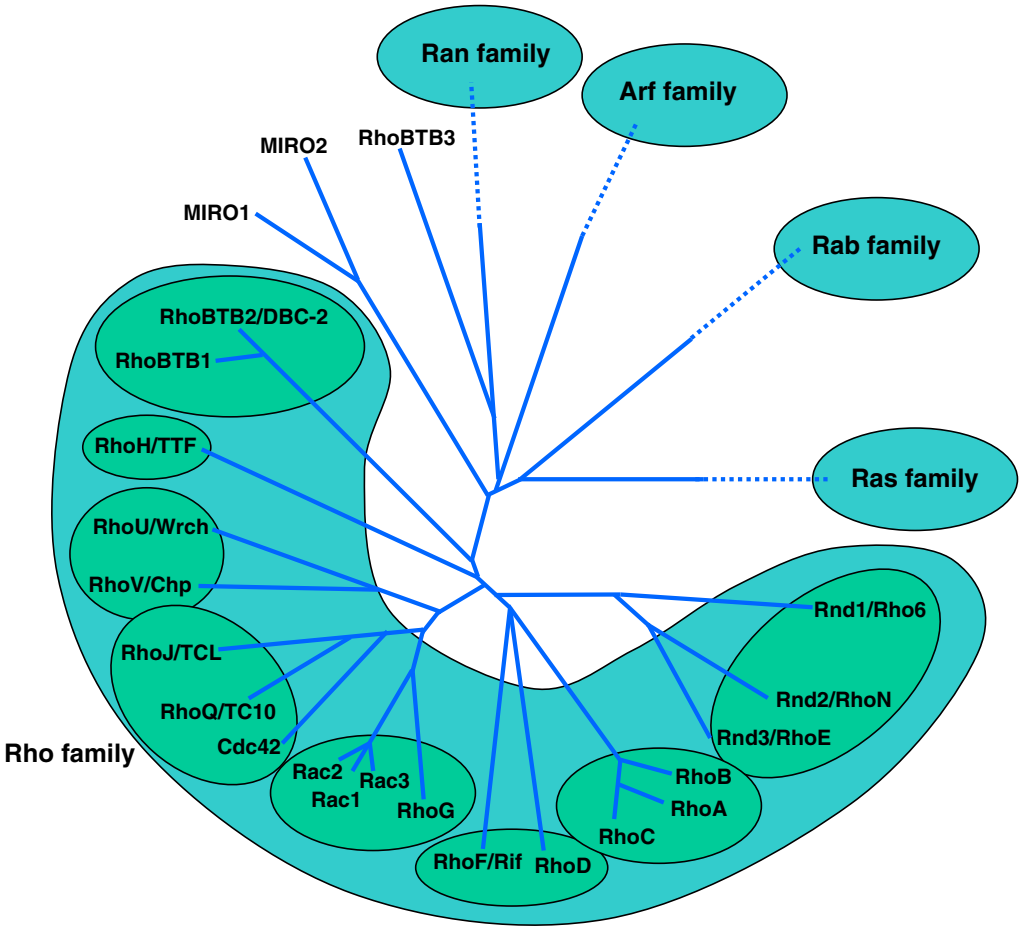

Fig. 1. Phylogenetic tree showing the mammalian Ras GTPase superfamily. The 20 Rho GTPase family members are grouped into eight subfamilies. Miro proteins and RhoBTB3 form independent branches within the superfamily, separate from the Rho GTPases.

will focus on the involvement of Rho GTPases in the cellular processes that contribute to cancer progression.

2. Steps in cancer progression

Rho GTPases have been reported to contribute to most steps of cancer initiation and progression including the acquisition of unlimited proliferation potential, survival and evasion from apoptosis, tissue invasion and the establishment of metastases.

Primary tumours generally arise as a consequence of multiple mutations and epigenetic changes affecting key genes that ultimately affect proliferation and survival. Activating mutations in the three Ras isoforms, Ki-Ras, N-Ras and Ha-Ras, are found in 15% of all human tumours. In contrast, Rho proteins are only rarely mutated in tumours, whereas their expression and/or activity are frequently altered. For example, several Rho GTPases are upregulated in some human tumours, including RhoA, RhoC, Rac1, Rac2, Rac3, Cdc42, Wrch2/RhoV and RhoF [11–13].

Uncontrolled proliferation, coupled to increased survival signals that permit tumour cells to escape from apoptosis, result in tumour growth. Some Rho GTPases stimulate cell cycle progression and regulate gene transcription, and this could in part explain their pro-oncogenic properties, for example in promoting Ras-induced transformation [14]. The induction of tumour vascularisation is essential for tumours to grow beyond a certain size and malignant cells release factors that promote angiogenesis from nearby pre-existing blood vessels. Some Rho GTPases are thought to be able to regulate the release of pro-angiogenic factors to promote neovascularisation [15].

Invasion of epithelial cancers is initiated when the integrity of the epithelium is disrupted and malignant cells disrupt the basement membrane and enter the underlying stroma (Fig. 4). This normally implies loosening of epithelial cell–cell contacts and acquisition of a more motile phenotype in a process frequently referred as epithelial to mesenchymal transition (EMT). Invasive epithelial cancer cells often have reduced expression of the cell–cell adhesion protein E-cadherin and start expressing markers of mesenchymal origin such as vimentin and N-cadherin [16]. Non-epithelial cancers also invade solid tissues [17].

Cancer cells can invade into tissues either as single cells or as cell groups (collective cell migration) (Fig. 5). Single cells have been described to use two alternative migratory mechanisms: mesenchymal and amoeboid migration. Cells using mesenchymal migration have an elongated morphology and extend long protrusions at the front. Integrin-based adhesions and strong traction are the driving forces for movement in addition to extracellular matrix (ECM) degradation by secreted and/or

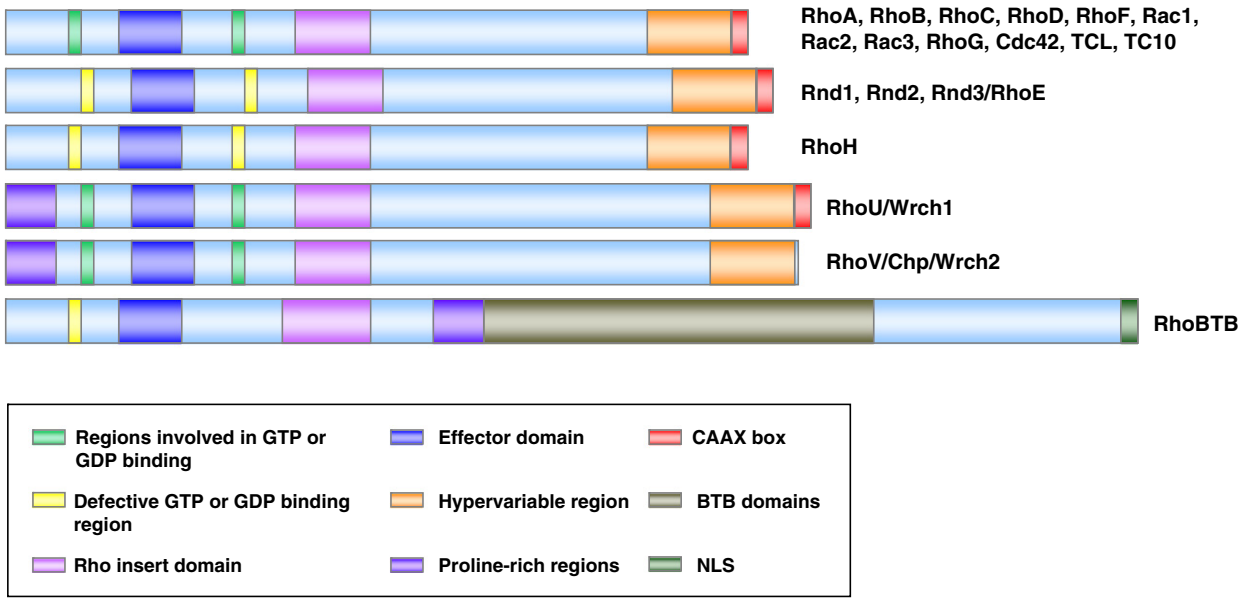

Fig. 2. Domain organization of the Rho GTPases. RhoA, RhoB, RhoC, Rac1, Rac2, Rac3, RhoG, RhoD, RhoF, Cdc42, TCL and TC10 have a similar basic protein structure. Rnd1, Rnd2, Rnd3/RhoE and RhoH are considered atypical Rho GTPases that have modifications in the GTP/GDP binding region that make them lack GTPase activity. Wrch1 and Wrch2 are characterized by the presence of a N-terminal proline-rich region. RhoBTB1 and 2 have the most divergent protein organization with two characteristic BTB domains NLS, nuclear localisation sequence.

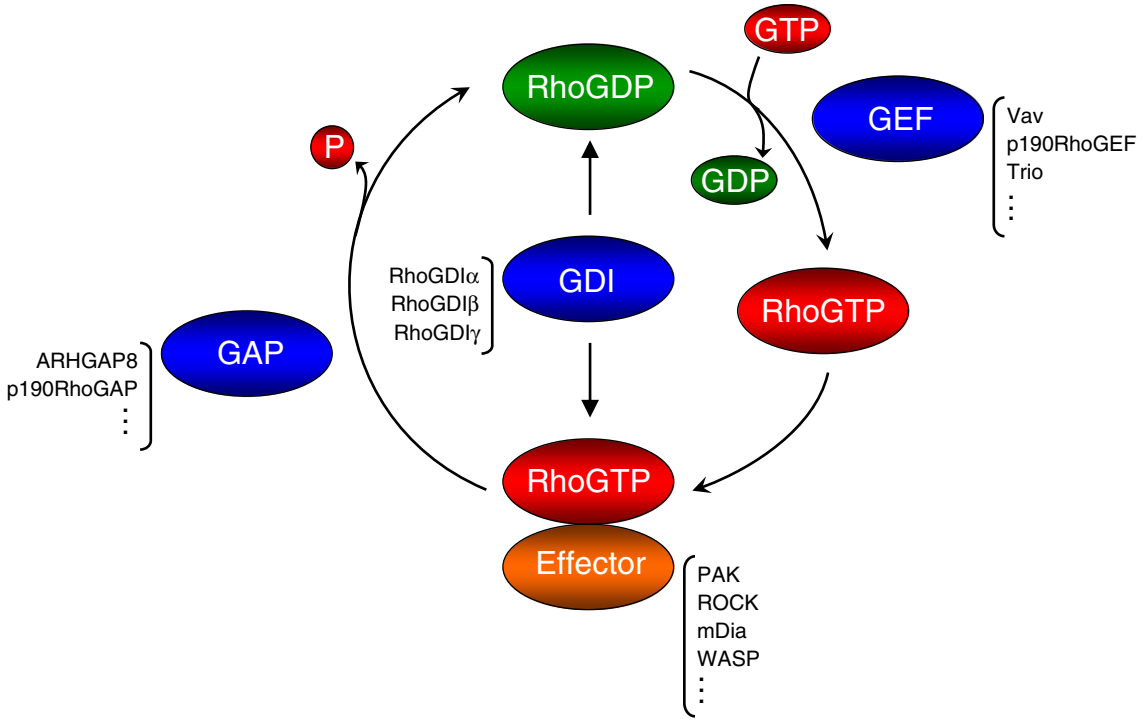

Fig. 3. Basic Rho GTPase activation cycle. The loading of the Rho GTPases with GTP and subsequent activation is catalyzed by GEFs. In their GTP form they bind to different effectors to perform their functions. GAP proteins promotes the hydrolysis of GTP to inactivate the protein. RhoGDI proteins constitute an additional step of regulation by sequestering the protein in the cytoplasm impairing its function. Some examples of GEFs, GAPs and effectors are depicted.

transmembrane proteases [17]. In contrast, amoeboid migration is generally independent of extracellular protease activity and is driven by actomyosin-based cortical contraction, and cells have a more rounded shape. In collective cell invasion, cells move in groups through the extracellular matrix and maintain cell–cell adhesions. A leading cell at the tip of the group generates the migratory traction necessary for movement and the cells at the back and middle of the group are mostly dragged passively. Degradation of the ECM by matrix metalloproteases (MMPs) is essential for this kind of collective

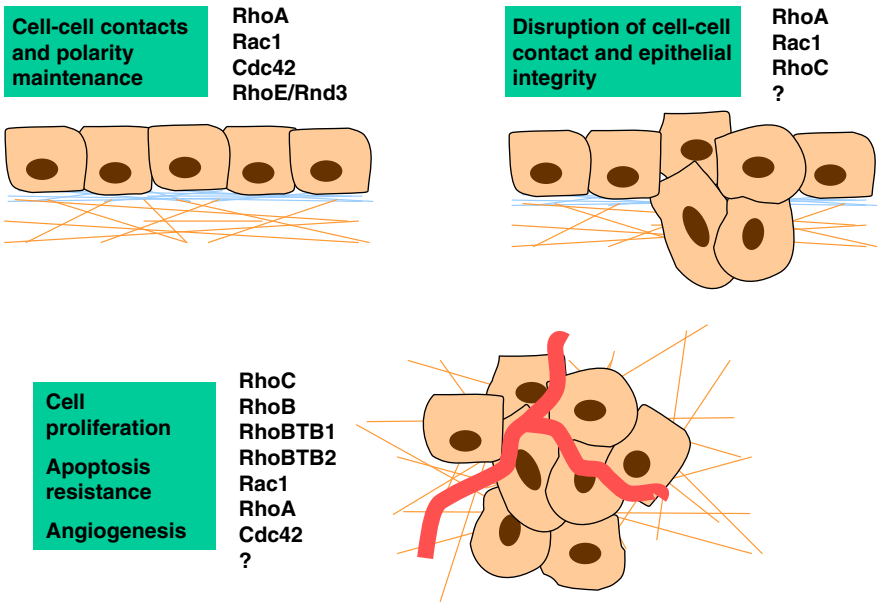

Fig. 4. Schematic model showing the potential roles of different Rho GTPases during various stages in cancer progression. See text for references.

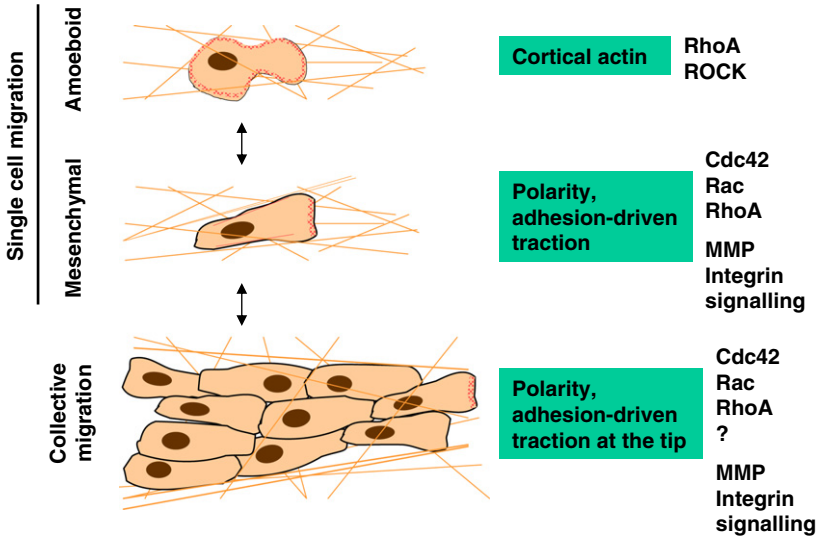

Fig. 5. Rho GTPases in tumour cell invasion. Different mechanisms of cancer cell invasion and the possible roles of some Rho GTPases are depicted. See text for references.

migration [18]. Cancer cells can convert from one type of migration to another depending on conditions [17,18].

To establish metastases in distant tissues, tumour cells have to enter the vascular or lymphatic system, then exit it and proliferate in the new tissue. The ability of Rho GTPase family members to regulate cytoskeletal dynamics, cell adhesion and cell migration [2] points to a central role in cancer cell invasion and metastasis. The possible roles of the different Rho GTPases in different steps of cancer progression are discussed below.

2.1. Rho subfamily

The Rho subfamily consists of the highly conserved RhoA, RhoB and RhoC proteins (Fig. 1). RhoA and RhoC expression and/or activity is frequently increased in human tumours [12,19], whereas RhoB is often downregulated [12].

RhoA has been implicated in virtually all stages of cancer progression. RhoA might play a role during tumour cell proliferation and survival: for example, in vitro, constitutively active RhoA can stimulate transformation [2]. In normal epithelia, RhoA contributes to the generation of epithelial polarity and junction assembly and function (reviewed in [20]) but also affects epithelial disruption during tumour progression. Rho activity can be inhibited downstream of cadherins leading to a more motile phenotype [21]. Different GEFs and GAPs influence how Rho proteins can act in different contexts either promoting epithelial organization and polarity or epithelial EMT,

as seen in studies of RhoA GEFs/GAPs in Drosophila models [22].

RhoA is important for both amoeboid and mesenchymal migration (Fig. 4). RhoA-ROCK signalling is proposed to induce actomyosin-based cortical contractility leading to amoeboid migration via blebbing, as well as tail retraction in mesenchymal migration [17]. A 3D in vitro invasion model using co-cultures of SSC12 carcinoma cells, that have not undergone a complete mesenchymal transformation retaining some epithelial markers, with stromal fibroblasts has shown how a fibroblast is the leading cell in this model of collective cell invasion [23]. This fibroblast generates the traction force and remodels the matrix through MMPs. Different Rho GTPases were found to be required in the leading fibroblast and the following carcinoma cells with a RhoA regulation of Myosin light chain (MLC) in the former and mainly Cdc42 and MRCK function in the latter. Rho GTPases can also regulate the production of MMPs, affecting matrix remodelling and tumour cell invasion. Rho can induce the expression or secretion of MMPs [24]. Nevertheless, studies on cells depleted of RhoA by RNAi are variable and show a strong dependence of cell background for its effect on migration and invasion [25,26]

In contrast to RhoA, RhoC has no apparent transforming activity and the involvement of RhoC in cancer progression appears to be restricted largely to metastasis [27]. RhoC was identified in a screen for genes upregulated in melanoma metastases [28], and has subsequently been proposed as a marker for poor prognosis in cancers of different origins [29]. Increased RhoC expression has been claimed as the possible cause for the induction in invasion and metastasis triggered by the overexpression of the microRNA-10b in breast cancer [30]. Studies of RhoC knock-out mice show that RhoC is dispensable for embryogenesis and tumour initiation but is required for metastasis [27]. RhoC expression is increased during EMT in a colon cancer model and contributes to EMT-induced migration, whereas RhoA levels go down [25]. Knockdown of RhoC expression by RNAi confirms that RhoC is important for invasion in vitro [25,26,31]. It is not yet clear how RhoC increases invasion and metastasis or why its effects differ from RhoA.

Some reports indicate that RhoA, RhoC and their downstream target ROCK are needed for cancer cell extravasation, but these studies are largely based on chemical inhibitors that are not completely specific [32]. Interestingly, RhoC can induce the production of angiogenic factors in breast cancer, and this could help promote entry into blood vessels and thereby metastasis [15]. More extensive work is needed to elucidate the possible contribution of Rho GTPases to the extravasation of cancer cells.

Unlike RhoA and RhoC, RhoB is often downregulated in human tumours and its expression inversely correlates with tumour aggressiveness [33]. It has been proposed that RhoB can work as a tumour suppressor as it is activated in response to several stress stimuli including DNA damage or hypoxia, and it has been reported to inhibit tumour growth, cell migration and invasion and have proapoptotic functions in cells [33]. RhoB knock-out mice develop normally but have enhanced carcinogen-induced skin tumour formation, in agreement with a role of RhoB as a tumour suppressor [34]. RhoB also suppresses invasion: for example it has been postulated to act downstream of PKCτ in the regulation of cancer cell invasion in vitro [35] and it was also reported to inhibit Ras-induced invasion and metastasis [36].

The exact mechanism whereby RhoB suppresses tumour growth and invasion is not clear, although its role in endosomal trafficking could be important. RhoB regulates the delivery of signalling proteins, including growth factor receptors and the tyrosine kinase Src, to specific intracellular compartments [37], and this could certainly influence proliferation and invasion.

2.2. Rac subfamily

The Rac subfamily of Rho GTPases comprises Rac1, Rac2, Rac3 and RhoG (Fig. 1). Rac1 is over-expressed in various tumours and accumulating evidence indicates that Rac1-dependent cell signalling is important for malignant transformation [12]. Rac1 is one of the few Rho GTPases mutated in some tumours, with mutations mainly affecting the effector domain that interacts with downstream targets. It was proposed that these mutations could increase the activity of the protein and the survival of the tumours [38].

A splice variant of Rac1, Rac1b, has an extra intron close to the GTP-binding region. It was initially identified to be upregulated in colon cancers [39]. It does not bind RhoGDI and thus is present predominantly in the GTP-bound state. Although Rac1b is defective in activating several Rac1-regulated signalling pathways, in some cell types it stimulates cell survival and cell cycle progression through NFκB, and is less susceptible to ubiquitination and degradation, which could explain its increased expression in cancers [40–42].

So far little is known about the role of Rac proteins in cancer progression in vivo. Rac1 knock-out in mice is embryonic lethal [43] but conditional knock-out mice have been studied extensively [44,45]. In a conditional lung cancer mouse model Rac1 function was required for K-Ras-driven proliferation and tumorigenicity [46]. Similarly, mice lacking the Rac-specific GEF Tiam1 are protected from Ras-induced skin cancer, developing fewer tumours, although the tumours that do form are more aggressive [47]. These results suggest that Rac proteins normally stimulate tumour cell proliferation but inhibit tumour dissemination.

Rac1 could contribute to cancer cell proliferation via regulation of the cell cycle: for example, it stimulates expression of cyclin D1, and induces cell transformation in vitro [2,14]. It is likely to inhibit cancer invasion through its ability to enhance epithelial cell-cell adhesion. However, active Rac can mediate the loss of adherens junction in some situations, promoting a more migratory phenotype, and thus Rac could promote or inhibit tumour cell invasion depending on the cell background [48–51]. Rac is necessary for the generation of lamellipodial protrusions during mesenchymal migration, as well as for the amoeboid migration of Ras-transformed cells (Fig. 5)[17]. Rac1 can also contribute to cancer cell invasion by regulating the production of MMPs and their natural inhibitors, the Tissue-specific inhibitors of MMP (TIMPs) [24].

Like Rac1, Rac2 and Rac3 are over-expressed in some tumours. Rac3 is hyperactive and/or deregulated in breast cancers [19,52,53]. The contribution of different Rac isoforms to migration is likely to depend on the cell type and their relative expression levels. Rac2 is required for neutrophil migration but whether it acts similarly in tumours is not known [54]. In contrast, Rac1 and Rac2 are dispensable for cell migration

in macrophages, although Rac1 is required for invasion [55]. Studies of Rac3-null mice indicate that Rac3 but not Rac1 or Rac2 specifically contributes to the development of Brc-Abl-induced lymphomas in vivo [56]. However, in fibroblasts, Rac1 but not Rac3 suppression by RNAi affects lamellipodium formation although cell invasion is reduced in both cases [57]. It is not yet clear how these results can be translated to cancer cell invasion in vivo.

Little is known about the role of RhoG in cancer, although it induces actin reorganization and cell migration via Rac in vitro [58], and thus it will be interesting to determine its role in cancer cell invasion.

2.3. Cdc42 subfamily

Cdc42 and the closely related RhoQ/TC10 and RhoJ/TCL form a distinct subfamily of Rho GTPases. Cdc42 expression is upregulated in some breast cancers [59], yet liver-specific knock-out indicates that loss of Cdc42 enhances liver cancer development [60], suggesting that the contribution of Cdc42 to cancer progression may be tissue-specific. This could reflect the multiple roles of Cdc42 in regulating cell polarity as well as cell cycle progression.

Both Cdc42 and RhoQ/TC10 stimulate transformation and contribute to Ras-induced transformation in vitro [2,12,14,61], and for Cdc42 this has been suggested to be due to its effect on receptor trafficking and degradation [62]. RhoQ/TC10 is also implicated in receptor trafficking, particularly of the glucose transporter [63], but whether this accounts for its role in transformation is not known. Another mechanism whereby Cdc42 could affect cell cycle progression is by regulating chromosome segregation during mitosis: only Cdc42 knockdown out of all Rho GTPases was found to induce chromosome misalignment during cell division, leading to multinucleate cells. RhoJ/TCL and RhoQ/TC10 enhance this effect when suppressed in conjunction with Cdc42 [64], pointing towards a possible redundancy in function of Cdc42 family proteins during spindle formation and cell division.

Cdc42 is involved in the establishment of normal epithelial polarity as well as migratory polarity via its interaction with the Par3/Par6/aPKC polarity complex, which in turn regulates Rac via Tiam1 [20]. Cdc42 is thus predicted to inhibit invasion by promoting epithelial polarity, yet conversely also stimulate migration. Indeed, it contributes to cancer cell invasion in single cells in vitro with a mesenchymal morphology although probably not with an amoeboid morphology [17,65]. It is also important for collective cancer cell invasion, where it acts through its target MRCK to stimulate actomyosin contractility [23] (Fig. 5). However, whether the Par3/Par6/aPKC complex is also involved in these processes is not yet known.

2.4. Wrch1 and Wrch2

Wrch1/RhoU and Wrch2/RhoV, also known as Chp, both have an N-terminal proline-rich domain that is not present in other Rho family members (Fig. 2), and which can bind to SH3 domain-containing proteins such as Nck2 and Grb2 [5]. Wrch1 is upregulated by the Wnt signalling pathway, and thus could be involved in Wnt-driven oncogenic transformation [66]. It can be upregulated or downregulated in some primary tumours [5,67], but it is not known whether this correlates with levels of Wnt signalling. Wrch1 stimulates cell cycle progression, and constitutively active Wrch1 is able to induce transformation of fibroblasts when overexpressed [66,67]. It is therefore possible that Wnt-induced Wrch1 expression contributes to cancer development by stimulating proliferation, although how it does this remains to be established.

Wrch2 is abundant in cancer cell lines and upregulated in some human cancers [11]. It differs from Wrch1 at the C-terminus: it lacks a CAAX box and instead has a unique 32 amino acid sequence (Fig. 2). It has been reported to stimulate the Jun N-terminal kinase (JNK) signalling pathway [11] but the relevance of this to cancer progression is not known.

Overexpression of Wrch1 and Wrch2 induces actin cytoskeletal reorganization including formation of filopodia and dissolution of stress fibers. Whether this reflects their physiological function or is just a consequence of sequence similarity to Cdc42 is not known, although, in the case of Wrch1, it does not bind to the Cdc42 effectors WASP or p21-activated kinase (PAK) [63]. However, Wrch interaction with Nck2 could allow them to recruit these proteins indirectly and thereby promote cell migration [68]. Future studies should delineate in more detail whether and how Wrch proteins contribute to cancer progression.

2.5. Rnd proteins

The Rnd (Round) proteins, Rnd1, Rnd2 and Rnd3/RhoE, received their name for the rounded morphology and loss of stress fibers observed in cells overexpressing Rnd1 or Rnd3. The three *Rnd* genes are regulated at the transcriptional level in response to a variety of stimuli. Rnd1 has been implicated in axon guidance and Rnd2 in neurite outgrowth and cytokinesis, but it is only Rnd3/RhoE that has been clearly linked to functions related with cancer progression [6].

Rnd3/RhoE has been reported to be downregulated in prostate cancer yet upregulated in other tumours [69,70]. The levels of Rnd3/RhoE in different tumours could reflect the fact that it is a p53-inducible gene and is induced by genotoxic stress [71]. Rnd3/RhoE could on the one hand suppress cancer cell proliferation since it can inhibit cell cycle progression and Ras-induced transformation, yet on the other hand it could enhance cancer progression by acting as a pro-survival factor [69,71,72]. Its effect would therefore depend on the cellular background.

Rnd3/RhoE also affects epithelial polarity and cell migration. Overexpression of Rnd3/RhoE in epithelial cells stimulates multilayering [20], which would be expected to enhance invasion. It also increases the migration speed of epithelial cells [6]. Given these multiple functions of Rnd3/RhoE on cell cycle, survival and morphology, it will be interesting to know how it affects different steps of cancer progression in vivo.

2.6. RhoD and RhoF/Rif

RhoD and RhoF have not so far been implicated in cancer cell proliferation or survival, but both of them can affect cell morphology. Overexpression of RhoF induce the generation of abundant long actin-rich Cdc42-independent filopodia and a moderate increase in stress fibers, but the relevance of these changes for cell migration and physiology is unknown [73]. Interestingly, RhoF is upregulated in malignant B-cell lymphomas [13]. Whether this overexpression leads to a change in adhesion or migration of lymphoma cells is not yet known.

RhoD regulates vesicle trafficking between intracellular compartments and endosomal motility [63]. RhoD can also in-

duce alterations to the actin cytoskeleton including loss of stress fibers, focal adhesion disassembly and actin-based protrusions at the plasma membrane, resulting in an inhibition of cell motility in some cases. The link between these two RhoD-regulated responses is so far speculative, but intracellular trafficking can mediate the delivery of signalling proteins to the plasma membrane affecting the formation of functional complexes that drive migration. No link so far has been found between cancer progression and RhoD expression.

2.7. RhoH

RhoH is frequently rearranged or mutated in B-cell lymphomas, and this is believed to contribute to lymphoma progression [5,74]. Consistent with this, RhoH knock-out mice show several deficiencies in T-cells [75]. Knockdown of RhoH by RNAi stimulates proliferation, survival and migration of hematopoietic progenitor cells [76]. Although RhoH by itself does not seem to exert a significant effect on actin reorganization, recent work places RhoH function as antagonistic to Rac1 in primary hematopoietic precursor cells: RhoH impairs migration, chemotaxis and cortical F-actin assembly by suppressing Rac1 activation and membrane targeting. Conversely, cells lacking RhoH have enhanced Rac1 activity and migration [77]. How RhoH regulates Rac1 at a molecular level is not yet known. Previous observations showed that RhoH can also inhibit NFκB and p38 activation by other Rho GTPases [78], suggesting that it may act to regulate multiple Rho family members. It is possible that this inhibitory effect of RhoH on Rac1 explains how loss of RhoH function by mutation contributes to malignant progression in lymphomas, since Rac1 has been implicated in lymphoma progression [79], although other mechanisms cannot be ruled out as the physiological function of RhoH has not been comprehensively studied.

2.8. RhoBTB subfamily

RhoBTB1 and RhoBTB2 (also known as deleted in breast cancer-2, DBC2) have an unusual domain structure, which includes an N-terminal GTP-binding domain followed by a proline-rich region and two BTB domains (Fig. 2). RhoBTB proteins have been suggested to be tumour suppressors, since RhoBTB2 was identified as a gene homozygously deleted in some breast and head and neck cancers and almost 50% of breast cancer cell lines [80]. RhoBTB1 has also recently been found to be deleted in some cancers, and in rare cases they are mutated with loss of function [81]. However, they have also been reported to be upregulated in some cancer cell lines [82]. Since very little is known of the molecular functions of RhoBTB proteins, it is only possible to speculate on their roles in cancer in vivo. RhoBTB2 is a substrate of cullin3-dependent ubiquitin ligase, and is implicated in the recruitment of cullin3, which promotes the ubiquitination and degradation of substrates implicated in cancer. RhoBTB2 might also regulate cell cycle progression and/or apoptosis as a target of the E2F1 transcription factor [83].

3. Rho regulators in cancer

The spatiotemporal activation of Rho GTPases is determined by which GEFs and GAPs are involved, and in turn these GEFs and GAPs can often act as scaffolds to bring the Rho GTPases and other signalling proteins together with their downstream targets [3]. This could explain the different, or even opposite in some cases, functions of closely related Rho GTPases in various aspects of cancer progression, for example RhoA versus RhoB.

Deregulation of expression or activity of some GEFs, GAPs and effector proteins has been observed in cancer. For example, Vav proteins are GEFs for Rac and probably other Rho proteins and have been described to have oncogenic properties. They are implicated in a variety of human malignancies such as neuroblastoma, melanoma, pancreatic tumours and leukaemia, although the oncogenic form has not been detected in human tumours [84]. The RhoGAP ARHGAP8 is frequently upregulated in colon and cervical tumours [85] and RhoGDIα expression is deregulated in various cancers [86]. Furthermore, effectors such as PAK and ROCK, downstream of Rac/Cdc42 and Rho, respectively, are upregulated in some cancers [87,88]. It is not clear how the altered expression of these various proteins influences Rho GTPase function in cancer and indeed whether the connection between these upregulated proteins and their partner Rho GTPases is relevant for tumour progression. Further studies analysing the consequences of these expression changes on Rho protein activity and also the specificity between different regulators, Rho isoforms and effectors will help to elucidate the particular functions of these proteins in the various steps of cancer progression.

4. Conclusions and perspective

Rho GTPases are involved in all stages during cancer progression. Although their initial discovery as regulators of cytoskeleton dynamics implied that they are most likely to contribute to cancer cell migration and invasion, it is now clear that the function of Rho GTPases is not restricted to these events and that they can affect tumour cells through modulation of gene transcription, cell division and survival, intracellular transport of signalling molecules or modifying the interaction of cancer cells with surrounding stromal cells. This makes the detailed analysis of how Rho GTPases work in cells and contribute to tumours very complex but at the same time promising for potential future therapeutical intervention. The involvement of specific GEFs or GAPs in defined processes regulated by Rho GTPases makes them particularly suitable as therapeutic targets [89].

Genetic models are so far available only for some of the Rho GTPases, including RhoB, RhoC, Rac1, Rac2, Rac3, Cdc42, RhoH and RhoG, and for a few regulators and effectors. The generation of cancer-specific models in mice will help to elucidate how these proteins work in cancer.

Most of what is known regarding the role of Rho GTPases in cancer cell invasion has come from the study of the prototypic members RhoA, Rac1 and Cdc42 and RhoC, but little is known regarding other members of the family although they are known to affect the actin cytoskeleton, and thus further studies are needed to clarify the roles of these less-characterized family members in tumourigenesis in vivo.

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