University of Groningen

Paving the Rho in cancer metastasis

Jansen, Sepp; Gosens, Reinoud; Wieland, Thomas; Schmidt, Martina

Published in:
Pharmacology & Therapeutics

DOI:
[10.1016/j.pharmthera.2017.09.002](https://doi.org/10.1016/j.pharmthera.2017.09.002)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Jansen, S., Gosens, R., Wieland, T., & Schmidt, M. (2018). Paving the Rho in cancer metastasis: Rho GTPases and beyond. *Pharmacology & Therapeutics*, 183, 1-21.
<https://doi.org/10.1016/j.pharmthera.2017.09.002>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Associate editor: M. Belvisi

Paving the Rho in cancer metastasis: Rho GTPases and beyond

Sepp Jansen^{a,b,c,*}, Reinoud Gosens^{a,c}, Thomas Wieland^b, Martina Schmidt^{a,c}^a Department of Molecular Pharmacology, University of Groningen, Groningen, the Netherlands^b Institute of Experimental and Clinical Pharmacology and Toxicology, Medical Faculty Mannheim, University of Heidelberg, Heidelberg, Germany^c Groningen Research Institute for Asthma and COPD, GRIAC, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

ARTICLE INFO

Keywords:

Cancer
Metastasis
GTPases
Rho
Rac

ABSTRACT

Malignant carcinomas are often characterized by metastasis, the movement of carcinoma cells from a primary site to colonize distant organs. For metastasis to occur, carcinoma cells first must adopt a pro-migratory phenotype and move through the surrounding stroma towards a blood or lymphatic vessel. Currently, there are very limited possibilities to target these processes therapeutically.

The family of Rho GTPases is an ubiquitously expressed division of GTP-binding proteins involved in the regulation of cytoskeletal dynamics and intracellular signaling. The best characterized members of the Rho family GTPases are RhoA, Rac1 and Cdc42. Abnormalities in Rho GTPase function have major consequences for cancer progression. Rho GTPase activation is driven by cell surface receptors that activate GTP exchange factors (GEFs) and GTPase-activating proteins (GAPs). In this review, we summarize our current knowledge on Rho GTPase function in the regulation of metastasis. We will focus on key discoveries in the regulation of epithelial-mesenchymal-transition (EMT), cell-cell junctions, formation of membrane protrusions, plasticity of cell migration and adaptation to a hypoxic environment. In addition, we will emphasize on crosstalk between Rho GTPase family members and other important oncogenic pathways, such as cyclic AMP-mediated signaling, canonical Wnt/ β -catenin, Yes-associated protein (YAP) and hypoxia inducible factor 1 α (Hif1 α) and provide an overview of the advancements and challenges in developing pharmacological tools to target Rho GTPase and the aforementioned crosstalk in the context of cancer therapeutics.

1. Introduction

Malignant cancer occurs when rapidly dividing tumor cells acquire the capacity to invade the surrounding tissue. While the primary tumor can often be removed by surgical resection, metastatic cancers are often associated with resistance to chemotherapeutic treatment, have higher post-treatment recurrence rates and are ultimately responsible for the

majority of cancer associated mortality (Steeg, 2016). Therefore, there is an urgent therapeutic need for suppression of metastasis. As prerequisite for occurrence of metastasis, carcinoma cells lose their epithelial phenotype and acquire a more motile, mesenchymal phenotype, a process commonly known as epithelial-mesenchymal transition (EMT). EMT allows cells to migrate from their primary site, through the surrounding tissue towards the blood or lymphatic system.

Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMT, amoeboid-mesenchymal transition; APC, adenomatous polyposis coli; Arp2/3, actin related protein 2/3; Cdc42, cell division control protein 42 homolog; CK1, casein kinase 1; COX-2, cyclooxygenase 2; CXCR4, C-X-C Motif Chemokine Receptor 4; DLC1/2/3, deleted in liver cancer 1/2/3; DOCK2, dedicator of cytokinesis 2; DVL, disheveled; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; EP1/2/3/4, E-type prostanoid receptor 1/2/3/4; Epac1/2, exchange protein directly activated by cyclic AMP 1/2; ET1, endothelin 1; FRET, fluorescence resonance energy transfer; GAP, GTPase activating protein; GDI, guanine nucleotide dissociation inhibitor; GDP, guanine diphosphate; GEF, guanine exchange factor; GPCR, G-protein coupled receptor; GSK-3, glycogen synthase kinase 3; GTP, guanine triphosphate; Hif1 α , hypoxia inducible factor 1 α ; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; HRE, hypoxia response element; IQGAP, IQ motif containing GAP; LARG, leukemia-associated Rho GEF; LATS1/2, large tumor suppressor kinase 1/2; LEF, lymphoid enhancer-binding factor 1; LIMK, Lin11, Isl-1 & Mec-3 (LIM) domain kinase; LPA, Lysophosphatidic acid; MAT, mesenchymal-amoeboid transition; mDia, diaphanous-related formin; MLC, myosin light chain; MLCK, MLC kinase; MMP, matrix metalloprotease; MRCK, myotonic dystrophy-related Cdc42-binding kinase; MRTF-A, myocardin-related transcription factor A; NET1, neuroepithelial cell transforming 1; NSCLC, non-small cell lung carcinoma; N-WASP, neural Wiskott-Aldrich syndrome protein; PAK, p21-activated kinase; PDK1, 3-phosphoinositide-dependent protein kinase 1; PDZ, PSD95, Dlg1, and ZO-1 domain; PGE₂, prostaglandin E₂; PKA, protein kinase A; PKC, protein kinase C; PLD1, phospholipase D1; pVHL, von Hippel-Lindau protein; RGS, regulator of G-protein signaling; Rac, Ras-related C3 botulinum toxin substrate; Rho, Ras homolog gene family; ROCK, rho-associated, coiled-coil-containing protein kinase; S1P, sphingosine 1 phosphate; SDF1, stromal cell-derived factor 1; Smurf1, SMAD specific E3 ubiquitin protein ligase 1; Sra1, steroid receptor RNA activator 1; SRF, serum response factor; TCF, transcription factor; TEAD, transcriptional enhancer factor domain; Tiam, T-cell lymphoma invasion and metastasis; Trio, Triple functional domain protein; VEGF, vascular endothelial growth factor; WAVE, WASP-family verprolin-homologous protein; WRC, WAVE regulatory complex; YAP, Yes-associated protein

* Corresponding author at: Institute for Experimental Pharmacology and Toxicology, Maybachstraße 14, 68169 Mannheim, Germany.

E-mail address: sepp.jansen@medma.uni-heidelberg.de (S. Jansen).

<http://dx.doi.org/10.1016/j.pharmthera.2017.09.002>

Available online 11 September 2017

0163-7258/ © 2017 Elsevier Inc. All rights reserved.

Fig. 1. GDP-GTP exchange cycle of Rho GTPases. Rho GTPases cycle between the GDP-bound inactive and GTP-bound active form, and have intrinsic GTPase activity that hydrolyses bound GTP to GDP and PO_4^{3-} . Activation is regulated by guanine nucleotide exchange factors (GEFs) that catalyze the exchange of GDP to GTP, and inactivation by GTPase-activating proteins (GAPs) that increase the rate of GTP hydrolysis to GDP. Guanosine-nucleotide dissociation inhibitors (GDIs) prevent the exchange of GDP to GTP, maintaining a GDP-bound pool in an inactive state in the cytosol. Rho GTPases also contain a C-terminal domain that undergoes post-translational modification by lipid groups, such as geranylgeranyl, palmitoyl, farnesyl and myristoyl moieties. Those lipid modifications are necessary for the binding of Rho GTPases to membranes and regulators, and thus for compartmentalization of the signal.

The Rho family of small GTPases comprises over 60 members and can be subdivided in multiple subfamilies, based on their structure and function and have a well-recognized role in the regulation of migration by acting on the cytoskeleton. The best characterized subfamilies are the Rho (RhoA, RhoB, RhoC), Rac (Rac1, Rac2, Rac3, RhoG) and Cdc42 (Cdc42, RhoQ, RhoJ) subfamilies. Small GTPases cycle between an inactive GDP-bound and an active GTP-bound bound state. Activation of small GTPases is achieved by guanine nucleotide exchange factors (GEFs) that catalyze the exchange of GDP to GTP (Cherfils & Zeghouf, 2013). Inactivation of small GTPase is achieved by guanine nucleotide activating proteins (GAPs) that stimulate the low intrinsic GTP-hydrolyzing activity of the small GTPases (Fig. 1). The guanine nucleotide dissociation inhibitors (GDIs) interact with the inactive GTPase domains and prevent the dissociation of guanine nucleotides from Rho GTPases (Cherfils & Zeghouf, 2013). Thus, GDIs preserve the inactive GTPase state by preventing GEF-mediated nucleotide exchange, or when they bind to the GTP bound state, they inhibit GTPase activity while maintaining interactions with downstream effectors. Rho proteins interact only in their active GTP-bound state with a range of different effectors and thereby regulate cellular functions. In addition, distinct intracellular localization of Rho proteins with overlapping effectors result in pronounced functional differences.

In this review we investigate the role of Rho family GTPases, their regulators and effectors, and other molecular interactions in regulating the invasion-metastasis cascade, and highlight pharmacological strategies to inhibit Rho GTPases, and their molecular interactions.

2. Rho protein networks in malignancy

It has long been recognized that Rho activity and expression are frequently elevated in tumors (Fritz, Just, & Kaina, 1999; Orgaz, Herraiz, & Sanz-Moreno, 2014). In this chapter we will introduce the most prominent Rho proteins, their regulators and effectors and provide an overview of their involvement in cancer. Increased expression of RhoA occurs in a variety of carcinomas such as hepatocellular, lung, and colorectal (Vega & Ridley, 2008). Similar, expression of RhoC positively correlates with cancer metastasis and invasion. Indeed, its deletion impairs such processes (Bellovin et al., 2006; Clark, Golub, Lander, & Hynes, 2000; van Golen et al., 2002; Vega, Fruhwirth, Ng, & Ridley, 2011; Xing et al., 2015) while overexpression enhances cancer cell migration and metastasis (Dietrich et al., 2009; Ma, Teruya-

Feldstein, & Weinberg, 2007). Conversely, expression of RhoB is downregulated in several malignancies (Huang & Prendergast, 2006) where its expression promotes apoptosis in cancer cell lines (Croft & Olson, 2011; Prendergast, 2001) and its depletion stimulates cancer cell migration (Vega, Colomba, Reymond, Thomas, & Ridley, 2012). Accordingly, miR-21, which is one of the most frequently up-regulated miRNAs in cancer, suppresses RhoB expression and thereby increases migration, proliferation and reduces apoptosis (Connolly, Van Doorslaer, Rogler, & Rogler, 2010; Liu et al., 2011). Therefore, it is generally believed that RhoB acts as a tumor suppressor while RhoA and RhoC acts as tumor promoters, although recent evidence is challenging this notion for RhoA for at least colorectal tumors (Rodrigues et al., 2014).

To date, mutations in Rho proteins have only been sparsely described in cancer and are mainly limited to subsets of gastric carcinoma (Kakiuchi et al., 2014; Kumar, Swamidass, & Bose, 2016). Nonetheless, activation levels of Rho proteins are often found altered. The activation or inactivation of Rho proteins is regulated by numerous signal transduction pathways, such as G-protein coupled receptors (GPCRs) and growth factor receptors. Of the different pathways that mediate GPCR-induced activation of Rho, signaling through $\text{G}\alpha_{12/13}$ is best understood. Activated $\text{G}\alpha_{12/13}$ interacts with the Rho exchange factors PDZ-RhoGEF, LARG, and p115RhoGEF (Aittaleb, Boguth, & Tesmer, 2010; Fukuhara, Chikumi, & Gutkind, 2001; Rossman, Der, & Sondek, 2005). Thereby, $\text{G}\alpha_{12/13}$ -coupled receptors activate RhoA and control cell migration (Dorsam & Gutkind, 2007). Therefore, it is important to recognize that, in addition to altered expression, deregulation of Rho proteins in cancer mainly occurs at the level of their activation.

An example of such deregulation in cancer that has recently received considerable attention is the Rho GAP deleted in liver 1 (DLC-1), of which downregulation is often observed in carcinomas (Braun & Olajoye, 2015) where it appears to be involved in the regulation of cell motility and migration as expression of DLC-1 reduces migration of carcinoma cells (Goodison et al., 2005; Wong et al., 2005). Strikingly, restoring DLC-1 expression in human cancer cells lacking the endogenous protein results in lower capacity to form tumors in mouse xenograft models, suggesting that DLC-1 functions as a tumor suppressor (Durkin, Ullmannova, Guan, & Popescu, 2007; Zhou, Thorgeirsson, & Popescu, 2004), while in breast cancer cells, silencing of DLC-1 enhances cell motility (Holeiter et al., 2008). Similarly, the other DLC members, DLC-2 and DLC-3, have comparable roles in cancer

progression (Durkin et al., 2007; Leung et al., 2005). Furthermore, in addition to altered expression, regulatory mechanisms exist that alter the function of DLC-1, such as phosphorylation by protein kinase A (PKA), a process enhancing the GAP activity of DLC-1 and thereby inhibiting motility of hepatocellular carcinoma in vitro and metastasis in vivo (Ko et al., 2013). For an overview over deregulated Rho GEFs, we refer to a recent publication by Cook and colleagues (Cook, Rossman, & Der, 2014).

Rho proteins exert their effects through activation of their downstream effectors, which include protein kinases, actin nucleation promoting molecules, and adaptor proteins. With regard to cancer, one interesting class of downstream Rho protein effectors, are the serine-threonine kinases, which can effectively be targeted with small-molecule inhibitors, making them interesting targets for cancer therapy. Activation of the Rho-associated protein kinases (ROCKs) regulates actomyosin contractility by increasing the phosphorylation of myosin light chain (MLC). This in turn stimulates myosin to interact with and move along actin filaments (Riento & Ridley, 2003). On the one hand, ROCKs stimulate the activity of MLC kinase (MLCK), while on the other hand ROCKs phosphorylate myosin binding subunit (MBS), which results in the inactivation of MLC phosphatase (MLCP). Increased ROCK activity and gene expression have been demonstrated in various tumors, with increased expression or activation of ROCKs being correlated with metastasis (Kale, Hengst, Desai, Amin, & Yun, 2015; Loirand, 2015; Rodriguez-Hernandez, Cantelli, Bruce, & Sanz-Moreno, 2016).

In addition to changes in the Rho isoforms, all Rac isoforms, including the constitutively active splice variant of Rac1, Rac1b, are overexpressed in various tumors and accumulating evidence indicates that they play an important role in the metastatic potential of cancer cells (Chan et al., 2005; Fritz et al., 1999; Jordan, Brazao, Boavida, Gespach, & Chastre, 1999; Orgaz et al., 2014; Zhou et al., 2013). Similarly, overexpression of Cdc42 occurs in several human tumors (Fritz et al., 1999; Orgaz et al., 2014). Cdc42 is involved in activation of signaling that regulates cell polarity, cytoskeletal remodeling, cell migration, and cell proliferation, and as such, Cdc42 can be regarded as pro-oncogenic (Stengel & Zheng, 2011). Nonetheless, this does not appear to be the case in the pediatric tumor neuroblastoma, where overexpression of N-Myc, an important inverse prognostic marker, is associated with a deletion of the chromosomal region that contains the Cdc42 gene, resulting in a reduction in Cdc42 expression (Valentijn et al., 2005). In neuroblastoma cells, expression of active Cdc42 is required for differentiation, suggesting that Cdc42 likely functions as a tumor suppressor in neuroblastoma. However, on the contrary, there are also indications that expression of Cdc42 correlates with undifferentiated neuroblastoma (Lee, Craig, Romain, Qiao, & Chung, 2014). Thus, the exact role of Cdc42 in tumor progression appears, at least for neuroblastoma, to be complicated and context dependent.

In a similar fashion as with the Rho isoforms, deregulation of Rac and Cdc42 occurs primarily at the level of activation. Examples of a deregulated Rac and Cdc42 GEF activity in cancers include the GEFs Tiam1, Vav2 and Trio, of which overexpression correlates with poor prognosis and enhanced metastatic behavior (Chen, Ding, et al., 2012; Menacho-Marquez et al., 2013; Patel et al., 2007; Schmidt & Debant, 2014). The kinase effectors of Rac and Cdc42 belong to the P21-activated kinase (PAK) family, which are often overexpressed in tumors and are inversely correlated with survival (Chow et al., 2012; Radu, Semenova, Kosoff, & Chernoff, 2014). For example, PAK1 exhibits overexpression or hyperactivation in 50% of breast tumors (Ong et al., 2011) while it is overexpressed in invasive prostate cancer cells compared to noninvasive cells (Goc et al., 2013).

From the findings discussed above it becomes clear that the best characterized Rho GTPases, i.e. RhoA, Rac1, and Cdc42, are often deregulated in cancer and are involved in tumor progression by contributing to pro-migratory and metastatic behavior of cancer cells. Deregulation of Rho GTPases primarily occurs at the level of activation and deactivation and the downstream effectors of Rho family GTPases.

3. Rho protein networks in epithelial-to-mesenchymal transition

For carcinoma cells to metastasize, the transformed cells need to lose their cell-cell contacts and adopt a more motile, migratory phenotype, a process commonly known as EMT. Normal epithelial cells are organized as two-dimensional layers with close contacts with neighboring cells and an apicobasal polarity axis. During EMT, epithelial cells lose their cell-cell adhesion complexes, rearrange their cytoskeleton and lose their apicobasal polarity. Actin dynamics during EMT are regulated by Rho proteins and the conversion from apicobasal polarity to front-rear polarity also involves Rho proteins. EMT is a key mechanism during normal development of organs and tissues, but over the past decade, the occurrence of EMT has become a well-documented event during tumor progression (Thiery, Acloque, Huang, & Nieto, 2009). Elucidating the exact molecular signature that drives this behavior is key to developing new therapeutic strategies to target EMT in cancer.

During embryogenesis, tumors such as neuroblastoma develop from the neural crest; an ectodermal cell population consisting of multipotent stem cells (Fort & Theveneau, 2014). In the neural crest of *Xenopus*, depletion of RhoV (an atypical Rho protein closely related to Rac and Cdc42) inhibits expression of EMT regulatory transcription factors, whereas overexpression of RhoV enhances the expression of such factors (Guemar et al., 2007). Similarly, inhibition of Rac1 or PAK1 inhibition results in an almost complete repression of EMT transcription factors in *Xenopus* (Bisson, Wedlich, & Moss, 2012), indicating that RhoV, Rac1, Cdc42, and their downstream effector PAK1, are required for EMT during development of the neural crest.

Cells undergoing EMT display a downregulation of E-cadherin and dissociation of their junctional complexes that associates cell-cell contacts with the actin cytoskeleton, known as the adherens junction (Berx & van Roy, 2009; Birchmeier & Behrens, 1994). Adherens junctions are specialized cell-cell adhesion complexes which help maintain apicobasal polarity in epithelial cells. In animal models with a loss of E-cadherin, epithelial tumor cells transform into highly invasive cells, suggesting that the loss of E-cadherin is a causal factor in metastasis (Derksen et al., 2006). Besides E-cadherin, the adherens junctional complex consists of several proteins involved in anchoring the transmembrane E-cadherin homodimers to the actin cytoskeleton, most notably the catenin family members β -catenin, α -catenin and p120-catenin.

Rho family members are both crucial in junctional assembly and disassembly (Akhtar & Hotchin, 2001; Shen, Hirsch, Sasiela, & Wu, 2008). The role of Rho proteins in the establishment and maintenance of epithelial cell polarity and junctional assembly has been the focus of a recent review, so will not be discussed in detail here (Mack & Georgiou, 2014).

Regarding disassembly of the adherens junctions, a crucial step during EMT, studies in pancreatic carcinoma cells demonstrate that overexpression of constitutively active Rac1 reduces E-cadherin expression, while conversely, dominant negative Rac1 increases E-cadherin levels (Hage, Meinel, Baum, Giehl, & Menke, 2009). Additionally, activation of Rac1 by the Rac GEF Tiam1 has been found to be required for adherens junctional disassembly (Knezevic et al., 2009; Rios-Doria, Kuefer, Ethier, & Day, 2004).

One mechanism by which Rac1 regulates stability of the adherens junction is through interaction with the Rac1 effector IQGAP1 (Fig. 2). Although IQGAP has high homology to other GAPs, it does not exhibit intrinsic GAP activity but binds with high affinity to GTP-bound Rac1. IQGAP1 associates with several proteins that constitute adherens junctions. In several carcinomas, IQGAP1 exhibits a high expression level with close correlation to metastatic potential, and increased cell membrane localization due to its association with adherens junctions (Jadeski, Mataraza, Jeong, Li, & Sacks, 2008; Nabeshima, Shimao, Inoue, & Koono, 2002; Nakamura et al., 2005). IQGAP1 expression is implicated in tumor progression via decreasing E-cadherin-mediated

Fig. 2. Rho protein signaling to the nucleus. Rho family GTPases are primarily known for regulating the actin cytoskeleton. However, several mechanisms exist in which activation of RhoA, Cdc42 and Rac1 results in altered gene transcription. At adherens junctions, β-catenin is sequestered with E-cadherin, thereby stabilizing cell-cell contacts. Free cytosolic β-catenin is under tight control by proteasomal degradation. Once stabilized, β-catenin translocates to the nucleus where it associates with transcription factors of the TCF/LEF family to activate gene transcription. Downstream of GPCRs (e.g. PGE₂, β-adrenoceptors), β-catenin stabilization and nuclear translocation can be enhanced by the cyclic AMP effector and Rap GEF Epac1, Rac1, and subsequent phosphorylation by the Rac1 effector PAK1. On the other hand, active Rac1 acts on its effector IQGAP1. IQGAP1 binds to proteins of the adherens junction, including β-catenin, thereby stabilizing the junctional complex. However, active Rac1 disrupts this interaction, thereby releasing β-catenin from the adherens junction, potentially making β-catenin available for nuclear translocation. The loss of adherens junctions also results in cytosolic localization of p120-catenin. There, p120-catenin interacts with RhoA, which leads to stabilization of the inactive form of RhoA, while on the other hand cytosolic p120-catenin leads to activation of Rac1 and Cdc42. High stiffness of the extracellular matrix, which is often the case in the tumor micro-environment, results in the translocation of the transcriptional co-activators MRTF-A and YAP. In the absence of active RhoA, MRTF-A sequesters with monomeric G-actin, while in the presence of active RhoA, G-actin polymerizes to F-actin filaments, thereby freeing MRTF-A to translocate to the nucleus where it acts as a transcriptional cofactor for SRF-mediated gene transcription. Similarly, nuclear translocation of YAP is also under control of RhoA. Inside the nucleus, YAP acts as a transcriptional cofactor for TEAD-domain containing transcription factors and interacts with β-catenin, thereby promoting β-catenin-dependent transcription.

cell adhesion (Hage et al., 2009; Kuroda et al., 1998). In the absence of active Rac1, IQGAP1 stabilizes the interaction between E-cadherin and β-catenin and thereby the junctional complex. Strikingly, transformation of pancreatic carcinoma cells with active Rac1, results in altered subcellular distribution of both E-cadherin and β-catenin and attenuated junctional stability (Hage et al., 2009). However, the effect of Rac1 on adherens junctional stability and the effect on cell-migration are still under debate and recent findings support the idea that activation of Rac1 might actually stabilize E-cadherin-mediated cell-cell contacts. The effect Rac1 has on junctional stability appears to be dependent on the mechanism of Rac1 activation. For instance, phosphorylation of the Rac GEF Tiam1, by the oncoprotein sarcoma-family kinase (Src), results in subsequent degradation of Tiam1 at adherens junctions, a step that is required for Src-mediated adherens junction disassembly and enhanced cell migration (Woodcock et al., 2009), possibly indicating that there is a requirement of suppressed Rac1 activity at adherens junctions during junctional disassembly. A recent study supports this idea by showing that expression of Rac1 enhanced

actin and E-cadherin localization at the adherens junctions, thereby promoting cellular aggregation with reduced cell migration (Marei et al., 2016). Intriguingly, while earlier studies have suggested IQGAP1 to disrupt the adherens junction via binding to β-catenin and thereby destabilizing the E-cadherin junctional complex (Hage et al., 2009; Kuroda et al., 1998), here, the authors suggest that Tiam1-mediated Rac1 activation results in IQGAP1 binding to Rac1 and Tiam1, in a complex in which Tiam1 acts as a scaffolding protein. This increased binding of active Rac1 to IQGAP1 inhibits its binding to β-catenin, thereby stabilizing E-cadherin-mediated cell-cell contacts (Fukata et al., 1999; Marei et al., 2016). In sharp contrast, the same study showed that expression of another Rac GEF, P-Rex1, is associated with cell-cell contact dissociation and a marked increase in cell migration (Marei et al., 2016). Here, the authors suggest that the underlying mechanism is the interaction between P-Rex1-activated Rac1 and a novel Rac1 interacting protein, FLII. Importantly, Tiam1-mediated Rac1 activation did not enhance this interaction, demonstrating that, beyond Rac1 activation, there are additional levels of regulation.

Fig. 3. Formation of invadopodia, lamellipodia, and filopodia. Upper panel). Invadopodia are actin-rich protrusions of the plasma membrane that are associated with degradation of the extracellular matrix. Upon integrin engagement, Src and ArhGEF5 interact leading to recruitment of ArhGEF5 to invadopodia, and subsequent activation of RhoA and Cdc42. Active Cdc42 releases the auto inhibitory conformation of the N-WASP complex, promoting actin polymerization via interaction with the Arp2/3 complex known to nucleate actin filaments. In addition Cdc42 is also involved in the trafficking of MMPs, such as MMP14 and the transmembrane MT1-MMP, to invadopodia, via the Cdc42 effector IQGAP1. Middle panel). At the leading edge of the cell, the highly dynamic lamellipodium is extended by Arp2/3 complex-mediated formation of new actin filaments from the sides of existing filaments. This leads to the assembly of a highly branched network of actin filaments. Upon integrin engagement, activated Rac1 promotes actin polymerization during lamellipodium formation through the WAVE complex, and subsequent activation of Arp2/3. Rac-mediated activation of PAK1 phosphorylates LIMK, which phosphorylates cofilin, thereby regulating actin-filament turnover. More recently, another function of cofilin was assigned to phospho-cofilin upstream of PLD1 activation. PLD1 synthesizes phosphatidic acid, which regulates DOCK2, thereby controlling Rac activation at the leading edge. Lower panel). Filopodia are thin protrusions that contain parallel bundles of actin filaments that extend from the leading edge migrating cells. Filopodia formation, in a similar fashion as invadopodia formation, is regulated by Cdc42, and subsequent activation of the WASP complex and Arp2/3. During filopodia formation Cdc42 also induces actin polymerization by activation of mDia2. Through PAK1 and LIMK, cofilin is inhibited thereby regulating actin-filament turnover.

Cdc42 is also linked to the adherens junction via IQGAP1, however, it appears to have an opposite effect on junctional stability. When Cdc42 is inactive, IQGAP1 binds to β -catenin, displacing α -catenin, resulting in the loss of α -catenin-linked actin filaments from the adherens junction and reduced cell-cell adhesion. Contrary, active Cdc42 binds to IQGAP1, thereby interfering with Cdc42/ β -catenin interaction (Kuroda et al., 1998).

With regard to regulation of RhoA activity at the adherens junction, research lines that focus on the role of p120-catenin in tumor progression are particularly of interest as p120-catenin regulates both the stability of the junctional complex and activates Rho proteins. p120-Catenin promotes clustering of cadherins which increases the stability and membrane localization of E-cadherin in carcinoma cells (Kowalczyk & Reynolds, 2004; Reynolds & Rocznik-Ferguson, 2004; Soto et al., 2008).

Upon expression of p120-catenin, E-cadherin and Rho, normal bronchial epithelium exhibit an intense membrane expression of p120-catenin and E-cadherin, while non-small cell lung carcinoma (NSCLC) cells show a reduced membrane expression pattern of both proteins. In addition, expression of RhoA, Cdc42, and Rac1 is higher in carcinoma

cells, a process associated with increased metastasis (Liu, Li, et al., 2009). Contrary, ectopic expression of E-cadherin in NSCLC cells negatively regulates the activation of RhoA and Cdc42 and is thereby able to reduce cell migration (Asnaghi et al., 2010). During EMT, p120-catenin dissociates from adherens junction and localizes in the cytoplasm where it can interact with Rho, contributing to increased migration and invasion (Noren, Liu, Burridge, & Kreft, 2000; Pieters, van Hengel, & van Roy, 2012). While p120-catenin lacks GAP activity, the direct interaction between p120-catenin and RhoA downregulates RhoA activity by stabilizing the inactive GDP-bound form of RhoA (Fig. 2) (Grosheva, Shtutman, Elbaum, & Bershadsky, 2001; Noren et al., 2000). On the other hand, p120-catenin activates Rac1 and Cdc42 indirectly by activating the GEF Vav2 (Anastasiadis & Reynolds, 2001; Cheung, Leung, & Wong, 2010; Johnson et al., 2010; Noren et al., 2000).

EMT is a complex cellular program that is not only characterized by loss of E-cadherin and adherens junctional complexes, but involves transcriptional programs that are under control of a group of EMT-inducing transcription factors such as Snail, Slug, Twist and Zeb (Kalluri, 2009). Although these transcription factors can be activated by a

plethora receptor-mediated pathways, they can also be activated as a consequence of destabilization of the adherens junction. For example, the constitutively active splice variant of Rac1, Rac1b, induces EMT in lung carcinoma and breast carcinoma through upregulation of EMT transcription factors, thereby decreasing expression of E-cadherin (Radisky et al., 2005; Stallings-Mann et al., 2012) and activation of the Rac1 kinase effector PAK1 directly phosphorylates Snail, thereby suppressing E-cadherin expression (Yang et al., 2005), suggesting the existence of positive feedback loops that, once triggered by either disassembly of adherens junctions or receptor-mediated pathways, help maintain the ongoing EMT process. An example of such a loop is the aforementioned Rac1 and Cdc42 effector IQGAP1, which can protect cytosolic β -catenin against degradation (Briggs, Li, & Sacks, 2002) suggesting that IQGAP1 is an important regulator of β -catenin. Once stabilized, β -catenin can translocate to the nucleus, where it activates transcriptional programs that could further reinforce EMT. The role of Rac1-regulated β -catenin in EMT is discussed in '10. YAP in relation to Epac1, Rac1 and β -catenin.'

4. Rho protein networks and polarized migration

Cell migration is a complex and dynamic process that involves continuous remodeling of the cellular architecture. Rho proteins are well known regulators of motility by controlling actin and microtubule dynamics. Mesenchymal migration is the best studied mode of migration, primarily in a two dimensional experimental setting. During mesenchymal migration, cells develop clearly defined polarity with a leading edge with characteristic membrane protrusions and a trailing end. There are three main types of membrane protrusions occurring during mesenchymal cell migration: invadopodia, lamellipodia, and filopodia, dependent on the spatiotemporal activation of Rac1 and Cdc42, respectively.

4.1. Rho protein networks and invadopodia

First we will describe the role of Rho proteins in actin-rich protrusions that extend into the extracellular matrix and are involved the breakdown of the extracellular matrix, known as invadopodia (Fig. 3, upper panel) (Spuul et al., 2014). Although activation of the cytosolic tyrosine kinase Src downstream of growth factor receptor signaling plays a prominent role in invadopodia formation, the precise molecular mechanisms that trigger invadopodia formation are currently poorly understood. In carcinoma cells, the crucial role of Cdc42 in this process is illustrated by expression of dominant active mutants of Cdc42, which promotes the formation of invadopodia, and conversely, by depletion of Cdc42, which prevents the formation of invadopodia (Pichot et al., 2010; Yamaguchi et al., 2005).

Although most studies on invadopodia formation have focused on Cdc42, a recent study shows that RhoA activation, by the Rho GEF ArhGEF5, in fact precedes the activation of Cdc42 at invadopodia (O'Connor & Chen, 2013). Interestingly, Src and ArhGEF5 interact and activate each other at focal adhesions, leading to recruitment of ArhGEF5 to invadopodia (Kuroiwa, Oneyama, Nada, & Okada, 2011). Together this suggests that RhoA and Cdc42 spatiotemporally interact with each other during the formation of invadopodia. Further recent work has revealed that binding of endothelin 1 (ET1) to its receptor induces direct interaction between the GPCR-associated scaffolding protein β -arrestin and PDZ-RhoGEF, a process driving the formation of invadopodia via PDZ-RhoGEF activated RhoC (Semprucci et al., 2015), indicating that both RhoA and RhoC indeed play important roles in invadopodia. The formin mDia2, which is activated by RhoA, further induces the formation of linear actin bundles, resulting in increased stability and elongation of invadopodia (Lizarraga et al., 2009).

In addition to existing as individual structures, invadopodia can also assemble into circular rosette-like structures that may display more efficient and localized ECM degradation (Pan, Chen, & Chen, 2011).

Recent findings from work studying the formation of invadopodia rosettes in melanoma cells expressing constitutively active Src demonstrate that the formation of such invadopodia rosettes is triggered by GPCR agonists, most notably lysophosphatidic acid (LPA) and endothelin, which induce the rapid formation of highly dynamic invadopodia rosettes (Kedziora et al., 2016). The LPA-induced reorganization of invadopodia was mediated through the LPA receptor 1 (LPA1), which can couple to $G\alpha_i$ and $G\alpha_{12/13}$. Pretreatment with the $G\alpha_i$ inhibitor pertussis toxin blocked rosette formation by LPA and endothelin, which indicates that this rosette formation critically depends on $G\alpha_i$ -linked signaling pathways. LPA treatment activates both Cdc42 and RhoA, whereas endothelin only activated Cdc42. The activation of Cdc42 was abolished by pertussis toxin, confirming the key role for $G\alpha_i$. However, RhoA activation demonstrated an inverse correlation with rosette formation, which suggests that RhoA activity might function as a suppressor. Indeed, treatment with the ROCK inhibitor Y27632 boosted rosette formation by LPA and endothelin. Therefore, it is suggested that the $G\alpha_{12/13}$ -linked RhoA-ROCK pathway counteracts $G\alpha_i$ -Cdc42-mediated rosette formation (Kedziora et al., 2016).

The role of Rac1 in invadopodia is more controversial and appears to be highly context dependent. Early studies have identified Rac1 to be required for the formation of invadopodia (Chuang et al., 2004). However, seemingly contrary, studies using fluorescence resonance energy transfer (FRET) for Rac1 activation, have shown that Rac1 regulates the disassembly of invadopodia through PAK1-dependent cortactin phosphorylation (Moshfegh, Bravo-Cordero, Miskolci, Condeelis, & Hodgson, 2014). The authors of this study argue that this mechanism facilitates the coordination of proteolytic activity occurring at invadopodia and the formation of locomotor protrusion (e.g. lamellipodia, filopodia), allowing for a proper turnover of invadopodia during cell invasion to achieve a highly invasive phenotype. In this regard, a recent study in melanoma cells shows that, while knockdown of wild-type Rac1 attenuates invadopodia function, knock-down of a hyperactive mutant Rac1 enhances the number of invadopodia forming cells (Revach, Winograd-Katz, Samuels, & Geiger, 2016), indicating that the a careful regulation of Rac1 activity in time and space at invadopodia is important for both assembly, functionality, and disassembly of invadopodia. In line with this notion, lysophosphatidic acid-induced invadopodia formation in fibrosarcoma cells require activation of Rac1 by the cyclic AMP-regulated GEF, Epac1 (Harper et al., 2010), which is discussed in more detail in relation to β -catenin in '10. YAP in relation to Epac1, Rac1 and β -catenin.'

In addition to regulating the assembly and disassembly of invadopodia, Cdc42 also plays an important role in the trafficking of matrix metalloproteases (MMPs) to the invadopodia (Fig. 3, upper panel). For example, the transmembrane MMP, MT1-MMP, accumulates at the invasive front of tumors in invadopodia (Poincloux, Lizarraga, & Chavrier, 2009). Silencing of IQGAP, which localizes at invadopodia upon Cdc42 activation, decreases levels of MT1-MMP at invadopodia, suggesting that Cdc42-induced IQGAP1 binding at invadopodia is crucial for the trafficking of MT1-MMP (Poincloux et al., 2009). Further, active Cdc42 triggers the interaction between IQGAP1 and the exocyst complex, the protein complex responsible for vesicle trafficking, an interaction which has been shown to be required for MMP14 accumulation at invadopodia (Sakurai-Yageta et al., 2008). Taken together, current studies indicate that a distinct subset of Rho GTPases and effectors are key to a tight control of invadopodia dynamics in time and space.

4.2. Rho protein networks and lamellipodia

Early studies by Hall, Ridley, and Nobes have demonstrated that Rho proteins are involved in rearranging the cellular architecture, and are thereby crucial in the process of cell migration. Importantly, these studies revealed that Rac promotes the formation of sheet-like

protrusions in response to the growth factor PDGF (Ridley & Hall, 1992; Ridley, Paterson, Johnston, Diekmann, & Hall, 1992). These protrusions, known as lamellipodia, exhibit branched actin networks that extend towards the leading edge (Fig. 3, middle panel). Essential for lamellipodium-driven migration are focal adhesion junctions where integrin engagement induces Rac activation (Lawson & Burridge, 2014). The structure of the branched actin network is the result of active Rac proteins interacting with the WAVE regulatory complex (WRC), consisting of WAVE1 and Sra1. This interaction induces a conformation change in WAVE1, leading to activation of the actin nucleation complex Arp2/3 and thus actin assembly (Chen, Borek, et al., 2010). In addition, Rac affects the phosphorylation of MLC in lamellipodia through its effector PAK1 (Kiosses, Shattil, Pampori, & Schwartz, 2001; van Leeuwen, van Delft, Kain, van der Kammen, & Collard, 1999). Downstream of PAK1, LIM kinase (LIMK) is activated which phosphorylates, and thereby primarily inactivates, cofilin (Yang et al., 1998). Cofilin underlies the rapid disassembly of long unbranched filaments in motile cells, while on the other hand the Arp2/3 complex mediates the branched extension of actin filaments typically observed in lamellipodia. Thus, the simultaneous disassembly of long actin filaments by cofilin, and the creation of branched extensions by the Arp2/3 complex, coordinate the formation of lamellipodia (Dan, Kelly, Bernard, & Minden, 2001; Delorme et al., 2007).

More recently, another cofilin function was assigned to phosphorylated cofilin, which so far has been considered inactive because it cannot bind actin. This phospho-cofilin mediates the activation of phospholipase D1 (PLD1) (Han et al., 2007), a process that appears to determine chemotaxis of cancer cells along a chemical gradient of phosphatidic acid. This in turn regulates the localization of the Rac GEF DOCK2, thereby controlling Rac activation at the leading edge (Nishikimi et al., 2009). Bernstein and Bamburg propose the interesting possibility that phospho-cofilin is part of positive feedback mechanism in which phosphatidic acid increases the activity of subsequently Rac1, PAK1, and LIMK, which further phosphorylates cofilin, thereby increasing levels of PLD1-generated phosphatidic acid, thus providing a mechanism of sustained migration along a chemical gradient (Fig. 3, middle panel) (Bernstein & Bamburg, 2010).

The balance between actin polymerization and integrin-mediated adhesion is crucial for lamellipodia-based migration. The PAK family of kinases plays a key role in promoting integrin-based adhesion turnover (Rane & Minden, 2014). For example, PAK1 phosphorylates GIT1, and this increases binding of GIT1 to the focal adhesion adaptor protein paxillin, a focal adhesion adaptor protein which is crucial for focal adhesion turnover (Hoefen & Berk, 2006). In a similar fashion, the less well characterized Rho protein RhoD and RhoJ promote focal adhesion turnover (Gad, Nehru, Ruusala, & Aspenstrom, 2012; Wilson et al., 2014). As such, RhoJ also interacts with GIT and activates PAK at focal adhesions, offering the possibility that RhoJ functions upstream of PAK to promote focal adhesion turnover (Vignal et al., 2000; Wilson et al., 2014).

Another mechanism regulating PAK activity at focal adhesions is by the Rac GEF β -Pix (Nayal et al., 2006). In addition to its role as a GEF, β -Pix is also a binding partner for PAK (Manser et al., 1998), an interaction that targets PAK to focal adhesion complexes (Stofega, Sanders, Gardiner, & Bokoch, 2004). Interestingly, although PAK is a well-known downstream effector of Rac1, activation of Rac1 is inhibited by PAK competing with Rac1 for β -Pix binding. Thus, in the absence of PAK, Rac1 activation is increased, leading to enhanced cell migration (ten Klooster, Jaffer, Chernoff, & Hordijk, 2006). However, in fibroblasts the reverse is also occurring. Here, PAK1 activation at the leading edge promotes β -Pix recruitment, leading to a local activation of Rac1 (Cau & Hall, 2005). In accordance, β -Pix remained localized at focal adhesions in lung carcinoma and depletion of β -Pix abolished cell migration (Yu et al., 2015). Together, these findings draw a picture in which activation and localization of Rac1 and PAK at focal adhesions are tightly organized in space and time by β -Pix.

In addition to a role for Rac, RhoA and its GEF p190RhoGEF also play a role in focal adhesion turnover. For example, silencing of p190RhoGEF in fibroblasts, or p190RhoGEF knockdown in mice, decreases RhoA activity and attenuates the formation of focal adhesions with a concomitant decrease in migration (Miller, Lawson, Chen, Lim, & Schlaepfer, 2012). Conversely, increased expression of p190RhoGEF in colorectal carcinoma further confirms the crucial role of p190RhoGEF and RhoA in focal adhesion turnover and cell migration (Yu et al., 2011). Further, p190RhoGEF is also able to activate RhoC at lamellipodia of migrating cells by controlling cofilin activity and thus the formation of the barbed end on actin filaments (Bravo-Cordero et al., 2011, 2013).

4.3. Rho protein networks and filopodia

Migrating cells also exhibit spike-like protrusions that extend beyond the leading edge of lamellipodia, which are known as filopodia (Fig. 3, bottom panel). Early studies have already identified Cdc42 to play a major role in filopodia formation (Nobes & Hall, 1995). Later, fibroblasts from Cdc42 knock-out mice were found unable to form filopodia while fibroblasts from Cdc42 GAP knock-out mice formed spontaneous filopodia (Yang, Wang, & Zheng, 2006), further establishing Cdc42 as the essential regulator of filopodia formation. Downstream of Cdc42, the formin mDia2 regulates actin nucleation and elongation of actin filaments (Ridley, 2011).

As discussed above, the cytoskeleton of lamellipodia consists of heavily branched actin filaments. In contrast, the cytoskeleton of filopodia is made up of straight, parallelly aligned actin filaments. Within filopodia, actin-bundling proteins tightly pack parallel actin filaments with their barbed ends facing toward the tip. Initiation of the actin filament formation is mediated by a nucleation complex. N-WASP functions as a nucleation promoting factor, which stimulates the ability of Arp2/3 to induce actin polymerization. In addition to N-WASP, formins such as mDia2, can also mediate actin nucleation. Studies on less well characterized Rho proteins have identified that RhoF stimulates the formation of filopodia, independent from the Cdc42/WASP/Arp2/3 pathway, but through interacting with mDia1 (Pellegrin & Mellor, 2005). Further, RhoD expression induces the formation of filopodia while silencing of RhoD decreases cell migration (Gad et al., 2012). In mesenchymal cells, Cdc42-induced and RhoD-induced filopodia are different in length, thickness, straightness and composition. This is suggested to be the result of focal adhesion formation in Cdc42-induced filopodia whereas focal adhesions collapse in the presence of activated RhoD (Koizumi et al., 2012).

The unidirectional organization of filopodial filaments allows molecular motors to transport various proteins to the tips where they can participate in filopodia maintenance and the establishment of cell–cell junctions, but also help and sensing of the local extracellular micro-environment for directional cues that underlie directional migration.

4.4. Rho protein networks in contraction and tail retraction

After cells have successfully polarized, formed lamellipodia and filopodia on the leading edge, and have formed strong focal adhesions anchoring the leading edge in place to the ECM, cells need to contract in order to move in the direction of the leading edge. For generating the contractile force, stress fibers are contracted by myosin II (Fig. 4). In short, at the trailing end RhoA regulates both actin polymerization and force generation via activation of the formin mDia and the kinase ROCK. ROCK phosphorylates and activates LIMK, which in turn inhibits the actin-depolymerizing factor cofilin. In addition, ROCK induces actomyosin contractility by inactivating MLCP (Narumiya & Watanabe, 2009). Simultaneously, at the trailing end, ROCK activates MLC2. The resulting force creates traction at the trailing end, thereby pulling the cell forward. Finally, the trailing end gets reattached to the ECM while at the same time at the leading edge, focal adhesion complexes are

Fig. 4. Plasticity of single cell migration. Plasticity in migration allows migrating tumor cells to adapt to changes in the tumor microenvironment. Mesenchymal cells require both the activity of Rac1 and Cdc42 for the turnover of actin filaments at the protruding edge, and the activation of RhoA/ROCK for generating the contractile force at the trailing end, where stress fibers are contracted by myosin II in order to pull the cell forward. For successful mesenchymal migration, the activity of RhoA, Rac1 and Cdc42 need to be spatiotemporally separated from one another. Indeed, subcellular domains where Rac1 is active show limited RhoA activity and vice versa, which is achieved by reciprocal interaction between Rac1/Cdc42 and RhoA. Downstream of Rac1 and Cdc42 activation, the ubiquitin ligase Smurf1 negatively regulates RhoA availability, while p190RhoGAP activation downregulates RhoA activity and PAK1 activation downregulates activity of RhoA GEFs. On the contrary, active ROCK inhibits the recruitment of β -Pix to focal adhesions, thus limiting Rac1 and Cdc42 activation. During amoeboid migration, cells show rounded morphology and use actomyosin contractility for propulsion. This type of migration is primarily dependent on cortical actin contractility, driven by RhoA and ROCK, and requires little activation of Rac1 and Cdc42. Amoeboid and mesenchymal migration are interchangeable. Generally, high activity of Rac1 and Cdc42 promote mesenchymal migration by inhibiting activation of RhoA/ROCK, while conversely, high activity of RhoA/ROCK promotes amoeboid migration by inactivating Rac1 and Cdc42 through ArhGAP22 and FilGAP. Therefore, inhibition of either pathway removes the block on the opposite pathway, thus inducing the opposite migratory phenotype.

disassembled and new protrusions are formed. Thus, the mesenchymal migratory cycle continues.

Interestingly, in the absence of Rho/ROCK signaling there appears to be a role for Cdc42 in regulating actomyosin contractility. Overexpression of ArhGEF9, a Cdc42 GEF, is commonly observed in hepatocellular carcinoma and correlates with a pro-migratory phenotype. Conversely, silencing of ArhGEF9 attenuates Cdc42 activation and restores an epithelial phenotype with a lower migratory capacity (Chen, Chan, et al., 2010). In fact, during mesenchymal cell movement of colorectal carcinoma cells, Cdc42 activation is necessary for actomyosin contractility through activation of the Cdc42 effector MRCK and subsequent MLC2 phosphorylation (Wilkinson, Paterson, & Marshall, 2005). Thus, while actomyosin contractility is

well-recognized to be regulated by RhoA and ROCK, these studies also suggest an important role for Cdc42 in polarized migration in addition to its well-known function in filopodia and invadopodia formation.

5. Spatiotemporal separation of Rho protein networks during polarized migration

In the traditional view, based almost exclusively on evidence gathered in 2D microenvironments, Rac1 is dominant at the leading edge (where it is required for actin filament extension, lamellipodia formation etc.), while RhoA dominant at the rear (where it mediates actomyosin contractility via ROCK (Ridley et al., 2003) (Fig. 4). More recent studies in cells in 3D environments has, however, revealed that

Rho GTPase signalling is far more complicated. The complexity of the role of Rho, Rac and Cdc42 in cancer cell migration is nicely illustrated by sphingosine-1-phosphate (S1P) and its ability to alter cell motility (Pyne & Pyne, 2010). Generally, S1P stimulates motility of cancer cells through the S1P receptor 1 and 3 known to couple to Rac1 and Cdc42 activation. On the other hand, S1P inhibits cancer cell motility via S1P receptor 2-dependent regulation of RhoA. Thus, the effect of S1P is dependent on the predominance of receptor subtype expression on the cancer cells. Therefore, for melanoma and glioblastoma cells that predominantly express S1P receptor 2, S1P functions as an inhibitor of cell motility through activating Rho while simultaneously inhibiting Rac1 (Arikawa et al., 2003). In fact, high levels of active Rho induce actomyosin-mediated retraction of lamellipodia and thereby inhibit mesenchymal migration. Generally, the involvement of Rho is different at the leading edge, where its activity needs to be reduced to allow for protrusion formation, compared to the tail, where its activity is required for contraction and retraction of the tail.

Subcellular domains where Rac is active show limited Rho activity and vice versa (Fig. 4). This distinctive patterning in Rho activity is achieved via the downstream Rac effector Par6, an important part of the so-called Par polarity complex. In primary epithelium, the cytoskeleton and associated Rho GTPases provide the initial polarity cue by directing the localization of the Par6/aPKC complex to the apical side of the cell and other polarity complexes to the lateral (e.g. Bazooka) and basolateral membranes (e.g. Dlg/Scrib/Lgl complex) (Chen & Zhang, 2013). The binding of Cdc42 to Par6 activates the associated aPKC (Horikoshi et al., 2009), which subsequently activates the Rho degrading ubiquitin ligase Smurf1, thereby reducing Rho availability at the leading edge (Wang et al., 2003). Alternatively, in response to integrin-engagement, Rac inactivates Rho via activating p190RhoGAP (Bustos, Forget, Settleman, & Hansen, 2008; Nimnual, Taylor, & Barsagi, 2003; Wildenberg et al., 2006). In addition, the Rac-activated kinase PAK1 phosphorylates, and thereby inactivates, a set of RhoA GEFs, such as PDZ-RhoGEF, GEF-H1, p115RhoGEF and NET1 (Alberts, Qin, Carr, & Frost, 2005; Barac et al., 2004; Callow, Zozulya, Gishizky, Jallal, & Smeal, 2005; Meiri et al., 2014; Nalbant, Chang, Birkenfeld, Chang, & Bokoch, 2009; Rosenfeldt, Castellone, Randazzo, & Gutkind, 2006; Zenke et al., 2004). However, while the role of RhoA at the trailing edge is well conserved in 3D cellular environments (Yamazaki, Kurisu, & Takenawa, 2009), it was suggested that the formation of protrusions at the leading edge of motile cells requires both Rac1 and RhoA activity in an oscillatory manner (Kim et al., 2015). In fact, the use of FRET-based biosensors revealed that Rho is also active at the leading edge (Pertz, Hodgson, Klemke, & Hahn, 2006), where it is activated immediately in front of Rac and Cdc42 in lamellipodia (Machacek et al., 2009). The recent development of optogenetic tools to alter subcellular distribution of RhoA to control cell and tissue mechanics will undoubtedly further strengthen our knowledge on subcellular domain of Rho GTPases during cell migration (Valon, Marin-Llaurado, Wyatt, Charras, & Treppe, 2017).

Active RhoA also limits Rac activation via ROCK through inhibition of the recruitment of β -Pix to focal adhesions, thereby locally limiting Rac activation (Kuo, Han, Hsiao, Yates, & Waterman, 2011; Sanz-Moreno et al., 2008). Interestingly, although RhoB has opposite effects compared to RhoA and RhoC on cancer progression, RhoB also affects Rac activity negatively. Loss of RhoB, which is often observed during lung cancer progression, results in the activation of Rac1 and EMT (Bousquet et al., 2015). However, the mechanism by which RhoB affects Rac1 activity is a multistep process and not a direct inhibitory effect of the one on the other. Here, downregulation of RhoB first results in the activation of Akt1 which further activates the Rac GEF Trio, thus increasing active Rac1. Thus, through mutually excluding cross-talk between RhoA and Rac, opposing functions in the regulation of focal adhesions, membrane protrusions and actomyosin contractility, are confined to subcellular compartments where their functions are required for effective cell migration.

6. Rho proteins in collective migration

Although it was generally assumed that cancer cells migrate as single cells, it has now become clear that cancer cells can migrate both collectively and individually and that cells dynamically and reversibly change their migratory behavior. At a cell biological level, most malignant cell types can invade as cohesive multicellular units in which cells migrate with intact cell-cell contacts and that loosening of cell-cell contacts is sufficient to permit this (Friedl & Gilmour, 2009). Mesenchymal migration of carcinoma cells is activated by extracellular factors that induce EMT. Within the tumor, where EMT inducing factors are less present, carcinoma cells migrate in such multicellular clusters, following tracks through the ECM created by cancer associated fibroblasts. By studying collective migration in a co-culture system of carcinoma cells and stromal fibroblasts, movement patterns of leading fibroblast were found to be driven by RhoA-dependent actomyosin contractility in concert with MMP activity, which creates tracks through the ECM. Interestingly, the trailing carcinoma cells use Cdc42-dependent actomyosin contractility, highlighting a new aspect of cooperation between Rho and Cdc42 (Gaggioli et al., 2007). While the leader cell of collective migration is often a cancer associated fibroblast, it could also be a cancer cell on the edge of an epithelial sheet. On the edge of a tumor, cells can undergo EMT, becoming leader cells that form membrane protrusions in the direction of cell movement, dependent on Rac and Cdc42 activity, and degrade ECM by secretion of proteases, similar to the mesenchymal movement described above. Cells that are further into a migrating sheet or strand maintain intact cell-cell junctions and do not undergo EMT. A correct front-rear polarity is important for collective cell migration and is maintained by upstream regulators of Rac such as the Scribble and Par polarity complexes. The Par complex consists of Par6, which upon Cdc42 binding recruits Par3 and aPKC. This complex is recruited to the leading edge where it regulates the activity of Rac via the Rac GEFs Tiam1 and Tiam2 (Chen & Macara, 2005; Nishimura et al., 2005; Wang et al., 2012). Importantly, while Rho/ROCK dependent actomyosin contractility is required for collective cell migration (Friedl, Wolf, & Zegers, 2014; Ridley, 2011; Zegers & Friedl, 2014), contractility needs to be limited to not disrupt cell-cell junctions. In addition to activating Rac, the Par complex also locally inhibits Rho near cell-cell junctions through regulating the localization of Rnd3, an atypical Rho GTPase devoid of GTP hydrolytic activity (Hidalgo-Carcedo et al., 2011), while it inhibits Rho at membrane protrusions through regulating Smurf1 (Wang et al., 2003). In pancreatic cancer cells, lower expression of Par3 correlates with a more invasive phenotype and lower patient survival. Thus, the loss of Par3 in pancreatic cancer might be the result of aberrant Rho activation and actomyosin contractility.

The Scribble complex localizes to the leading edge in response to integrin engagement. There, Scribble controls the activation Rac and PAK via the Rac GEF β -Pix (Bahri et al., 2010), which is required for collective cell migration (Omelchenko et al., 2014). However, on the rear edge, Scribble can be recruited to adherens junctions by associating with E-cadherin, where it contributes to maintenance of cell-cell junctional stability by regulating β -Pix, Rac and PAK, which is crucial for collective cell migration (Nola et al., 2011; Qin, Capaldo, Gumbiner, & Macara, 2005; Tay, Ng, & Manser, 2010; Yates et al., 2013). E-cadherin was considered to be the main cadherin involved in collective cell migration, but recently a key role for P-cadherin is emerging, driven by observations that P-cadherin depletion impairs collective cell migration in two and three dimensional *in vitro* experimental settings (Ng, Besser, Danuser, & Brugge, 2012; Nguyen-Ngoc et al., 2012). However, the role of P-cadherin has remained elusive until the observation that P-cadherin expression predicts the amount of intercellular force, while E-cadherin expression predicts the build-up of intercellular force (Bazellieres et al., 2015). Importantly, a recent study identified that P-cadherin recruits β -Pix and thereby activates Cdc42, which underlies the cellular reorganizations observed in collective cell

migration (Plutoni et al., 2016).

Together, these findings draw a picture in which activation of Rho GTPases needs to be finely tuned to allow for adequate actomyosin contractility between multiple cells. A recent study applied a screening approach in to identify which exchange factors are involved in coordinating intercellular communication during collective cell migration and identified several RhoGEFs that are involved in this process, most notably ArhGEF3, ArhGEF18, PDZ-RhoGEF and p190-RhoGEF (Zaritsky et al., 2017). The findings in the abovementioned study suggest that, although necessary for generation of motility forces, actomyosin contractility acts as an inhibitor for cell-to-cell communication. This implies that during collective migration, contractility must be balanced between generation of motile forces and transmission of mechanical guidance cues between cells (Zaritsky et al., 2017).

7. Rho proteins in amoeboid migration

Most studies on the molecular mechanisms that underlie migration and tumor cell invasion have been carried out in *in vitro* two dimensional settings. However, tumor cells invade in a three dimensional extracellular space where cell movement is limited by surrounding ECM. Tumor cells can exploit the ECM degrading machinery to overcome this barrier. Therefore, many therapeutic strategies have explored the possibilities to inhibit ECM degrading proteases, however, to date only weak beneficial effects have been observed in animal models and clinical trials alike. In response to inhibition of ECM degrading proteases, carcinoma cells can exploit a different type of motility that does not rely on degradation of ECM. This type of migration is known as amoeboid migration and is characterized by flexible shape changes and a rounded cell morphology. Although earlier studies have proposed this type of migration to be a widespread phenomenon in cancer cell migration, the notion that protease-independent single cell migration is only plausible when the ECM is devoid of collagen cross-links, has put this under debate. Therefore, Weiss and colleagues noted that the design of studies on amoeboid migration insufficiently reproduce structural characteristics of collagen networks (Sabeh, Shimizu-Hirota, & Weiss, 2009).

Amoeboid migrating cells use actomyosin contractility, stress fibers, and focal adhesions for propulsion, without the need for Rac/Cdc42-driven actin polymerization (Fig. 4). During amoeboid migration, RhoA/ROCK driven cortical actin contractility promotes the rapid remodeling of the cell cortex characteristic of this type of migration (Friedl & Wolf, 2003; Lammernann & Sixt, 2009; Wolf et al., 2003). Since an early observation on the role of ROCK in tumor cell motility (Itoh et al., 1999), many studies have reported that inhibition of ROCK activity leads to decreased tumor cell invasion and metastasis (de Toledo, Anguille, Roger, Roux, & Gadea, 2012; Jeong et al., 2012; Liu, Goldstein, Scepansky, & Rosenblatt, 2009; Zhu et al., 2011). The atypical Rnd family of Rho proteins are constitutively GTP-bound. Rnd1 and Rnd3 (also known as RhoE) contribute to loss of stress fibers and induce cell rounding, potentially by antagonizing Rho/ROCK-driven actomyosin contractility. Both Rnd1 and Rnd3 can interact with p190RhoGAP, thereby increasing the GAP activity of p190RhoGAP towards RhoA, resulting in decreased actomyosin contractility (Wennerberg et al., 2003). Such a process can be observed during formation and retraction of membrane blebs during amoeboid migration. A recent study elegantly demonstrates that in the early stages of bleb formation, Rnd3 localizes to the inner membrane of the blebs (Aoki et al., 2016). Here, the interaction with p190RhoGAP prevents the local activation of RhoA and actomyosin contractility. While the blebs grow in size, the concentration of Rnd3/p190RhoGAP complexes decreases, allowing to some degree of RhoA/ROCK activation. Subsequently, ROCK can phosphorylates Rnd3, thereby preventing the interaction with p190RhoGAP. This will eventually lead to RhoA/ROCK overcoming the Rnd3/p190RhoGAP brake on RhoA activation, and thereby promotes retraction of the bleb (Ikenouchi & Aoki, 2017).

To date, very little is known about the GEFs that regulate RhoA during amoeboid migration. GEF-H1, a RhoA GEF, is activated response to microtubule destabilization and promotes amoeboid migration (Eitaki, Yamamori, Meike, Yasui, & Inanami, 2012). Interestingly, the majority of cells depleted of another RhoA GEF, NET1, switch to mesenchymal cell migration as their means of motility (Carr, Zuo, Oh, & Frost, 2013), indicating that tumor cells bear some degree of plasticity in their means of migration and have the ability to switch from one type of single cell migration to the other.

8. Rho proteins regulate plasticity of single cell migration

For metastatic dissemination of tumor cells, plasticity of cell migration is a prerequisite. This allows migrating cells to adapt to changes in the tumor microenvironment (Sahai, 2007). As mentioned above, the amoeboid and mesenchymal types of migration are interchangeable (Fig. 4). The suppression or activation of certain molecular pathways allows cells to transition from one type to the other, known as mesenchymal-amoeboid transition (MAT) or amoeboid-mesenchymal transition (AMT). The first observations of the involvement of Rho proteins in AMT were from Sahai and colleagues, who have shown that silencing of Rho/ROCK in amoeboid migrating melanoma cells induces a switch to mesenchymal migration (Sahai & Marshall, 2003). In follow-up studies, they have demonstrated that ROCK regulates the formation of actomyosin bundles in the cell cortex, just behind the invading edge (Wyckoff, Pinner, Gschmeissner, Condeelis, & Sahai, 2006). Later, 3-phosphoinositide-dependent protein kinase 1 (PDK1) was shown to activate ROCK1 at the plasma membrane by competing with the atypical GTPase and negative ROCK1 regulator, Rnd3. As such, silencing of PDK1 results in impaired amoeboid migration and a mesenchymal phenotype (Pinner & Sahai, 2008).

Interestingly, just as in polarized migration, Rac and Rho negatively regulate each other and thereby regulate plasticity in migration. For example, active Rac promotes mesenchymal movement by activating WAVE2, thereby inhibiting the ROCK-dependent MLC2 phosphorylation required for amoeboid migration (Sanz-Moreno et al., 2008). Conversely, ROCK inactivates Rac by activating a Rac GAP, ArhGAP22 (Fig. 4). In addition, ROCK phosphorylates and thereby activates the Rac GAP FilGAP (Fig. 4) (Saito, Ozawa, Hibino, & Ohta, 2012). In breast carcinoma cells, depletion of FilGAP induces a mesenchymal phenotype; while conversely, overexpression of FilGAP induced an amoeboid phenotype. Since ROCK and Rac negatively regulate each other, inhibition of either pathway removes the block on the opposite pathway, thus promoting the opposite phenotype. Experimentally, such theory is further supported by studies on ROCK and Rac1 inhibition in different cancer cell models. However, while inhibition of ROCK in colorectal carcinoma cells induces AMT and inhibition of Rac1 in fibrosarcoma cells induces mesenchymal-amoeboid transition (MAT), inhibition of Rac1 in glioblastoma cells, which primarily exhibit mesenchymal migration, did not induce MAT but rather blocked overall migration (Yamazaki et al., 2009). This indicates that the effects of Rac1 and RhoA inhibition are dependent on the preferred type of migration exhibited by different cell types. Similar to Rac1, Cdc42 interacts with Rho/ROCK signaling and thereby regulates plasticity in cell migration. For example, cellular levels of RhoA are regulated by Smurf1. Cdc42 activates and recruits Smurf1 to the leading edge, thereby helping to maintain a mesenchymal phenotype via reduction of ROCK activity at the leading edge (Sahai, 2007). Inhibition of Cdc42 in mesenchymal cells induces Rho/ROCK-dependent MAT, while conversely, inhibition of Rho/ROCK resulted in AMT in amoeboid cells (Wilkinson et al., 2005). However, Cdc42 is also required for maintenance of amoeboid morphology and inhibition or knock-down of the DOCK10, a Cdc42 GEF, or the Cdc42 effectors N-WASP or PAK2 promotes AMT (Gadea, Sanz-Moreno, Self, Godi, & Marshall, 2008). Nonetheless, when Cdc42 is inhibited, both mesenchymal and amoeboid cell movement are blocked, indicating that Cdc42 is important in both manners of single

cell migration.

Although historically, the two ROCK isoforms have been viewed as redundant, recent observations show that the two isoforms of ROCK might have different functions (Newell-Litwa et al., 2015). Knockdown of ROCK1 was found to result in cell rounding and a decrease in cell size while cells with knockdown of ROCK2 maintained a spread and elongated morphology (Jerrell, Leih, & Parekh, 2017).

Observation like these underscore that regulation of plasticity of cell migration is far greater as initially might have been anticipated.

9. Rho proteins in mechanotransduction

For metastasis to occur, a series of sequential steps is required that ultimately result in the migration of a malignant cell from its primary site towards a new site where it could adhere and form a secondary tumor. These sequential steps are collectively known as the metastatic cascade or the invasion-metastasis cascade (Poste & Fidler, 1980; Valastyan & Weinberg, 2011). Early during the metastatic cascade, tumor cells invade locally through the surrounding ECM and stromal cell layers. However, the tissue architecture of normal epithelium provides a barrier to invasiveness that first must be overcome by malignant cells before they can develop a migratory phenotype. In this section we highlight the role of the ECM in the tumor microenvironment in promoting tumor progression and discuss how Rho GTPases help integrate the signals coming from the ECM and change tumor cell behavior.

Local invasion begins with tumor cells breaching the basement membrane. A compromised integrity of the basement membrane allows malignant cells to come in contact with stromal components, which results in aberrant cell polarity and invasion of stromal compartments by malignant cells (Cox & Ertler, 2011). ECM components are degraded by a large variety of proteolytic enzymes of which MMPs are the best studied and therapeutically under investigation (Butcher, Alliston, & Weaver, 2009; Vandenbroucke & Libert, 2014). Considering the importance of the ECM for cancer progression, correct understanding of the interplay between the ECM and cancer cells might hold the key to better cancer treatment and the molecular mechanisms that drive this are only recently beginning to emerge (Fang & Declerck, 2013). The physical microenvironment within a solid tumor differs from that of normal tissue in that there is increased mechanical compression due to tumor cell proliferation and increased deposition of stiff ECM (Paszek & Weaver, 2004). These changes in the tumor microenvironment alter the behavior of cancer cells through mechanotransduction pathways, which involve both the external environment and internal signaling (Huang & Ingber, 2005). Communication between the ECM and the cytoskeleton is mediated by conformational changes in proteins that respond to physical changes in the microenvironment, and translate these into chemical signals inside the cell (Ingber, 2006). Collectively, such proteins are known as mechanosensors of which integrins, are the most well studied in tumor progression (Guo & Giancotti, 2004).

The perceived force promotes malignant transformation and motility through modulation of and actin dynamics by Rho GTPases (McBeath, Pirone, Nelson, Bhadriraju, & Chen, 2004). Moreover, mechanical stimuli such as high matrix stiffness can induce the translocation of the transcriptional co-activator myocardin-related transcription factor A (MRTF-A) in a RhoA/ROCK-dependent manner (Fig. 2) (Zhao et al., 2007). In the cytosol, MRTF-A sequesters with monomeric G-actin but activation of RhoA by increased matrix stiffness induces polymerization of G-actin to form F-actin filaments, thereby setting MRTF-A free to translocate to the nucleus where it acts as a transcriptional cofactor for serum response factor (SRF)-mediated gene transcription (Guettler, Vartiainen, Miralles, Larijani, & Treisman, 2008; Miralles, Posern, Zaromytidou, & Treisman, 2003; Posern, Miralles, Guettler, & Treisman, 2004; Vartiainen, Guettler, Larijani, & Treisman, 2007). The importance of this pathway in tumor progression is

demonstrated by the observation that depletion of MRTF-A or SRF attenuates cell motility and invasion of breast carcinoma and melanoma cells (Hermann et al., 2016; Medjkane, Perez-Sanchez, Gaggioli, Sahai, & Treisman, 2009). In a similar fashion, a stiff ECM can induce nuclear accumulation of Yes-associated protein (YAP) (Fig. 2) (Aragona et al., 2013; Dupont et al., 2011), which is often hyperactivated in tumors (Harvey, Zhang, & Thomas, 2013; Johnson & Halder, 2014). Much like MRTF-A, YAP is a transcriptional co-activator of the transcriptional enhancer factor domain (TEAD)-containing transcription factors, although a number of other transcription factors have been reported to interact with YAP (Zhao, Lei, & Guan, 2008; Zhao, Ye, et al., 2008). YAP is a downstream mediator of the Hippo pathway and is functionally inhibited through phosphorylation by the large tumor suppressor kinases 1 and 2 (LATS1/2). Studies on YAP activation in cells exposed to a rigid ECM revealed that treatment with a RhoA inhibitor abolishes YAP nuclear accumulation while inhibition of LATS1/2 had no effect, indicating that RhoA and not LATS1/2 regulates YAP activation in mechanotransduction (Aragona et al., 2013; Dupont et al., 2011; Nardone et al., 2017). The discovery that RhoA is involved in activation of YAP was further extended upon by studies showing that YAP activation is regulated via GPCRs and downstream activation of RhoA (Mo, Yu, Gong, Brown, & Guan, 2012; Yu, Mo, & Guan, 2012). Importantly, expression of dominant negative RhoA was effective in blocked YAP activation, whereas constitutively active RhoA was sufficient to induce YAP nuclear translocation and tumor progression (Feng et al., 2014; Yu et al., 2012, 2014). A striking example of RhoA control over YAP nuclear translocation comes from the work on validating optogenetic tools to study RhoA in controlling cytoskeletal remodeling. Here, the authors show that by inducing RhoA membrane localization using a light sensitive subcellular localization switch, RhoA is cortically activated by RhoGEFs where it promotes cortical contractility (Valon et al., 2017). These changes in contractility are accompanied by a rapid and reversible nuclear YAP translocation. The reverse, in which RhoA can be inactivated by inducing mitochondrial localization using a light sensitive switch, is accompanied by a rapid and transient decrease in nuclear YAP localization. As such, optogenetic RhoA provides a new tool to locally study the mechanistic relationship between mechanical forces and YAP localization.

Interestingly, studies from Sahai and colleagues in cancer-associated fibroblasts have revealed that YAP is required for the acquirement of a stiff ECM in the tumor microenvironment (Calvo et al., 2013). Subsequently, this stiffening of the matrix can activate YAP, thus creating a feed-forward loop. Importantly, inhibition of ROCK was sufficient to break this loop (Calvo et al., 2011, 2013).

10. YAP in relation to Epac1, Rac1 and β -catenin

In the previous section, we have discussed the role of the Hippo pathway effector YAP in mechanotransduction. In recent years, knowledge on the complexity of YAP regulation has expanded with the identification that YAP is involved in other pathways that play important roles in cancer as well, especially GPCRs and the β -catenin pathway. YAP can be sequestered in the cytoplasm as part of the APC/Axin/GSK3 β /CK1 destruction complex that normally controls levels of free cytosolic β -catenin. Upon Wnt ligand-receptor engagement, cytoplasmic sequestration of YAP by this complex is blocked, leading to nuclear translocation of YAP as well as β -catenin stabilization (Azzolin et al., 2014; Imajo, Miyatake, Iimura, Miyamoto, & Nishida, 2012). Inside the nucleus, YAP can interact with β -catenin, thereby promoting β -catenin-dependent transcription (Fig. 2) (Heallen et al., 2011; Rosenbluh et al., 2012). On the other hand, DVL is an elementary factor in transducing the signal from a Wnt ligand binding to one of its receptors and thereby facilitates the Wnt/ β -catenin pathway. Cytoplasmic presence of YAP sequesters DVL in the cytoplasm, thereby suppressing Wnt signaling (Barry et al., 2013; Varelas et al., 2010). Thus, it is reasonable to suggest that cytoplasmic YAP functions as an inhibitor of

β -catenin, whereas nuclear YAP functions as a positive modulator of β -catenin-dependent transcription (Azzolin et al., 2014), suggesting that β -catenin might also play a role in mechanotransduction.

As discussed earlier, cells undergoing EMT show downregulation of adherens junctions and the junctional protein E-cadherin. The molecular pathways that are activated upon E-cadherin loss are not adequately understood. However, disruption of adherens junctions can lead to nuclear accumulation of the adherens junctional component β -catenin (Coluccia et al., 2006; Koenig, Mueller, Hasel, Adler, & Menke, 2006; Morali et al., 2001; Onder et al., 2008). β -catenin, in addition to its role in stabilizing adherens junctions, also functions as the primary mediator of the canonical Wnt pathway, an important developmental and oncogenic pathway (Clevers & Nusse, 2012). Regulation of Wnt signaling and the molecular machinery involved have multiple degrees of complexity and describing these in detail, is far beyond the scope of this review. It is important to note, that free cytosolic β -catenin is rapidly degraded by a multiprotein destruction complex when this pathway is off. However, once turned on, the destruction complex is disrupted, allowing β -catenin to translocate to the nucleus. Inside the nucleus, β -catenin acts as a transcriptional co-activator by associating with several transcription factors, most prominently to the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors. Nuclear translocation and induction of β -catenin-dependent gene transcription are often associated with enhanced tumor progression (Clevers & Nusse, 2012).

Interestingly, translocation of β -catenin can also occur independent of Wnt signaling. In this regard, studies focusing on the inflammatory mediator prostaglandin E_2 (PGE_2) and cyclooxygenase-2 (COX-2), which is often found overexpressed in carcinomas, are of particular interest. By binding to E-type prostanoid (EP) receptors, PGE_2 exerts its effects on cell behavior. The different EP receptors, EP1–4, are GPCRs that couple to distinct intracellular pathways based on the G-protein they associate with. It is now widely recognized that PGE_2 promotes tumor progression (Buchanan et al., 2006; Kim et al., 2011; Wang & Dubois, 2006). Importantly, downstream of PGE_2 binding to its receptors β -catenin and TCF-dependent transcriptional programs can be activated (Castellone, Teramoto, Williams, Druey, & Gutkind, 2005; Ho, Liang, Hung, & Liang, 2013; Shao, Jung, Liu, & Sheng, 2005), through a mechanism involving cyclic AMP (Brudvik, Paulsen, Aandahl, Roald, & Tasken, 2011; Jansen et al., 2015; Shao et al., 2005). In endothelial cells, the cyclic AMP regulated GEF Epac1 can function as an atypical GEF for Rac1, downstream of PGE_2 (Birukova et al., 2007, 2010; Maillet et al., 2003). Recent studies performed by our group on the role of β -catenin in EMT and migration of NSCLC cells, have further identified the involvement of the cyclic AMP regulated GEF Epac1 (Jansen et al., 2016). Herein, we show that PGE_2 induces EMT, nuclear β -catenin accumulation and transcription of β -catenin target genes. Downregulation, pharmacological inhibition, or expression of a mutant Epac1 with a defunct nuclear pore localization signal (Parnell, Smith, & Yarwood, 2015), abolishes the effects of PGE_2 on either β -catenin or EMT. Although we have not yet elaborated on the exact molecular mechanisms by which Epac1 is involved in β -catenin-dependent transcription, we anticipate that there is a critical involvement of Rho proteins (Fig. 2). Activation of Rac1 has been recognized as a driving factor in β -catenin stabilization, nuclear import, and target gene transcription (Fig. 2) (Wu et al., 2008). Although the underlying mechanisms leading to the nuclear β -catenin by Rac1 are not fully understood, it has been proposed that Rac1 facilitates nuclear import of β -catenin (Esufali & Bapat, 2004), thus opening up the possibility that PGE_2 promotes nuclear import through a mechanism involving Epac1-mediated activation of Rac1.

Over the last few years, experimental evidence for the involvement of Epac1 in cancer progression is beginning to emerge (Almahariq, Mei, & Cheng, 2015; Banerjee & Cheng, 2015; Parnell, Palmer, & Yarwood, 2015; Parnell, Smith, et al., 2015; Schmidt, Dekker, & Maarsingh, 2013). For example in melanoma cells, silencing

of Epac1 attenuates migration *in vitro* and metastasis *in vivo* (Baljinnyam et al., 2009, 2010, 2011, 2014; Gao et al., 2006). Similarly, in pancreatic adenocarcinoma, activation of Epac1 enhances migration while inhibition or silencing of Epac1 attenuates migration (Almahariq et al., 2013; Burdya et al., 2013). Indeed, mice deficient for Epac1 exhibit a lower level of metastasis as shown by *in vivo* imaging of xenografts (Almahariq, Chao, et al., 2015; Almahariq, Mei, et al., 2015). Seemingly on the contrary, pharmacological activation of Epac1 decreases cell migration in prostate cancer cells (Grandoch et al., 2009). However, later studies suggested that inhibition of migration was the result of nonspecific PKA activation rather than Epac activation as silencing of Epac had no effect on the Epac agonist-induced decrease in cell migration, while simultaneous application of the Epac agonist with a PKA inhibitor reversed the effect (Menon et al., 2012). In line with these findings, several studies in multiple different cancer cell lines have shown that PKA and Epac exert opposite effects on cell migration (Burdya et al., 2013; Lee, Lee, & Moon, 2014). Crosstalk between Epac1 and Rac1 in cancer cells has been described in cervical carcinoma and fibrosarcoma in which Epac1 activation results in enhanced migration in a Rac1-dependent manner (Harper et al., 2010; Lee, Lee, et al., 2014).

The Rac effector PAK1 has been shown in recent studies to induce β -catenin phosphorylation on serine 675, a residue that is once phosphorylated, stabilizes β -catenin and enhances nuclear β -catenin accumulation (Fig. 2) (Arias-Romero, Villamar-Cruz, Huang, Hoeflich, & Chernoff, 2013; Zhu et al., 2012). Thus, crosstalk between Epac1 and Rac1 could result in increased stability of free cytosolic β -catenin and nuclear accumulation.

11. Hypoxia-driven metastasis

Inside a tumor, carcinoma cells also undergo EMT, however in contrast to cells that are more in the periphery of the tumor, the transition from an epithelial-like to a mesenchymal-like cell depends on different cues. For example, inside the tumor, hypoxia drives EMT through regulation of the EMT transcriptional regulator Twist (Peinado & Cano, 2008; Yang et al., 2008). Therefore, the occurrence of hypoxia is linked to increased metastatic potential (Gilkes, Semenza, & Wirtz, 2014; Gilkes, Xiang, et al., 2014). Generally, hypoxia leads to the induction of the α subunit of the heterodimeric transcription factor Hif-1 (Brown & Wilson, 2004). Under conditions of abundant oxygen availability, cytosolic Hif1 α levels are under control by the sequential action of prolyl hydroxylases, which hydroxylate Hif1 α , and Von Hippel-Lindau protein (pVHL), an ubiquitin ligase that targets Hif1 α for proteasomal degradation. However, under hypoxic conditions, prolyl hydroxylases become inactive and Hif1 α can accumulate within the cytosol and translocate to the nucleus, where it forms a heterodimer with the β subunit and regulates transcriptional adaptations to the hypoxic environment.

When hepatocellular carcinoma cells are exposed to hypoxic conditions, Rac1 and Cdc42 are activated (Hirota & Semenza, 2001). Interestingly, expression of dominant negative Rac1 or Cdc42 prevents Hif1 α transcriptional activity, while expression of constitutively active Rac1 enhances Hif1 α transcriptional activity (Xue et al., 2006), while conversely, dominant negative Rac1 and Cdc42 increase the expression of pVHL and thereby decrease Hif1 α under hypoxia (Xue et al., 2006).

In addition, studies in renal cell carcinoma have revealed that under hypoxic conditions RhoA and ROCK are also involved in the regulation of pVHL (Turcotte, Desrosiers, & Beliveau, 2004), while on the other hand, Hif1 α regulates the expression and activity of RhoA and ROCK (Gilkes, Xiang, et al., 2014). Importantly, this regulation of RhoA and ROCK under hypoxia was found to promote the formation of focal adhesions, enhance actomyosin contractility and increases overall cell motility (Gilkes, Xiang, et al., 2014). The importance of this crosstalk between RhoA/ROCK and Hif1 α is nicely illustrated by a recent study showing that renal cell carcinoma cells, which are often deficient in

pVHL and consequently have high levels of Hif1 α , are synthetically lethal to pharmacological ROCK inhibition, a process which was dependent on upregulation of Hif1 α (Thompson, Landman, & Razorenova, 2017; Thompson, Nguyen, et al., 2017). Additionally, the stabilization of Hif1 α or overexpression also results in formation of invadopodia, which is accompanied by an increase in Cdc42 and β -Pix expression. When expression of β -Pix is reduced, the formation of invadopodia is decreased, suggesting the fundamental requirement for β -Pix in hypoxia-induced invadopodia formation (Md Hashim et al., 2013).

Similar to Rac1, Cdc42, and RhoA, expression of Rnd3 is also increased under hypoxic conditions, involving Hif1 α mediated transcriptional activity. Here, downregulation of Rnd3 abolishes hypoxia-induced cell migration via a mechanism involving the chemokine receptor C-X-C motif chemokine receptor 4 (CXCR4) (Feng et al., 2013), a GPCR that is often aberrantly expressed in malignant cells (Balkwill, 2004). Expression of both CXCR4 receptor and its ligand stromal cell derived factor-1 (SDF1) are under control by Hif1 α mediated transcription (Burger & Kipps, 2006). Indeed, tumors with inactivating mutations in pVHL express simultaneously high levels of SDF1 and CXCR4 (Staller et al., 2003; Zagzag et al., 2005). Functionally, binding of SDF1 to CXCR4 induces expression of genes involved in cell invasion, and as such mediate metastatic behavior of cancer cells (Teicher & Fricker, 2010). Ultimately, this enables tumor cells to escape from hypoxic areas by migrating towards a gradient of SDF1. Indeed, silencing of either CXCR4 or Hif1 α decreases migration of colorectal carcinoma cells (Romain et al., 2014). The mechanisms of Hif1 α /CXCR4 interaction are still under investigation, but recent reports suggest the involvement of PDZ-RhoGEF-mediated activation of RhoA (Guo et al., 2016; Struckhoff et al., 2013).

One of the best recognized target genes of Hif1 α is vascular endothelial growth factor (VEGF), one of the major growth factors regulating angiogenesis and as such, adaptation to hypoxia. Expression of VEGF is essential in cancer progression by affecting both endothelial cells as well as being part of autocrine signaling cascades involved in regulating important characteristics of cancer cells, including EMT (Goel & Mercurio, 2013). Early studies in breast cancer have shown that overexpression of RhoC increased VEGF expression and secretion (van Golen et al., 2002; van Golen, Wu, Qiao, Bao, & Merajver, 2000a, 2000b). Similarly, expression of constitutively active RhoC increases expression of VEGF in breast carcinoma (Wu, Wu, Kumar-Sinha, Chinnaiyan, & Merajver, 2004). Of particular interest are findings that show that RhoC is crucial for the interaction between malignant cells and endothelial cells, a process which is required for transendothelial cell migration, underlying metastasis (Reymond, Im, et al., 2012; Reymond et al., 2015; Reymond, Riou, & Ridley, 2012).

Activation of Hif1 α is linked to EMT in cancer cells and as such downregulation of Hif1 α suppresses cell migration and invasion (Krishnamachary et al., 2003; Sahlgren, Gustafsson, Jin, Poellinger, & Lendahl, 2008). Earlier we discussed the involvement of PGE₂, Rho proteins and its effectors in dissociation of the adherens junctional complex and in activating β -catenin-dependent transcription. In this regard, studies on β -catenin transcriptional activity under hypoxic conditions are of particular interest. Expression of COX-2 is regulated by Hif1 α through Hif1 α binding to the hypoxia response elements (HRE) in the COX-2 promoter region, leading to increased PGE₂ levels (Csiki et al., 2006; Kaidi, Qualtrough, Williams, & Paraskeva, 2006). Silencing of Hif1 α negatively affects stability and transcriptional activity of β -catenin and results in the restoration of β -catenin to E-cadherin at the adherens junctions (Santoyo-Ramos, Likhatcheva, Garcia-Zepeda, Castaneda-Patlan, & Robles-Flores, 2014). Moreover, silencing of β -catenin reversed the hypoxia-induced EMT features and attenuated invasion and metastasis (Liu et al., 2010), indicating that the interaction between β -catenin and Hif1 α allows for the increased invasive capacity of carcinoma cells (Zhang et al., 2013).

On the other hand, hypoxia inhibits β -catenin/TCF complex formation and thereby inhibits β -catenin transcriptional activity (Kaidi,

Williams, & Paraskeva, 2007). It is believed that this is the consequence of Hif1 α and TCF competing for direct binding of β -catenin. The interaction between Hif1 α and β -catenin occurs at the promoter region of Hif1 α target genes and as such, β -catenin can promote Hif1 α -mediated gene transcription, promoting cellular adaptation to hypoxia. Conversely, β -catenin associating with Hif1 α may override the normal proliferative effect of PGE₂ on carcinoma cells, which is dependent on TCF-mediated gene transcription, and shifts towards Hif1 α -mediated adaptation to hypoxia (Greenhough et al., 2009).

Recently, it was found that hypoxia results in the phosphorylation of β -catenin at Y654 in a Src-dependent manner (Xi et al., 2013). All β -catenin phosphorylated at this residue was found complexed with Hif1 α and it was demonstrated that this β -catenin phosphorylation was required for Src to promote Hif1 α transcriptional activity and hypoxia-induced EMT. In mice with hypoxic pancreatic carcinoma, Hif1 α and pY654- β -catenin complexes accumulate which coincided with an invasive phenotype (Xi et al., 2013).

A series of recent studies on the nuclear orphan receptor Nur77, which is directly regulated by Hif1 α under hypoxic conditions (Choi, Park, Kang, Liu, & Youn, 2004), have identified how Hif1 α and β -catenin can enhance each other's activity under hypoxic conditions. Hypoxia increases expression of both β -catenin and Nur77 in colorectal carcinoma cells, where expression of Nur77 correlates with invasive potential and MMP9 expression and shows an inverse correlation with E-cadherin expression (Wang et al., 2014). Interestingly, Nur77 and β -catenin confer a mutual feedback mechanism in which active β -catenin increases the expression of Nur77 through Hif1 α , and hypoxia-induced Nur77 enhances β -catenin transcriptional activation. The interaction between β -catenin, Hif1 α and Nur77 regulates hypoxia-induced EMT and invasive capacity of colorectal cancer cells (To, Zeng, Zeng, & Wong, 2014).

Together, these findings support the interesting interplay between hypoxia and inflammatory mediators (e.g. PGE₂), that together with intracellular signaling mediators such as Rho GTPases and β -catenin, determine cellular adaptations to the hypoxic environment, including expression of angiogenic factors that stimulate the formation of new vasculature to overcome the hypoxic environment. In the context of cancer metastasis, this can become problematic, as the newly formed vasculature often displays poor maturation and is very leaky and easily accessible for disseminating cells. As the abovementioned molecular interactions under hypoxic conditions are also driving factors in EMT and the adoption of a pro-migratory, pro-invasive phenotype, these might ultimately be primary driving mechanisms of metastasis (Weis & Cheresh, 2011).

12. Therapeutic perspective: targeting Rho protein networks in cancer

As outlined in this review, Rho GTPase pathways regulate multiple aspects important for dissemination of malignant cells. Therefore, key components in these pathways are attractive targets for therapeutic intervention. Here, we highlight possible pharmacological possibilities to interfere with deregulated Rho GTPase networks in cancer.

All members of the small GTPase superfamily are generally considered to be undruggable as they lack stable cavities beside the nucleotide binding pockets. The bacterial exoenzyme C3 inhibits RhoA, RhoB and RhoC by adding an ADP-ribose moiety on asparagine residues, thereby inactivating the small GTPase (Vogelsgesang, Pautsch, & Aktories, 2007). Because of their high specificity for Rho GTPases, C3-like exoenzymes are widely used as Rho inhibitors in fundamental and preclinical studies. However, C3-like ADP-ribosyltransferases have poor cell accessibility and introduce covalent modifications, thus it is unlikely these compounds have therapeutic applications.

To elicit their effects, Rho GTPases have to be anchored to the plasma membrane by means of isoprenylation. Statins are inhibitors of

3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the key enzyme for cholesterol biosynthesis. By inhibiting HMGCR, statins deplete the cell of the lipids required for membrane anchoring and thus induce the retention of Rho GTPases in the cytosol, thereby inhibiting their function (Gazzerro et al., 2012). Therapeutically, statins are widely used in cardiovascular disease, but statins also display promising anti-cancer capabilities by preventing tumor angiogenesis and metastasis (Demierre, Higgins, Gruber, Hawk, & Lippman, 2005; Thurnher, Nussbaumer, & Gruenbacher, 2012; Yeganeh et al., 2014).

As different Rho GTPases are activated in response to distinct signals by specific GEFs, targeting GEFs could provide better selectivity. One example targeting a specific RhoGEF is the development of inhibitors for the RhoA GEF LARG. The compound Y16 was identified through high-throughput screening and was shown to inhibit RGS-containing RhoGEFs. In breast cancer cells, Y216 inhibits RhoA and attenuated mammary sphere formation in breast cancer cells (Evelyn et al., 2009; Shang et al., 2013). Another GEF inhibitor, Rhosin, prevents binding of RhoA to multiple RhoGEFs, including LARG, Dbl, p115RhoGEF and PDZ-RhoGEF. Recently, it was shown that Rhosin blocks chemotherapy resistance in cancer stem cells by inhibition of RhoA (Yoon et al., 2016).

Considering its role in regulating cytoskeletal dynamics, the Rho kinase effector ROCK has been particularly implicated in tumor metastasis. The ROCK inhibitors Y27632 and fasudil have been extensively studied in cancer and beneficial effects have been observed in many types of cancers (Kale et al., 2015; Rath & Olson, 2012). This supports the development of selective ROCK inhibitors for cancer therapy. Several recently developed ROCK inhibitors have been proven to be more potent and/or selective compared to Y27632 or fasudil. For example, OXA-06 and PT262 inhibit migration and invasion of NSCLC cells (Tsai et al., 2011; Vigil et al., 2012), RKI-1447 inhibits breast cancer cell invasion (Patel et al., 2012; Patel, Liu, Wang, Li, & Sebt, 2014), and CCT129253 and AT13148 impaired melanoma cell invasion and metastasis *in vivo* (Sadok et al., 2015). However, targeting ROCK also comes with the liability of playing an important role in the contraction of blood vessels. As such, ROCK inhibitors have a high likelihood of inducing hypotension (Kale et al., 2015). Further, inhibition of ROCK can also increase survival of cancer stem cells (Castro, Maurer, Hebbard, & Oshima, 2013; Ohata et al., 2012). Importantly, and rather counterintuitive, there are also reports of ROCK inhibition resulting in increased pro-invasive behavior (Bhandary et al., 2015; Zhang et al., 2011). As such, despite the significant promise ROCK inhibitors exhibited in experimental studies, no clinical trials on ROCK inhibition in solid tumors have been completed to date although a phase 1 trial on AT13148 was initiated in 2012 (NCT01585701) and is expected to complete in 2017. For a more extensive overview of ROCK inhibitors we refer to (Loirand, 2015).

Inhibition of Rac signaling has provided promising *in vitro* results. The Rac inhibitor NSC23766 effectively inhibits Rac1 activation by blocking a surface groove that is critical for the interaction with the Rac GEFs Tiam1 and Trio (Gao, Dickerson, Guo, Zheng, & Zheng, 2004). In lymphomas, NSC23766 blocks dissemination in xenograft studies (Thomas et al., 2007). However, the potency of NSC23766 is insufficient to be considered for clinical application. Other, more potent, inhibitors have been developed using NSC23766 as a starting point, such as EHOp-016, which blocks the interaction between the GEF Vav2 and Rac1 (Montalvo-Ortiz et al., 2012). In addition, non-competitive inhibitors have been developed, such as EHT 1864 (Shutes et al., 2007). Both EHOp-016 and EHT 1864 decrease migration of metastatic breast cancer cells (Katz et al., 2012; Montalvo-Ortiz et al., 2012). Several novel compounds that target both activation of Rac1 and Cdc42 have been developed in recent years, using the structure of NSC23766 as a starting point. For example, AZA1 inhibits cellular migration of prostate cancer cells in xenograft models via inhibition of Rac1 and Cdc42 but not RhoA GTPase activity (Zins, Lucas, Reichl, Abraham, & Aharinejad, 2013), while a Cdc42-selective small-molecule

inhibitor, AZA197, suppresses colorectal carcinoma cell migration (Zins, Gunawardhana, Lucas, Abraham, & Aharinejad, 2013; Zins, Lucas, et al., 2013). Other small molecule modulators targeting Cdc42 interaction with the GEF intersectin have also been developed such as Casin (Florian et al., 2012) and ZCL278, which suppresses actin-based motility and migration in prostate cancer (Friesland et al., 2013). Recently, ML141 was identified as a noncompetitive allosteric inhibitor with excellent selectivity towards Cdc42 (Hong et al., 2013) and was shown to interfere with collective migration downstream of P-cadherin/ β -Pix (Plutoni et al., 2016).

Downstream of Rac and Cdc42, PAKs are increasingly recognized as plausible therapeutic targets. To date, several PAK inhibitors have been developed. The non-competitive inhibitor IPA-3 was designed to target PAKs independent of ATP by adding a sulfhydryl moiety to the N-terminal region, thereby promoting the inhibitory conformation (Deacon et al., 2008). IPA-3 reduces tumor progression in hepatocellular carcinoma and prostate cancer xenograft models (Al-Azayzih, Missaoui, Cummings, & Somanath, 2016; Wong et al., 2013). However, the downside of promoting the inhibitory conformation of PAKs is that IPA-3 is ineffective in inhibiting pre-activated PAK. Thus, the use IPA-3 against cancer cells with elevated PAK activity may not be effective. In addition, the continuous reduction of the sulfhydryl moiety makes IPA-3 unsuitable for further clinical development. Recently, new 1,4-naphthohydroquinone-based compounds have been identified that allosterically interact with PAKs but do not induce the formation of covalent modifications (Kim et al., 2016). The only ATP-competitive PAK inhibitor that has progressed to a clinical trial is PF-3758309 (Murray et al., 2010). Although it remains difficult to ascribe the effects solely to PAK inhibition due to its poor target selectivity, the compound showed promising results and *in vitro* and *in vivo* studies in preclinical cancer models (Bradshaw-Pierce et al., 2013; Chow et al., 2012; Murray et al., 2010; Pitts et al., 2013), however, due to undesirable pharmacokinetics, the lack of a dose-response relationship, and adverse effects, the trial was prematurely terminated. More recently, the pyridopyrimidinone FRAX597 was identified by high-throughput screening as a potent inhibitor of PAKs and displays potent anti-tumor activity *in vitro* and *in vivo* (Chow et al., 2012, 2015; Licciulli et al., 2013; Mortazavi et al., 2015; Yeo et al., 2016). Other compounds identified in these screens, FRAX486 and FRAX1036, similarly exhibit excellent PAK1 potency, however all of these compounds demonstrated poor permeability and adverse inhibition of hERG potassium channels. Addressing these issues, the G-5555 compound was developed based on the structure of FRAX1036 (Ndubaku et al., 2015). While the compound shows desirable *in vivo* pharmacokinetics and demonstrated excellent *in vitro* growth inhibition in cancer cell lines, a xenograft study revealed that G-5555 was associated with acute cardiovascular toxicity (Rudolph et al., 2016), suggesting that G-5555 is not suitable for further clinical development. Several other ATP-competitive PAK1 inhibiting compounds have recently been disclosed (McCoull et al., 2016), but have until now not been studied as anticancer agents.

The role of the cyclic AMP regulated exchange factor Epac1 is emerging in cancer (Almahariq, Mei, et al., 2015; Banerjee & Cheng, 2015; Parnell, Palmer, et al., 2015; Parnell, Smith, et al., 2015; Schmidt et al., 2013). Interestingly, in cancer cell migration, activation of Epac1 can be linked to activation of Rac. As such, Epac1 could prove to be an interesting target for the treatment of cancer metastasis. Considerable interest has recently been given to the development of inhibitors of Epac1. By high-throughput screening for Epac antagonists, the Cheng and colleagues identified several novel Epac subtype-specific inhibitors (Almahariq et al., 2013; Chen, Tsalkova, et al., 2012; Tsalkova, Mei, & Cheng, 2012; Tsalkova, Mei, Li, et al., 2012). Especially the ESI-09, which inhibits both Epac subtypes, has high bioavailability and no toxicity when administered *in vivo* to mice (Almahariq, Chao, et al., 2015; Almahariq, Mei, et al., 2015; Gong et al., 2013; Yan et al., 2013; Zhu et al., 2015). Another Epac antagonist, CE3F4, shows high selectivity for Epac1 compared to Epac2 (Courilleau et al., 2012;

Courilleau, Bouyssou, Fischmeister, Lezoualc'h, & Blondeau, 2013). The development of more specific Epac subtype inhibitors is currently underway (Wang et al., 2017). Observations from our group show that this inhibitor attenuates PGE₂-induced EMT and migration of NSCLC cells in a β -catenin-dependent manner (Jansen et al., 2016). Thus, the notion put forward earlier that Rho proteins, Epac1 and β -catenin cooperate in driving carcinoma cell migration might be a therapeutically targetable interaction that could decrease dissemination of malignant cells. While further investigation is required to better understand the role of Epac1 in cancer cell migration, both *in vitro* and *in vivo*, there is substantial evidence suggesting that Epac1 inhibitors might provide a novel approach for the treatment of cancer metastasis (Almahariq, Mei, et al., 2015).

This summary of available strategies to target Rho protein networks on several levels, demonstrates that considerable efforts are underway to develop novel tools that greatly support research on Rho proteins in the context of cell migration and cancer metastasis and as such of undeniable value. However, considering the importance of these signaling pathways in cancer, a surprisingly low number of these compounds have been developed beyond an early preclinical stage. Poor pharmacokinetics and target specificity, as well as the often very context dependent and ambivalent findings, are seriously limiting chances to develop these tools into a clinical setting. Here, we have highlighted how changes in Rho protein networks often result in changes in cell-cell contacts and cell-ECM contacts and, thereby, in perceived cellular force. These changes often result in abrogated activation and localization of secondary signaling intermediates that have the ability to affect transcriptional programs, thereby translating short-term changes in cellular dynamics in long-lasting effects and as such could be interesting therapeutic targets in cancer. Two of these intermediates have gained a lot of attention over the past decade, namely β -catenin (Clevers & Nusse, 2012) and YAP (Moroishi, Hansen, & Guan, 2015; Zanconato, Cordenonsi, & Piccolo, 2016). Of the plenty of the pharmacological tools inhibiting β -catenin interacting with TCF or other transcriptional co-activators, one made it recently to a phase 1 clinical trial. Based on the promising results in preclinical cancer models of ICG-001, an inhibitor of the β -catenin/CBP interaction (Le, McDermott, & Jimeno, 2015; Masuda, Sawa, & Yamada, 2015), the second generation β -catenin/CBP antagonist PRI-724 was developed of which the clinical phase I trial in advanced solid tumors has been carried out (NCT01302405). Although the trial was terminated due to low patient enrollment, it revealed that PRI-724 has an acceptable toxicity (Lenz & Kahn, 2014) and as such, trials in subjects with acute and chronic myelogenous leukemia (AML/CML) are underway (NCT01606579). On the other hand, targeting of transcriptional regulation by YAP has proven to be difficult with verteporfin being the only compound able to inhibit the physical association between YAP and TEAD (Liu-Chittenden et al., 2012), although results obtained from a recently developed peptide that mimics VGLL4, a TEAD interacting protein, are encouraging (Jiao et al., 2014). However, one of the most promising therapeutic strategies going forward, might be tankyrase inhibitors, such as XAV939, G007-LK, and JW55. These compounds have been proven effective in inhibiting both β -catenin-mediated and YAP/TEAD-mediated gene transcription in cancer (S. M. Huang et al., 2009; Wang et al., 2015), and have excellent pharmacokinetic characteristic in mice (Lau et al., 2013; Waaler et al., 2012).

Acknowledgment

We would like to thank the Netherlands Organization for Scientific Research (Vidi grant: 016.116.309), the Van der Meer-Boerema Foundation and the Groningen University Institute for Drug Exploration (GUIDE) for financial support.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

- Aittaleb, M., Boguth, C. A., & Tesmer, J. J. (2010). Structure and function of heterotrimeric G protein-regulated rho guanine nucleotide exchange factors. *Molecular Pharmacology*, 77(2), 111–125.
- Akhtar, N., & Hotchin, N. A. (2001). RAC1 regulates adherens junctions through endocytosis of E-cadherin. *Molecular Biology of the Cell*, 12(4), 847–862.
- Al-Azayzih, A., Missaoui, W. N., Cummings, B. S., & Somanath, P. R. (2016). Liposome-mediated delivery of the p21 activated kinase-1 (PAK-1) inhibitor IPA-3 limits prostate tumor growth in vivo. *Nanomedicine: Nanotechnology, Biology and Medicine*, 12(5), 1231–1239.
- Alberts, A. S., Qin, H., Carr, H. S., & Frost, J. A. (2005). PAK1 negatively regulates the activity of the rho exchange factor NET1. *The Journal of Biological Chemistry*, 280(13), 12152–12161.
- Almahariq, M., Chao, C., Mei, F. C., Hellmich, M. R., Patrikeev, I., Motamedi, M., et al. (2015). Pharmacological inhibition and genetic knockdown of exchange protein directly activated by cAMP 1 reduce pancreatic cancer metastasis in vivo. *Molecular Pharmacology*, 87(2), 142–149.
- Almahariq, M., Mei, F. C., & Cheng, X. (2015). The pleiotropic role of exchange protein directly activated by cAMP 1 (EPAC1) in cancer: Implications for therapeutic intervention. *Acta Biochimica et Biophysica Sinica*, 48(1), 75–81.
- Almahariq, M., Tsalkova, T., Mei, F. C., Chen, H., Zhou, J., Sastry, S. K., et al. (2013). A novel EPAC-specific inhibitor suppresses pancreatic cancer cell migration and invasion. *Molecular Pharmacology*, 83(1), 122–128.
- Anastasiadis, P. Z., & Reynolds, A. B. (2001). Regulation of rho GTPases by p120-catenin. *Current Opinion in Cell Biology*, 13(5), 604–610.
- Aoki, K., Maeda, F., Nagasako, T., Mochizuki, Y., Uchida, S., & Ikenouchi, J. (2016). A RhoA and Rnd3 cycle regulates actin reassembly during membrane blebbing. *Proceedings of the National Academy of Sciences of the United States of America*, 113(13), E1863–71.
- Aragona, M., Panciera, T., Manfrin, A., Giulitti, S., Michielin, F., Elvassore, N., et al. (2013). A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. *Cell*, 154(5), 1047–1059.
- Arias-Romero, L. E., Villamar-Cruz, O., Huang, M., Hoefflich, K. P., & Chernoff, J. (2013). Pak1 kinase links ErbB2 to beta-catenin in transformation of breast epithelial cells. *Cancer Research*, 73(12), 3671–3682.
- Arikawa, K., Takuwa, N., Yamaguchi, H., Sugimoto, N., Kitayama, J., Nagawa, H., et al. (2003). Ligand-dependent inhibition of B16 melanoma cell migration and invasion via endogenous SIP2 G protein-coupled receptor requirement of inhibition of cellular RAC activity. *The Journal of Biological Chemistry*, 278(35), 32841–32851.
- Asnaghi, L., Vass, W. C., Quadri, R., Day, P. M., Qian, X., Braverman, R., et al. (2010). E-cadherin negatively regulates neoplastic growth in non-small cell lung cancer: Role of rho GTPases. *Oncogene*, 29(19), 2760–2771.
- Azzolin, L., Panciera, T., Soligo, S., Enzo, E., Bicciato, S., Dupont, S., et al. (2014). YAP/TAZ incorporation in the beta-catenin destruction complex orchestrates the wnt response. *Cell*, 158(1), 157–170.
- Bahri, S., Wang, S., Conder, R., Choy, J., Vlachos, S., Dong, K., et al. (2010). The leading edge during dorsal closure as a model for epithelial plasticity: Pak is required for recruitment of the scribble complex and septate junction formation. *Development (Cambridge, England)*, 137(12), 2023–2032.
- Baljinnyam, E., De Lorenzo, M. S., Xie, L. H., Iwatsubo, M., Chen, S., Goydos, J. S., et al. (2010). Exchange protein directly activated by cyclic AMP increases melanoma cell migration by a Ca²⁺-dependent mechanism. *Cancer Research*, 70(13), 5607–5617.
- Baljinnyam, E., Iwatsubo, K., Kurotani, R., Wang, X., Ulucan, C., Iwatsubo, M., et al. (2009). Epac increases melanoma cell migration by a heparan sulfate-related mechanism. *American Journal of Physiology. Cell Physiology*, 297(4), C802–C813.
- Baljinnyam, E., Umemura, M., Chuang, C., De Lorenzo, M. S., Iwatsubo, M., Chen, S., et al. (2014). Epac1 increases migration of endothelial cells and melanoma cells via FGF2-mediated paracrine signaling. *Pigment Cell & Melanoma Research*, 27(4), 611–620.
- Baljinnyam, E., Umemura, M., De Lorenzo, M. S., Iwatsubo, M., Chen, S., Goydos, J. S., et al. (2011). Epac1 promotes melanoma metastasis via modification of heparan sulfate. *Pigment Cell & Melanoma Research*, 24(4), 680–687.
- Balkwill, F. (2004). Cancer and the chemokine network. *Nature Reviews. Cancer*, 4(7), 540–550.
- Banerjee, U., & Cheng, X. (2015). Exchange protein directly activated by cAMP encoded by the mammalian rapgef3 gene: Structure, function and therapeutics. *Gene*, 570(2), 157–167.
- Barac, A., Basile, J., Vazquez-Prado, J., Gao, Y., Zheng, Y., & Gutkind, J. S. (2004). Direct interaction of p21-activated kinase 4 with PDZ-RhoGEF, a G protein-linked rho guanine exchange factor. *The Journal of Biological Chemistry*, 279(7), 6182–6189.
- Barry, E. R., Morikawa, T., Butler, B. L., Shrestha, K., de la Rosa, R., Yan, K. S., et al. (2013). Restriction of intestinal stem cell expansion and the regenerative response by YAP. *Nature*, 493(7430), 106–110.
- Bazellieres, E., Conte, V., Elosegui-Artola, A., Serra-Picamal, X., Bintanel-Morcillo, M., Roca-Cusachs, P., et al. (2015). Control of cell-cell forces and collective cell dynamics by the intercellular adhesions. *Nature Cell Biology*, 17(4), 409–420.
- Bellovin, D. I., Simpson, K. J., Danilov, T., Maynard, E., Rimm, D. L., Oettgen, P., et al. (2006). Reciprocal regulation of RhoA and RhoC characterizes the EMT and identifies RhoC as a prognostic marker of colon carcinoma. *Oncogene*, 25(52), 6959–6967.
- Bernstein, B. W., & Bamberg, J. R. (2010). ADF/cofilin: A functional node in cell biology. *Trends in Cell Biology*, 20(4), 187–195.
- Berx, G., & van Roy, F. (2009). Involvement of members of the cadherin superfamily in cancer. *Cold Spring Harbor Perspectives in Biology*, 1(6), a003129.

- Bhandary, L., Whipple, R. A., Vitolo, M. I., Charpentier, M. S., Boggs, A. E., Chakrabarti, K. R., et al. (2015). ROCK inhibition promotes microtentacles that enhance re-attachment of breast cancer cells. *Oncotarget*, 6(8), 6251–6266.
- Birchmeier, W., & Behrens, J. (1994). Cadherin expression in carcinomas: Role in the formation of cell junctions and the prevention of invasiveness. *Biochimica et Biophysica Acta*, 1198(1), 11–26.
- Birukova, A. A., Burdette, D., Moldobaeva, N., Xing, J., Fu, P., & Birukov, K. G. (2010). Rac GTPase is a hub for protein kinase A and epac signaling in endothelial barrier protection by cAMP. *Microvascular Research*, 79(2), 128–138.
- Birukova, A. A., Zagranichnaya, T., Fu, P., Alekseeva, E., Chen, W., Jacobson, J. R., et al. (2007). Prostaglandins PGE(2) and PGI(2) promote endothelial barrier enhancement via PKA- and Epac1/Rap1-dependent rac activation. *Experimental Cell Research*, 313(11), 2504–2520.
- Bisson, N., Wedlich, D., & Moss, T. (2012). The p21-activated kinase Pak1 regulates induction and migration of the neural crest in xenopus. *Cell Cycle (Georgetown, Texas)*, 11(7), 1316–1324.
- Bousquet, E., Calvayrac, O., Mazieres, J., Lajoie-Mazenc, I., Boubekeur, N., Favre, G., et al. (2015). RhoB loss induces Rac1-dependent mesenchymal cell invasion in lung cells through PP2A inhibition. *Oncogene*, 35(14), 1760–1769.
- Bradshaw-Pierce, E. L., Pitts, T. M., Tan, A. C., McPhillips, K., West, M., Gustafson, D. L., et al. (2013). Tumor P-glycoprotein correlates with efficacy of PF-3758309 in vitro and in vivo models of colorectal cancer. *Frontiers in Pharmacology*, 4, 22.
- Braun, A. C., & Olayioye, M. A. (2015). Rho regulation: DLC proteins in space and time. *Cellular Signalling*, 27(8), 1643–1651.
- Bravo-Cordero, J. J., Oser, M., Chen, X., Eddy, R., Hodgson, L., & Condeelis, J. (2011). A novel spatiotemporal RhoC activation pathway locally regulates cofilin activity at invadopodia. *Current Biology: CB*, 21(8), 635–644.
- Bravo-Cordero, J. J., Sharma, V. P., Roh-Johnson, M., Chen, X., Eddy, R., Condeelis, J., et al. (2013). Spatial regulation of RhoC activity defines protrusion formation in migrating cells. *Journal of Cell Science*, 126(Pt 15), 3356–3369.
- Briggs, M. W., Li, Z., & Sacks, D. B. (2002). IQGAP1-mediated stimulation of transcriptional co-activation by beta-catenin is modulated by calmodulin. *The Journal of Biological Chemistry*, 277(9), 7453–7465.
- Brown, J. M., & Wilson, W. R. (2004). Exploiting tumour hypoxia in cancer treatment. *Nature Reviews. Cancer*, 4(6), 437–447.
- Brudvik, K. W., Paulsen, J. E., Aandahl, E. M., Roald, B., & Tasken, K. (2011). Protein kinase A antagonist inhibits beta-catenin nuclear translocation, c-myc and COX-2 expression and tumor promotion in apc(min/+) mice. *Molecular Cancer*, 10 (149–4598–10–149).
- Buchanan, F. G., Gorden, D. L., Matta, P., Shi, Q., Matrisian, L. M., & DuBois, R. N. (2006). Role of beta-arrestin 1 in the metastatic progression of colorectal cancer. *Proceedings of the National Academy of Sciences of the United States of America*, 103(5), 1492–1497.
- Burdys, A., Conant, A., Haynes, L., Zhang, J., Jalink, K., Sutton, R., et al. (2013). cAMP inhibits migration, ruffling and paxillin accumulation in focal adhesions of pancreatic ductal adenocarcinoma cells: Effects of PKA and EPAC. *Biochimica et Biophysica Acta*, 1833(12), 2664–2672.
- Burger, J. A., & Kipps, T. J. (2006). CXCR4: A key receptor in the crosstalk between tumor cells and their microenvironment. *Blood*, 107(5), 1761–1767.
- Bustos, R. I., Forget, M. A., Sletten, J. E., & Hansen, S. H. (2008). Coordination of rho and rac GTPase function via p190B RhoGAP. *Current Biology: CB*, 18(20), 1606–1611.
- Butcher, D. T., Alliston, T., & Weaver, V. M. (2009). A tense situation: Forcing tumour progression. *Nature Reviews. Cancer*, 9(2), 108–122.
- Callow, M. G., Zozulya, S., Gishizky, M. L., Jallal, B., & Smeal, T. (2005). PAK4 mediates morphological changes through the regulation of GEF-H1. *Journal of Cell Science*, 118(Pt 9), 1861–1872.
- Calvo, F., Ege, N., Grande-Garcia, A., Hooper, S., Jenkins, R. P., Chaudhry, S. I., et al. (2013). Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. *Nature Cell Biology*, 15(6), 637–646.
- Calvo, F., Sanz-Moreno, V., Agudo-Ibanez, L., Wallberg, F., Sahai, E., Marshall, C. J., et al. (2011). RasGRF suppresses Cdc42-mediated tumour cell movement, cytoskeletal dynamics and transformation. *Nature Cell Biology*, 13(7), 819–826.
- Carr, H. S., Zuo, Y., Oh, W., & Frost, J. A. (2013). Regulation of focal adhesion kinase activation, breast cancer cell motility, and amoeboid invasion by the RhoA guanine nucleotide exchange factor Net1. *Molecular and Cellular Biology*, 33(14), 2773–2786.
- Castellone, M. D., Teramoto, H., Williams, B. O., Druey, K. M., & Gutkind, J. S. (2005). Prostaglandin E2 promotes colon cancer cell growth through a gs-axin-beta-catenin signaling axis. *Science (New York, N.Y.)*, 310(5753), 1504–1510.
- Castro, D. J., Maurer, J., Hebbard, L., & Oshima, R. G. (2013). ROCK1 inhibition promotes the self-renewal of a novel mouse mammary cancer stem cell. *Stem Cells (Dayton, Ohio)*, 31(1), 12–22.
- Cau, J., & Hall, A. (2005). Cdc42 controls the polarity of the actin and microtubule cytoskeletons through two distinct signal transduction pathways. *Journal of Cell Science*, 118(Pt 12), 2579–2587.
- Chan, A. Y., Coniglio, S. J., Chuang, Y. Y., Michaelson, D., Knaus, U. G., Philips, M. R., et al. (2005). Roles of the Rac1 and Rac3 GTPases in human tumor cell invasion. *Oncogene*, 24(53), 7821–7829.
- Chen, Z., Borek, D., Padrick, S. B., Gomez, T. S., Metlagel, Z., Ismail, A. M., et al. (2010). Structure and control of the actin regulatory WAVE complex. *Nature*, 468(7323), 533–538.
- Chen, L., Chan, T. H., Yuan, Y. F., Hu, L., Huang, J., Ma, S., et al. (2010). CHD1L promotes hepatocellular carcinoma progression and metastasis in mice and is associated with these processes in human patients. *The Journal of Clinical Investigation*, 120(4), 1178–1191.
- Chen, B., Ding, Y., Liu, F., Ruan, J., Guan, J., Huang, J., et al. (2012). Tiam1, over-expressed in most malignancies, is a novel tumor biomarker. *Molecular Medicine Reports*, 5(1), 48–53.
- Chen, X., & Macara, I. G. (2005). Par-3 controls tight junction assembly through the rac exchange factor Tiam1. *Nature Cell Biology*, 7(3), 262–269.
- Chen, H., Tsalkova, T., Mei, F. C., Hu, Y., Cheng, X., & Zhou, J. (2012). 5-cyano-6-oxo-1,6-dihydro-pyrimidines as potent antagonists targeting exchange proteins directly activated by cAMP. *Bioorganic & Medicinal Chemistry Letters*, 22(12), 4038–4043.
- Chen, J., & Zhang, M. (2013). The Par3/Par6/aPKC complex and epithelial cell polarity. *Experimental Cell Research*, 319(10), 1357–1364.
- Cherfils, J., & Zeghouf, M. (2013). Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiological Reviews*, 93(1), 269–309.
- Cheung, L. W., Leung, P. C., & Wong, A. S. (2010). Cadherin switching and activation of p120 catenin signaling are mediators of gonadotropin-releasing hormone to promote tumor cell migration and invasion in ovarian cancer. *Oncogene*, 29(16), 2427–2440.
- Choi, J. W., Park, S. C., Kang, G. H., Liu, J. O., & Youn, H. D. (2004). Nur77 activated by hypoxia-inducible factor-1alpha overproduces proopiomelanocortin in von hippel-lindau-mutated renal cell carcinoma. *Cancer Research*, 64(1), 35–39.
- Chow, H. Y., Dong, B., Duron, S. G., Campbell, D. A., Ong, C. C., Hoeflich, K. P., et al. (2015). Group I paks as therapeutic targets in NF2-deficient meningioma. *Oncotarget*, 6(4), 1981–1994.
- Chow, H. Y., Jubb, A. M., Koch, J. N., Jaffer, Z. M., Stepanova, D., Campbell, D. A., et al. (2012). p21-activated kinase 1 is required for efficient tumor formation and progression in a ras-mediated skin cancer model. *Cancer Research*, 72(22), 5966–5975.
- Chuang, Y. Y., Tran, N. L., Rusk, N., Nakada, M., Berens, M. E., & Symons, M. (2004). Role of synaptojanin 2 in glioma cell migration and invasion. *Cancer Research*, 64(22), 8271–8275.
- Clark, E. A., Golub, T. R., Lander, E. S., & Hynes, R. O. (2000). Genomic analysis of metastasis reveals an essential role for RhoC. *Nature*, 406(6795), 532–535.
- Clevers, H., & Nusse, R. (2012). Wnt/beta-catenin signaling and disease. *Cell*, 149(6), 1192–1205.
- Coluccia, A. M., Benati, D., Dekhil, H., De Filippo, A., Lan, C., & Gambacorti-Passerini, C. (2006). SKI-606 decreases growth and motility of colorectal cancer cells by preventing pp60(c-src)-dependent tyrosine phosphorylation of beta-catenin and its nuclear signaling. *Cancer Research*, 66(4), 2279–2286.
- Connolly, E. C., Van Doorslaer, K., Rogler, L. E., & Rogler, C. E. (2010). Overexpression of miR-21 promotes an in vitro metastatic phenotype by targeting the tumor suppressor RHO. *Molecular Cancer Research: MCR*, 8(5), 691–700.
- Cook, D. R., Rossman, K. L., & Der, C. J. (2014). Rho guanine nucleotide exchange factors: Regulators of rho GTPase activity in development and disease. *Oncogene*, 33(31), 4021–4035.
- Courilleau, D., Bissier, M., Jullian, J. C., Lucas, A., Bouyssou, P., Fischmeister, R., et al. (2012). Identification of a tetrahydroquinoline analog as a pharmacological inhibitor of the cAMP-binding protein epac. *The Journal of Biological Chemistry*, 287(53), 44192–44202.
- Courilleau, D., Bouyssou, P., Fischmeister, R., Lezoualc'h, F., & Blondeau, J. P. (2013). The (R)-enantiomer of CE3F4 is a preferential inhibitor of human exchange protein directly activated by cyclic AMP isoform 1 (Epac1). *Biochemical and Biophysical Research Communications*, 440(3), 443–448.
- Cox, T. R., & Erler, J. T. (2011). Remodeling and homeostasis of the extracellular matrix: Implications for fibrotic diseases and cancer. *Disease Models & Mechanisms*, 4(2), 165–178.
- Croft, D. R., & Olson, M. F. (2011). Transcriptional regulation of rho GTPase signaling. *Transcription*, 2(5), 211–215.
- Csiki, I., Yanagisawa, K., Haruki, N., Nadaf, S., Morrow, J. D., Johnson, D. H., et al. (2006). Thioredoxin-1 modulates transcription of cyclooxygenase-2 via hypoxia-inducible factor-1alpha in non-small cell lung cancer. *Cancer Research*, 66(1), 143–150.
- Dan, C., Kelly, A., Bernard, O., & Minden, A. (2001). Cytoskeletal changes regulated by the PAK4 serine/threonine kinase are mediated by LIM kinase 1 and cofilin. *The Journal of Biological Chemistry*, 276(34), 32115–32121.
- de Toledo, M., Anguille, C., Roger, L., Roux, P., & Gadea, G. (2012). Cooperative anti-invasive effect of Cdc42/Rac1 activation and ROCK inhibition in SW620 colorectal cancer cells with elevated blebbing activity. *PLoS One*, 7(11), e48344.
- Deacon, S. W., Beeser, A., Fukui, J. A., Rennefahrt, U. E., Myers, C., Chernoff, J., et al. (2008). An isoform-selective, small-molecule inhibitor targets the autoregulatory mechanism of p21-activated kinase. *Chemistry & Biology*, 15(4), 322–331.
- Delorme, V., Machacek, M., DerMardirossian, C., Anderson, K. L., Wittmann, T., Hanein, D., et al. (2007). Cofilin activity downstream of Pak1 regulates cell protrusion efficiency by organizing lamellipodium and lamella actin networks. *Developmental Cell*, 13(5), 646–662.
- Demierre, M. F., Higgins, P. D., Gruber, S. B., Hawk, E., & Lippman, S. M. (2005). Statins and cancer prevention. *Nature Reviews. Cancer*, 5(12), 930–942.
- Derksen, P. W., Liu, X., Saridin, F., van der Gulden, H., Zevenhoven, J., Evers, B., et al. (2006). Somatic inactivation of E-cadherin and p53 in mice leads to metastatic lobular mammary carcinoma through induction of anoikis resistance and angiogenesis. *Cancer Cell*, 10(5), 437–449.
- Dietrich, K. A., Schwarz, R., Liska, M., Grass, S., Menke, A., Meister, M., et al. (2009). Specific induction of migration and invasion of pancreatic carcinoma cells by RhoC, which differs from RhoA in its localisation and activity. *Biological Chemistry*, 390(10), 1063–1077.
- Dorsam, R. T., & Gutkind, J. S. (2007). G-protein-coupled receptors and cancer. *Nature Reviews. Cancer*, 7(2), 79–94.
- Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., et al. (2011). Role of YAP/TAZ in mechanotransduction. *Nature*, 474(7350), 179–183.
- Durkin, M. E., Ullmannova, V., Guan, M., & Popescu, N. C. (2007). Deleted in liver cancer 3 (DLC-3), a novel rho GTPase-activating protein, is downregulated in cancer and inhibits tumor cell growth. *Oncogene*, 26(31), 4580–4589.
- Eitaki, M., Yamamori, T., Meike, S., Yasui, H., & Inanami, O. (2012). Vincristine enhances amoeboid-like motility via GEF-H1/RhoA/ROCK/Myosin light chain signaling in MKN45 cells. *BMC Cancer*, 12 (469–2407–12–469).
- Esfali, S., & Bapat, B. (2004). Cross-talk between Rac1 GTPase and dysregulated wnt signaling pathway leads to cellular redistribution of beta-catenin and TCF/LEF-mediated transcriptional activation. *Oncogene*, 23(50), 8260–8271.
- Evelyn, C. R., Feng, T., Rojas, R. J., Larsen, M. J., Sondek, J., & Neubig, R. R. (2009). High-throughput screening for small-molecule inhibitors of LARG-stimulated RhoA nucleotide binding via a novel fluorescence polarization assay. *Journal of Biomolecular Screening*, 14(2), 161–172.
- Fang, H., & Declercq, Y. A. (2013). Targeting the tumor microenvironment: From

- understanding pathways to effective clinical trials. *Cancer Research*, 73(16), 4965–4977.
- Feng, X., Degese, M. S., Iglesias-Bartolome, R., Vaque, J. P., Molinolo, A. A., Rodrigues, M., et al. (2014). Hippo-independent activation of YAP by the GNAQ uveal melanoma oncogene through a trio-regulated rho GTPase signaling circuitry. *Cancer Cell*, 25(6), 831–845.
- Feng, B., Li, K., Zhong, H., Ren, G., Wang, H., Shang, Y., et al. (2013). RhoE promotes metastasis in gastric cancer through a mechanism dependent on enhanced expression of CXCR4. *PLoS One*, 8(11), e81709.
- Florian, M. C., Dorr, K., Niebel, A., Daria, D., Schrezenmeier, H., Rojewski, M., et al. (2012). Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. *Cell Stem Cell*, 10(5), 520–530.
- Fort, P., & Theveneau, E. (2014). PleiotRHOpic: Rho pathways are essential for all stages of neural crest development. *Small GTPases*, 5, e27975.
- Friedl, P., & Gilmour, D. (2009). Collective cell migration in morphogenesis, regeneration and cancer. *Nature Reviews. Molecular Cell Biology*, 10(7), 445–457.
- Friedl, P., & Wolf, K. (2003). Tumour-cell invasion and migration: Diversity and escape mechanisms. *Nature Reviews. Cancer*, 3(5), 362–374.
- Friedl, P., Wolf, K., & Zegers, M. M. (2014). Rho-directed forces in collective migration. *Nature Cell Biology*, 16(3), 208–210.
- Friesland, A., Zhao, Y., Chen, Y. H., Wang, L., Zhou, H., & Lu, Q. (2013). Small molecule targeting Cdc42-intersectin interaction disrupts golgi organization and suppresses cell motility. *Proceedings of the National Academy of Sciences of the United States of America*, 110(4), 1261–1266.
- Fritz, G., Just, I., & Kaina, B. (1999). Rho GTPases are over-expressed in human tumors. *International Journal of Cancer. Journal International Du Cancer*, 81(5), 682–687.
- Fukata, M., Kuroda, S., Nakagawa, M., Kawajiri, A., Itoh, N., Shoji, I., et al. (1999). Cdc42 and Rac1 regulate the interaction of IQGAP1 with beta-catenin. *The Journal of Biological Chemistry*, 274(37), 26044–26050.
- Fukuhara, S., Chikumi, H., & Gutkind, J. S. (2001). RGS-containing RhoGEFs: The missing link between transforming G proteins and rho? *Oncogene*, 20(13), 1661–1668.
- Gad, A. K., Nehru, V., Ruusala, A., & Aspenstrom, P. (2012). RhoD regulates cytoskeletal dynamics via the actin nucleation-promoting factor WASp homologue associated with actin golgi membranes and microtubules. *Molecular Biology of the Cell*, 23(24), 4807–4819.
- Gadea, G., Sanz-Moreno, V., Self, A., Godi, A., & Marshall, C. J. (2008). DOCK10-mediated Cdc42 activation is necessary for amoeboid invasion of melanoma cells. *Current Biology: CB*, 18(19), 1456–1465.
- Gaggioli, C., Hooper, S., Hidalgo-Carcedo, C., Grosse, R., Marshall, J. F., Harrington, K., et al. (2007). Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. *Nature Cell Biology*, 9(12), 1392–1400.
- Gao, Y., Dickerson, J. B., Guo, F., Zheng, J., & Zheng, Y. (2004). Rational design and characterization of a rac GTPase-specific small molecule inhibitor. *Proceedings of the National Academy of Sciences of the United States of America*, 101(20), 7618–7623.
- Gao, L., Feng, Y., Bowers, R., Becker-Hapak, M., Gardner, J., Council, L., et al. (2006). Ras-associated protein-1 regulates extracellular signal-regulated kinase activation and migration in melanoma cells: Two processes important to melanoma tumorigenesis and metastasis. *Cancer Research*, 66(16), 7880–7888.
- Gazzerro, P., Proto, M. C., Gangemi, G., Malfitano, A. M., Ciaglia, E., Pisanti, S., et al. (2012). Pharmacological actions of statins: A critical appraisal in the management of cancer. *Pharmacological Reviews*, 64(1), 102–146.
- Gilkes, D. M., Semenza, G. L., & Wirtz, D. (2014). Hypoxia and the extracellular matrix: Drivers of tumor metastasis. *Nature Reviews. Cancer*, 14(6), 430–439.
- Gilkes, D. M., Xiang, L., Lee, S. J., Chaturvedi, P., Hubbi, M. E., Wirtz, D., et al. (2014). Hypoxia-inducible factors mediate coordinated RhoA-ROCK1 expression and signaling in breast cancer cells. *Proceedings of the National Academy of Sciences of the United States of America*, 111(3), E384–E393.
- Goc, A., Al-Azayzih, A., Abdalla, M., Al-Husein, B., Kavuri, S., Lee, J., et al. (2013). P21 activated kinase-1 (Pak1) promotes prostate tumor growth and microinvasion via inhibition of transforming growth factor beta expression and enhanced matrix metalloproteinase 9 secretion. *The Journal of Biological Chemistry*, 288(5), 3025–3035.
- Goel, H. L., & Mercurio, A. M. (2013). VEGF targets the tumour cell. *Nature Reviews. Cancer*, 13(12), 871–882.
- Gong, B., Shelite, T., Mei, F. C., Ha, T., Hu, Y., Xu, G., et al. (2013). Exchange protein directly activated by cAMP plays a critical role in bacterial invasion during fatal rickettsiosis. *Proceedings of the National Academy of Sciences of the United States of America*, 110(48), 19615–19620.
- Goodison, S., Yuan, J., Sloan, D., Kim, R., Li, C., Popescu, N. C., et al. (2005). The RhoGAP protein DLC-1 functions as a metastasis suppressor in breast cancer cells. *Cancer Research*, 65(14), 6042–6053.
- Grandoch, M., Rose, A., ter Braak, M., Jendrossek, V., Rubben, H., Fischer, J. W., et al. (2009). Epac inhibits migration and proliferation of human prostate carcinoma cells. *British Journal of Cancer*, 101(12), 2038–2042.
- Greenhough, A., Smartt, H. J., Moore, A. E., Roberts, H. R., Williams, A. C., Paraskeva, C., et al. (2009). The COX-2/PGE2 pathway: Key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis*, 30(3), 377–386.
- Grosheva, I., Shtutman, M., Elbaum, M., & Bershadsky, A. D. (2001). p120 catenin affects cell motility via modulation of activity of rho-family GTPases: A link between cell-cell contact formation and regulation of cell locomotion. *Journal of Cell Science*, 114(Pt 4), 695–707.
- Guemar, L., de Santa Barbara, P., Vignal, E., Maurel, B., Fort, P., & Faure, S. (2007). The small GTPase RhoV is an essential regulator of neural crest induction in xenopus. *Developmental Biology*, 310(1), 113–128.
- Guettler, S., Vartiainen, M. K., Miralles, F., Larjani, B., & Treisman, R. (2008). RPEL motifs link the serum response factor C/EBPβ but not myocardin to rho signaling via actin binding. *Molecular and Cellular Biology*, 28(2), 732–742.
- Guo, W., & Giancotti, F. G. (2004). Integrin signalling during tumour progression. *Nature Reviews. Molecular Cell Biology*, 5(10), 816–826.
- Guo, F., Wang, Y., Liu, J., Mok, S. C., Xue, F., & Zhang, W. (2016). CXCL12/CXCR4: A symbiotic bridge linking cancer cells and their stromal neighbors in oncogenic communication networks. *Oncogene*, 35(7), 816–826.
- Hage, B., Meinel, K., Baum, I., Giehl, K., & Menke, A. (2009). Rac1 activation inhibits E-cadherin-mediated adherens junctions via binding to IQGAP1 in pancreatic carcinoma cells. *Cell Communication and Signaling: CCS*, 7 (23-811X-7-23).
- Han, L., Stope, M. B., de Jesus, M. L., Oude Weernink, P. A., Urban, M., Wieland, T., et al. (2007). Direct stimulation of receptor-controlled phospholipase D1 by phospho-cofilin. *The EMBO Journal*, 26(19), 4189–4202.
- Harper, K., Arsenault, D., Boulay-Jean, S., Lauzier, A., Lucien, F., & Dubois, C. M. (2010). Autotaxin promotes cancer invasion via the lysophosphatidic acid receptor 4: Participation of the cyclic AMP/EPAC/Rac1 signaling pathway in invadopodia formation. *Cancer Research*, 70(11), 4634–4643.
- Harvey, K. F., Zhang, X., & Thomas, D. M. (2013). The hippo pathway and human cancer. *Nature Reviews. Cancer*, 13(4), 246–257.
- Heallen, T., Zhang, M., Wang, J., Bonilla-Claudio, M., Klysis, E., Johnson, R. L., et al. (2011). Hippo pathway inhibits wnt signaling to restrain cardiomyocyte proliferation and heart size. *Science (New York, N.Y.)*, 332(6028), 458–461.
- Hermann, M. R., Jakobson, M., Colo, G. P., Rognoni, E., Jakobson, M., Kupatt, C., et al. (2016). Integrins synergise to induce expression of the MRTF-A-SRF target gene ISG15 for promoting cancer cell invasion. *Journal of Cell Science*, 129(7), 1391–1403.
- Hidalgo-Carcedo, C., Hooper, S., Chaudhry, S. I., Williamson, P., Harrington, K., Leitinger, B., et al. (2011). Collective cell migration requires suppression of actomyosin at cell-cell contacts mediated by DDR1 and the cell polarity regulators Par3 and Par6. *Nature Cell Biology*, 13(1), 49–58.
- Hirota, K., & Semenza, G. L. (2001). Rac1 activity is required for the activation of hypoxia-inducible factor 1. *The Journal of Biological Chemistry*, 276(24), 21166–21172.
- Ho, M. Y., Liang, S. M., Hung, S. W., & Liang, C. M. (2013). MIG-7 controls COX-2/PGE2-mediated lung cancer metastasis. *Cancer Research*, 73(1), 439–449.
- Hoefen, R. J., & Berk, B. C. (2006). The multifunctional GIT family of proteins. *Journal of Cell Science*, 119(Pt 8), 1469–1475.
- Holeiter, G., Heering, J., Erlmann, P., Schmid, S., Jahne, R., & Olayioye, M. A. (2008). Deleted in liver cancer 1 controls cell migration through a Dial1-dependent signaling pathway. *Cancer Research*, 68(21), 8743–8751.
- Hong, L., Kenney, S. R., Phillips, G. K., Simpson, D., Schroeder, C. E., Noth, J., et al. (2013). Characterization of a Cdc42 protein inhibitor and its use as a molecular probe. *The Journal of Biological Chemistry*, 288(12), 8531–8543.
- Horikoshi, Y., Suzuki, A., Yamanaka, T., Sasaki, K., Mizuno, K., Sawada, H., et al. (2009). Interaction between PAR-3 and the aPKC-PAR-6 complex is indispensable for apical domain development of epithelial cells. *Journal of Cell Science*, 122(Pt 10), 1595–1606.
- Huang, S., & Ingber, D. E. (2005). Cell tension, matrix mechanics, and cancer development. *Cancer Cell*, 8(3), 175–176.
- Huang, S. M., Mishina, Y. M., Liu, S., Cheung, A., Stegmeier, F., Michaud, G. A., et al. (2009). Tankyrase inhibition stabilizes axin and antagonizes wnt signalling. *Nature*, 461(7264), 614–620.
- Huang, M., & Prendergast, G. C. (2006). RhoB in cancer suppression. *Histology and Histopathology*, 21(2), 213–218.
- Ikenouchi, J., & Aoki, K. (2017). Membrane bleb: A seesaw game of two small GTPases. *Small GTPases*, 8(2), 85–89.
- Imajo, M., Miyatake, K., Iimura, A., Miyamoto, A., & Nishida, E. (2012). A molecular mechanism that links hippo signalling to the inhibition of Wnt/beta-catenin signaling. *The EMBO Journal*, 31(5), 1109–1122.
- Ingber, D. E. (2006). Cellular mechanotransduction: Putting all the pieces together again. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, 20(7), 811–827.
- Itoh, K., Yoshioka, K., Akedo, H., Uehata, M., Ishizaki, T., & Narumiya, S. (1999). An essential part for rho-associated kinase in the transcellular invasion of tumor cells. *Nature Medicine*, 5(2), 221–225.
- Jadeski, L., Mataraza, J. M., Jeong, H. W., Li, Z., & Sacks, D. B. (2008). IQGAP1 stimulates proliferation and enhances tumorigenesis of human breast epithelial cells. *The Journal of Biological Chemistry*, 283(2), 1008–1017.
- Jansen, S. R., Holman, R., Hedemann, I., Frankes, E., Elzinga, C. R., Timens, W., et al. (2015). Prostaglandin E2 promotes MYCN non-amplified neuroblastoma cell survival via beta-catenin stabilization. *Journal of Cellular and Molecular Medicine*, 19(1), 210–226.
- Jansen, S. R., Poppinga, W. J., de Jager, W., Lezoualc'h, F., Cheng, X., Wieland, T., et al. (2016). Epac1 links prostaglandin E2 to beta-catenin-dependent transcription during epithelial-to-mesenchymal transition. *Oncotarget*, 7(29), 46354–46370.
- Jeong, K. J., Park, S. Y., Cho, K. H., Sohn, J. S., Lee, J., Kim, Y. K., et al. (2012). The Rho/ROCK pathway for lysophosphatidic acid-induced proteolytic enzyme expression and ovarian cancer cell invasion. *Oncogene*, 31(39), 4279–4289.
- Jerrell, R. J., Leih, M. J., & Parekh, A. (2017). The ROCK isoforms differentially regulate the morphological characteristics of carcinoma cells. *Small GTPases*, 0.
- Jiao, S., Wang, H., Shi, Z., Dong, A., Zhang, W., Song, X., et al. (2014). A peptide mimicking VGLL4 function acts as a YAP antagonist therapy against gastric cancer. *Cancer Cell*, 25(2), 166–180.
- Johnson, R., & Halder, G. (2014). The two faces of hippo: Targeting the hippo pathway for regenerative medicine and cancer treatment. *Nature Reviews. Drug Discovery*, 13(1), 63–79.
- Johnson, E., Seachrist, D. D., DeLeon-Rodriguez, C. M., Lozada, K. L., Miedler, J., Abdul-Karim, F. W., et al. (2010). HER2/Erbb2-induced breast cancer cell migration and invasion require p120 catenin activation of Rac1 and Cdc42. *The Journal of Biological Chemistry*, 285(38), 29491–29501.
- Jordan, P., Brazao, R., Boavida, M. G., Gespach, C., & Chastre, E. (1999). Cloning of a novel human Rac1b splice variant with increased expression in colorectal tumors. *Oncogene*, 18(48), 6835–6839.
- Kaidi, A., Qualtrough, D., Williams, A. C., & Paraskeva, C. (2006). Direct transcriptional up-regulation of cyclooxygenase-2 by hypoxia-inducible factor (HIF)-1 promotes colorectal tumor cell survival and enhances HIF-1 transcriptional activity during hypoxia. *Cancer Research*, 66(13), 6683–6691.
- Kaidi, A., Williams, A. C., & Paraskeva, C. (2007). Interaction between beta-catenin and HIF-1 promotes cellular adaptation to hypoxia. *Nature Cell Biology*, 9(2), 210–217.
- Kakiuchi, M., Nishizawa, T., Ueda, H., Gotoh, K., Tanaka, A., Hayashi, A., et al. (2014).

- Recurrent gain-of-function mutations of RHOA in diffuse-type gastric carcinoma. *Nature Genetics*, 46(6), 583–587.
- Kale, V. P., Hengst, J. A., Desai, D. H., Amin, S. G., & Yun, J. K. (2015). The regulatory roles of ROCK and MRCK kinases in the plasticity of cancer cell migration. *Cancer Letters*, 361(2), 185–196.
- Kalluri, R. (2009). EMT: When epithelial cells decide to become mesenchymal-like cells. *The Journal of Clinical Investigation*, 119(6), 1417–1419.
- Katz, E., Sims, A. H., Sproul, D., Caldwell, H., Dixon, M. J., Meehan, R. R., et al. (2012). Targeting of rac GTPases blocks the spread of intact human breast cancer. *Oncotarget*, 3(6), 608–619.
- Kedziora, K. M., Leyton-Puig, D., Argenzio, E., Boumeester, A. J., van Butselar, B., Yin, T., et al. (2016). Rapid remodeling of invadosomes by gi-coupled receptors: DISSECTING THE ROLE OF rho GTPases. *The Journal of Biological Chemistry*, 291(9), 4323–4333.
- Kim, D. J., Choi, C. K., Lee, C. S., Park, M. H., Tian, X., Kim, N. D., et al. (2016). Small molecules that allosterically inhibit p21-activated kinase activity by binding to the regulatory p21-binding domain. *Experimental & Molecular Medicine*, 48, e229.
- Kim, T. H., Monsefi, N., Song, J. H., von Kriegsheim, A., Vandamme, D., Pertz, O., et al. (2015). Network-based identification of feedback modules that control RhoA activity and cell migration. *Journal of Molecular Cell Biology*, 7(3), 242–252.
- Kim, S. H., Park, Y. Y., Kim, S. W., Lee, J. S., Wang, D., & DuBois, R. N. (2011). ANGPTL4 induction by prostaglandin E2 under hypoxic conditions promotes colorectal cancer progression. *Cancer Research*, 71(22), 7010–7020.
- Kiosses, W. B., Shattil, S. J., Pampori, N., & Schwartz, M. A. (2001). Rac recruits high-affinity integrin alphavbeta3 to lamellipodia in endothelial cell migration. *Nature Cell Biology*, 3(3), 316–320.
- Knezevic, I. I., Predescu, S. A., Neamu, R. F., Gorovoy, M. S., Knezevic, N. M., Easington, C., et al. (2009). Tiam1 and Rac1 are required for platelet-activating factor-induced endothelial junctional disassembly and increase in vascular permeability. *The Journal of Biological Chemistry*, 284(8), 5381–5394.
- Ko, F. C., Chan, L. K., Sze, K. M., Yeung, Y. S., Tse, E. Y., Lu, P., et al. (2013). PKA-induced dimerization of the RhoGAP DLC1 promotes its inhibition of tumorigenesis and metastasis. *Nature Communications*, 4, 1618.
- Koenig, A., Mueller, C., Hasel, C., Adler, G., & Menke, A. (2006). Collagen type I induces disruption of E-cadherin-mediated cell-cell contacts and promotes proliferation of pancreatic carcinoma cells. *Cancer Research*, 66(9), 4662–4671.
- Koizumi, K., Takano, K., Kaneyasu, A., Watanabe-Takano, H., Tokuda, E., Abe, T., et al. (2012). RhoD activated by fibroblast growth factor induces cytoneme-like cellular protrusions through mDia3C. *Molecular Biology of the Cell*, 23(23), 4647–4661.
- Kowalczyk, A. P., & Reynolds, A. B. (2004). Protecting your tail: Regulation of cadherin degradation by p120-catenin. *Current Opinion in Cell Biology*, 16(5), 522–527.
- Krishnamachary, B., Berg-Dixon, S., Kelly, B., Agani, F., Feldser, D., Ferreira, G., et al. (2003). Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. *Cancer Research*, 63(5), 1138–1143.
- Kumar, R. D., Swamidass, S. J., & Bose, R. (2016). Unsupervised detection of cancer driver mutations with parsimony-guided learning. *Nature Genetics*, 48(10), 1288–1294.
- Kuo, J. C., Han, X., Hsiao, C. T., Yates, J. R., III, & Waterman, C. M. (2011). Analysis of the myosin-II-responsive focal adhesion proteome reveals a role for beta-pix in negative regulation of focal adhesion maturation. *Nature Cell Biology*, 13(4), 383–393.
- Kuroda, S., Fukata, M., Nakagawa, M., Fujii, K., Nakamura, T., Ookubo, T., et al. (1998). Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of E-cadherin-mediated cell-cell adhesion. *Science (New York, N.Y.)*, 281(5378), 832–835.
- Kuriwaki, M., Oneyama, C., Nada, S., & Okada, M. (2011). The guanine nucleotide exchange factor Arhgef5 plays crucial roles in src-induced podosome formation. *Journal of Cell Science*, 124(Pt 10), 1726–1738.
- Lammermann, T., & Sixt, M. (2009). Mechanical modes of ‘amoeboid’ cell migration. *Current Opinion in Cell Biology*, 21(5), 636–644.
- Lau, T., Chan, E., Callow, M., Waaler, J., Boggs, J., Blake, R. A., et al. (2013). A novel tankyrase small-molecule inhibitor suppresses APC mutation-driven colorectal tumor growth. *Cancer Research*, 73(10), 3132–3144.
- Lawson, C. D., & Burridge, K. (2014). The on-off relationship of rho and rac during integrin-mediated adhesion and cell migration. *Small GTPases*, 5, e27958.
- Le, P. N., McDermott, J. D., & Jimeno, A. (2015). Targeting the wnt pathway in human cancers: Therapeutic targeting with a focus on OMP-54F28. *Pharmacology & Therapeutics*, 146, 1–11.
- Lee, S., Craig, B. T., Romain, C. V., Qiao, J., & Chung, D. H. (2014). Silencing of CDC42 inhibits neuroblastoma cell proliferation and transformation. *Cancer Letters*, 355(2), 210–216.
- Lee, J. W., Lee, J., & Moon, E. Y. (2014). HeLa human cervical cancer cell migration is inhibited by treatment with dibutyryl-cAMP. *Anticancer Research*, 34(7), 3447–3455.
- Lenz, H. J., & Kahn, M. (2014). Safely targeting cancer stem cells via selective catenin coactivator antagonism. *Cancer Science*, 105(9), 1087–1092.
- Leung, T. H., Ching, Y. P., Yam, J. W., Wong, C. M., Yau, T. O., Jin, D. Y., et al. (2005). Deleted in liver cancer 2 (DLC2) suppresses cell transformation by means of inhibition of RhoA activity. *Proceedings of the National Academy of Sciences of the United States of America*, 102(42), 15207–15212.
- Licciulli, S., Maksimoska, J., Zhou, C., Troutman, S., Kota, S., Liu, Q., et al. (2013). FRAAX597, a small molecule inhibitor of the p21-activated kinases, inhibits tumorigenesis of neurofibromatosis type 2 (NF2)-associated schwannomas. *The Journal of Biological Chemistry*, 288(40), 29105–29114.
- Liu, S., Goldstein, R. H., Scepanky, E. M., & Rosenblatt, M. (2009). Inhibition of rho-associated kinase signaling prevents breast cancer metastasis to human bone. *Cancer Research*, 69(22), 8742–8751.
- Liu, Y., Li, Q. C., Miao, Y., Xu, H. T., Dai, S. D., Wei, Q., et al. (2009). Ablation of p120-catenin enhances invasion and metastasis of human lung cancer cells. *Cancer Science*, 100(3), 441–448.
- Liu, M., Tang, Q., Qiu, M., Lang, N., Li, M., Zheng, Y., et al. (2011). miR-21 targets the tumor suppressor RhoB and regulates proliferation, invasion and apoptosis in colorectal cancer cells. *FEBS Letters*, 585(19), 2998–3005.
- Liu, L., Zhu, X. D., Wang, W. Q., Shen, Y., Qin, Y., Ren, Z. G., et al. (2010). Activation of beta-catenin by hypoxia in hepatocellular carcinoma contributes to enhanced metastatic potential and poor prognosis. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, 16(10), 2740–2750.
- Liu-Chittenden, Y., Huang, B., Shim, J. S., Chen, Q., Lee, S. J., Anders, R. A., et al. (2012). Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP. *Genes & Development*, 26(12), 1300–1305.
- Lizarraga, F., Poincloux, R., Romao, M., Montagnac, G., Le Dez, G., Bonne, I., et al. (2009). Diaphanous-related formins are required for invadopodia formation and invasion of breast tumor cells. *Cancer Research*, 69(7), 2792–2800.
- Loirand, G. (2015). Rho kinases in health and disease: From basic science to translational research. *Pharmacological Reviews*, 67(4), 1074–1095.
- Ma, L., Teruya-Feldstein, J., & Weinberg, R. A. (2007). Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature*, 449(7163), 682–688.
- Machacek, M., Hodgson, L., Welch, C., Elliott, H., Pertz, O., Nalbant, P., et al. (2009). Coordination of rho GTPase activities during cell protrusion. *Nature*, 461(7260), 99–103.
- Mack, N. A., & Georgiou, M. (2014). The interdependence of the rho GTPases and apical-basal cell polarity. *Small GTPases*, 5(2), 10.
- Maillet, M., Robert, S. J., Cacquevel, M., Gastineau, M., Vivien, D., Bertoglio, J., et al. (2003). Crosstalk between Rap1 and rac regulates secretion of sAPPalpha. *Nature Cell Biology*, 5(7), 633–639.
- Manser, E., Loo, T. H., Koh, C. G., Zhao, Z. S., Chen, X. Q., Tan, L., et al. (1998). PAK kinases are directly coupled to the PIX family of nucleotide exchange factors. *Molecular Cell*, 1(2), 183–192.
- Mareli, H., Carpy, A., Woroniuk, A., Vennin, C., White, G., Timpson, P., et al. (2016). Differential Rac1 signalling by guanine nucleotide exchange factors implicates FLN1 in regulating Rac1-driven cell migration. *Nature Communications*, 7, 10664.
- Masuda, M., Sawa, M., & Yamada, T. (2015). Therapeutic targets in the wnt signaling pathway: Feasibility of targeting TNK1 in colorectal cancer. *Pharmacology & Therapeutics*, 156, 1–9.
- McBeath, R., Pirone, D. M., Nelson, C. M., Bhadriraju, K., & Chen, C. S. (2004). Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Developmental Cell*, 6(4), 483–495.
- McCull, W., Hennessy, E. J., Blades, K., Chuaqui, C., Dowling, J. E., Ferguson, A. D., et al. (2016). Optimization of highly kinase selective bis-anilino pyrimidine PAK1 inhibitors. *ACS Medicinal Chemistry Letters*, 7(12), 1118–1123.
- MD Hashim, N. F., Nicholas, N. S., Dart, A. E., Kirakiadis, S., Paleolog, E., & Wells, C. M. (2013). Hypoxia-induced invadopodia formation: A role for beta-PIX. *Open Biology*, 3(6), 120159.
- Medjkane, S., Perez-Sanchez, C., Gaggioli, C., Sahai, E., & Treisman, R. (2009). Myocardin-related transcription factors and SRF are required for cytoskeletal dynamics and experimental metastasis. *Nature Cell Biology*, 11(3), 257–268.
- Meiri, D., Marshall, C. B., Mokady, D., LaRose, J., Mullin, M., Gingras, A. C., et al. (2014). Mechanistic insight into GPCR-mediated activation of the microtubule-associated RhoA exchange factor GEF-H1. *Nature Communications*, 5, 4857.
- Menacho-Marquez, M., Garcia-Escudero, R., Ojeda, V., Abad, A., Delgado, P., Costa, C., et al. (2013). The rho exchange factors Vav2 and Vav3 favor skin tumor initiation and promotion by engaging extracellular signaling loops. *PLoS Biology*, 11(7), e1001615.
- Menon, J., Doebele, R. C., Gomes, S., Bevilacqua, E., Reindl, K. M., & Rosner, M. R. (2012). A novel interplay between Rap1 and PAK regulates induction of angiogenesis in prostate cancer. *PLoS One*, 7(11), e49893.
- Miller, N. L., Lawson, C., Chen, X. L., Lim, S. T., & Schlaepfer, D. D. (2012). Rgnf (p190rhoGEF) knockout inhibits RhoA activity, focal adhesion establishment, and cell motility downstream of integrins. *PLoS One*, 7(5), e37830.
- Miralles, F., Posern, G., Zaromytidou, A. I., & Treisman, R. (2003). Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell*, 113(3), 329–342.
- Mo, J. S., Yu, F. X., Gong, R., Brown, J. H., & Guan, K. L. (2012). Regulation of the hippo-YAP pathway by protease-activated receptors (PARs). *Genes & Development*, 26(19), 2138–2143.
- Montalvo-Ortiz, B. L., Castillo-Pichardo, L., Hernandez, E., Humphries-Bickley, T., De la Mota-Peynado, A., Cubano, L. A., et al. (2012). Characterization of EHop-016, novel small molecule inhibitor of rac GTPase. *The Journal of Biological Chemistry*, 287(16), 13228–13238.
- Morali, O. G., Delmas, V., Moore, R., Jeanney, C., Thiery, J. P., & Larue, L. (2001). IGF-II induces rapid beta-catenin relocation to the nucleus during epithelium to mesenchyme transition. *Oncogene*, 20(36), 4942–4950.
- Moroishi, T., Hansen, C. G., & Guan, K. L. (2015). The emerging roles of YAP and TAZ in cancer. *Nature Reviews. Cancer*, 15(2), 73–79.
- Mortazavi, F., Lu, J., Phan, R., Lewis, M., Trinidad, K., Aljlani, A., et al. (2015). Significance of KRAS/PAK1/Crk pathway in non-small cell lung cancer oncogenesis. *BMC Cancer*, 15 (381-015-1360-4).
- Moshfegh, Y., Bravo-Cordero, J. J., Miskolci, V., Condeelis, J., & Hodgson, L. (2014). A trio-Rac1-Pak1 signalling axis drives invadopodia disassembly. *Nature Cell Biology*, 16(6), 574–586.
- Murray, B. W., Guo, C., Piraino, J., Westwick, J. K., Zhang, C., Lamerdin, J., et al. (2010). Small-molecule p21-activated kinase inhibitor PF-3758309 is a potent inhibitor of oncogenic signaling and tumor growth. *Proceedings of the National Academy of Sciences of the United States of America*, 107(20), 9446–9451.
- Nabeshima, K., Shimao, Y., Inoue, T., & Kono, M. (2002). Immunohistochemical analysis of IQGAP1 expression in human colorectal carcinomas: Its overexpression in carcinomas and association with invasion fronts. *Cancer Letters*, 176(1), 101–109.
- Nakamura, H., Fujita, K., Nakagawa, H., Kishi, F., Takeuchi, A., Aute, I., et al. (2005). Expression pattern of the scaffold protein IQGAP1 in lung cancer. *Oncology Reports*, 13(3), 427–431.
- Nalbant, P., Chang, Y. C., Birkenfeld, J., Chang, Z. F., & Bokoch, G. M. (2009). Guanine nucleotide exchange factor-H1 regulates cell migration via localized activation of RhoA at the leading edge. *Molecular Biology of the Cell*, 20(18), 4070–4082.
- Nardone, G., Oliver-De La Cruz, J., Vrbysky, J., Martini, C., Pribyl, J., Skladal, P., et al. (2017). YAP regulates cell mechanics by controlling focal adhesion assembly. *Nature Communications*, 8, 15321.
- Narumiya, S., & Watanabe, N. (2009). Migration without a clutch. *Nature Cell Biology*,

- 11(12), 1394–1396.
- Nayal, A., Webb, D. J., Brown, C. M., Schaefer, E. M., Vicente-Manzanares, M., & Horwitz, A. R. (2006). Paxillin phosphorylation at Ser273 localizes a GIT1-PIX-PAK complex and regulates adhesion and protrusion dynamics. *The Journal of Cell Biology*, 173(4), 587–589.
- Ndubaku, C. O., Crawford, J. J., Drobnick, J., Aliagas, I., Campbell, D., Dong, P., et al. (2015). Design of selective PAK1 inhibitor G-5555: Improving properties by employing an unorthodox low-pK a polar moiety. *ACS Medicinal Chemistry Letters*, 6(12), 1241–1246.
- Newell-Litwa, K. A., Badoual, M., Asmussen, H., Patel, H., Whitmore, L., & Horwitz, A. R. (2015). ROCK1 and 2 differentially regulate actomyosin organization to drive cell and synaptic polarity. *The Journal of Cell Biology*, 210(2), 225–242.
- Ng, M. R., Besser, A., Danuser, G., & Brugge, J. S. (2012). Substrate stiffness regulates cadherin-dependent collective migration through myosin-II contractility. *The Journal of Cell Biology*, 199(3), 545–563.
- Nguyen-Ngoc, K. V., Cheung, K. J., Brenot, A., Shamir, E. R., Gray, R. S., Hines, W. C., et al. (2012). ECM microenvironment regulates collective migration and local dissemination in normal and malignant mammary epithelium. *Proceedings of the National Academy of Sciences of the United States of America*, 109(39), E2595–604.
- Nimnual, A. S., Taylor, L. J., & Bar-Sagi, D. (2003). Redox-dependent downregulation of rho by rac. *Nature Cell Biology*, 5(3), 236–241.
- Nishikimi, A., Fukuhara, H., Su, W., Hongu, T., Takasuga, S., Mihara, H., et al. (2009). Sequential regulation of DOCK2 dynamics by two phospholipids during neutrophil chemotaxis. *Science (New York, N.Y.)*, 324(5925), 384–387.
- Nishimura, T., Yamaguchi, T., Kato, K., Yoshizawa, M., Nabeshima, Y., Ohno, S., et al. (2015). PAR-6-PAR-3 mediates Cdc42-induced rac activation through the rac GEFs STEF/Tiam1. *Nature Cell Biology*, 17(3), 270–277.
- Nobes, C. D., & Hall, A. (1995). Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell*, 81(1), 53–62.
- Nola, S., Daigaku, R., Smolarczyk, K., Carstens, M., Martin-Martin, B., Longmore, G., et al. (2011). Ajuba is required for rac activation and maintenance of E-cadherin adhesion. *The Journal of Cell Biology*, 195(5), 855–871.
- Noren, N. K., Liu, B. P., Burridge, K., & Krefl, B. (2000). p120 catenin regulates the actin cytoskeleton via rho family GTPases. *The Journal of Cell Biology*, 150(3), 567–580.
- O'Connor, K., & Chen, M. (2013). Dynamic functions of RhoA in tumor cell migration and invasion. *Small GTPases*, 4(3), 141–147.
- Ohata, H., Ishiguro, T., Aihara, Y., Sato, A., Sakai, H., Sekine, S., et al. (2012). Induction of the stem-like cell regulator CD44 by rho kinase inhibition contributes to the maintenance of colon cancer-initiating cells. *Cancer Research*, 72(19), 5101–5110.
- Omelchenko, T., Rabadan, M. A., Hernandez-Martinez, R., Grego-Bessa, J., Anderson, K. V., & Hall, A. (2014). Beta-pix directs collective migration of anterior visceral endoderm cells in the early mouse embryo. *Genes & Development*, 28(24), 2764–2777.
- Onder, T. T., Gupta, P. B., Mani, S. A., Yang, J., Lander, E. S., & Weinberg, R. A. (2008). Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. *Cancer Research*, 68(10), 3645–3654.
- Ong, C. C., Jubb, A. M., Haverly, P. M., Zhou, W., Tran, V., Truong, T., et al. (2011). Targeting p21-activated kinase 1 (PAK1) to induce apoptosis of tumor cells. *Proceedings of the National Academy of Sciences of the United States of America*, 108(17), 7177–7182.
- Orgaz, J. L., Herraz, C., & Sanz-Moreno, V. (2014). Rho GTPases modulate malignant transformation of tumor cells. *Small GTPases*, 5, e29019.
- Pan, Y. R., Chen, C. L., & Chen, H. C. (2011). FAK is required for the assembly of podosome rosettes. *The Journal of Cell Biology*, 195(1), 113–129.
- Parnell, E., Palmer, T. M., & Yarwood, S. J. (2015). The future of EPAC-targeted therapies: Agonism versus antagonism. *Trends in Pharmacological Sciences*, 36(4), 203–214.
- Parnell, E., Smith, B. O., & Yarwood, S. J. (2015). The cAMP sensors, EPAC1 and EPAC2, display distinct subcellular distributions despite sharing a common nuclear pore localization signal. *Cellular Signalling*, 27(5), 989–996.
- Paszek, M. J., & Weaver, V. M. (2004). The tension mounts: Mechanics meets morphogenesis and malignancy. *Journal of Mammary Gland Biology and Neoplasia*, 9(4), 325–342.
- Patel, R. A., Forinash, K. D., Pireddu, R., Sun, Y., Sun, N., Martin, M. P., et al. (2012). Rki-1447 is a potent inhibitor of the rho-associated ROCK kinases with anti-invasive and antitumor activities in breast cancer. *Cancer Research*, 72(19), 5025–5034.
- Patel, R. A., Liu, Y., Wang, B., Li, R., & Sefti, S. M. (2014). Identification of novel ROCK inhibitors with anti-migratory and anti-invasive activities. *Oncogene*, 33(5), 550–555.
- Patel, V., Rosenfeldt, H. M., Lyons, R., Servitja, J. M., Bustelo, X. R., Siroff, M., et al. (2007). Persistent activation of Rac1 in squamous carcinomas of the head and neck: Evidence for an EGFR/Vav2 signaling axis involved in cell invasion. *Carcinogenesis*, 28(6), 1145–1152.
- Peinado, H., & Cano, A. (2008). A hypoxic twist in metastasis. *Nature Cell Biology*, 10(3), 253–254.
- Pellegrin, S., & Mellor, H. (2005). The rho family GTPase rif induces filopodia through mDia2. *Current Biology: CB*, 15(2), 129–133.
- Pertz, O., Hodgson, L., Klemke, R. L., & Hahn, K. M. (2006). Spatiotemporal dynamics of RhoA activity in migrating cells. *Nature*, 440(7087), 1069–1072.
- Pichot, C. S., Arvanitis, C., Hartig, S. M., Jensen, S. A., Bechill, J., Marzouk, S., et al. (2010). Cdc42-interacting protein 4 promotes breast cancer cell invasion and formation of invadopodia through activation of N-WASP. *Cancer Research*, 70(21), 8347–8356.
- Pieters, T., van Hengel, J., & van Roy, F. (2012). Functions of p120ctn in development and disease. *Frontiers in Bioscience (Landmark Edition)*, 17, 760–783.
- Pinner, S., & Sahai, E. (2008). PDK1 regulates cancer cell motility by antagonising inhibition of ROCK1 by RhoE. *Nature Cell Biology*, 10(2), 127–137.
- Pitts, T. M., Kulikowski, G. N., Tan, A. C., Murray, B. W., Arcaroli, J. J., Tentler, J. J., et al. (2013). Association of the epithelial-to-mesenchymal transition phenotype with responsiveness to the p21-activated kinase inhibitor, PF-3758309, in colon cancer models. *Frontiers in Pharmacology*, 4, 35.
- Plutoni, C., Bazellieres, E., Le Borgne-Rochet, M., Comunale, F., Bruges, A., Seveno, M., et al. (2016). P-cadherin promotes collective cell migration via a Cdc42-mediated increase in mechanical forces. *The Journal of Cell Biology*, 212(2), 199–217.
- Poincloux, R., Lizarraga, F., & Chavrier, P. (2009). Matrix invasion by tumour cells: A focus on MT1-MMP trafficking to invadopodia. *Journal of Cell Science*, 122(Pt 17), 3015–3024.
- Posern, G., Miralles, F., Guettler, S., & Treisman, R. (2004). Mutant actins that stabilise F-actin use distinct mechanisms to activate the SRF coactivator MAL. *The EMBO Journal*, 23(20), 3973–3983.
- Poste, G., & Fidler, I. J. (1980). The pathogenesis of cancer metastasis. *Nature*, 283(5743), 139–146.
- Prendergast, G. C. (2001). Actin' up: RhoB in cancer and apoptosis. *Nature Reviews. Cancer*, 1(2), 162–168.
- Pyne, N. J., & Pyne, S. (2010). Sphingosine 1-phosphate and cancer. *Nature Reviews. Cancer*, 10(7), 489–503.
- Qin, Y., Capaldo, C., Gumbiner, B. M., & Macara, I. G. (2005). The mammalian scribble polarity protein regulates epithelial cell adhesion and migration through E-cadherin. *The Journal of Cell Biology*, 171(6), 1061–1071.
- Radisky, D. C., Levy, D. D., Littlepage, L. E., Liu, H., Nelson, C. M., Fata, J. E., et al. (2005). Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature*, 436(7047), 123–127.
- Radu, M., Semenova, G., Kosoff, R., & Chernoff, J. (2014). PAK signalling during the development and progression of cancer. *Nature Reviews. Cancer*, 14(1), 13–25.
- Rane, C. K., & Minden, A. (2014). P21 activated kinases: Structure, regulation, and functions. *Small GTPases*, 5. <http://dx.doi.org/10.4161/sgtp.28003> (Epub 2014 Mar 21).
- Rath, N., & Olson, M. F. (2012). Rho-associated kinases in tumorigenesis: Re-considering ROCK inhibition for cancer therapy. *EMBO Reports*, 13(10), 900–908.
- Revach, O. Y., Winograd-Katz, S. E., Samuels, Y., & Geiger, B. (2016). The involvement of mutant Rac1 in the formation of invadopodia in cultured melanoma cells. *Experimental Cell Research*, 343(1), 82–88.
- Reymond, N., Im, J. H., Garg, R., Cox, S., Soyer, M., Riou, P., et al. (2015). RhoC and ROCKs regulate cancer cell interactions with endothelial cells. *Molecular Oncology*, 9(6), 1043–1055.
- Reymond, N., Im, J. H., Garg, R., Vega, F. M., Borda d'Agua, B., Riou, P., et al. (2012). Cdc42 promotes transendothelial migration of cancer cells through beta1 integrin. *The Journal of Cell Biology*, 199(4), 653–668.
- Reymond, N., Riou, P., & Ridley, A. J. (2012). Rho GTPases and cancer cell transendothelial migration. *Methods in Molecular Biology (Clifton, N.J.)*, 827, 123–142.
- Reynolds, A. B., & Roczniak-Ferguson, A. (2004). Emerging roles for p120-catenin in cell adhesion and cancer. *Oncogene*, 23(48), 7947–7956.
- Ridley, A. J. (2011). Life at the leading edge. *Cell*, 145(7), 1012–1022.
- Ridley, A. J., & Hall, A. (1992). The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell*, 70(3), 389–399.
- Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D., & Hall, A. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell*, 70(3), 401–410.
- Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., et al. (2003). Cell migration: Integrating signals from front to back. *Science (New York, N.Y.)*, 302(5651), 1704–1709.
- Riento, K., & Ridley, A. J. (2003). Rocks: Multifunctional kinases in cell behaviour. *Nature Reviews. Molecular Cell Biology*, 4(6), 446–456.
- Rios-Doria, J., Kuefer, R., Ethier, S. P., & Day, M. L. (2004). Cleavage of beta-catenin by calpain in prostate and mammary tumor cells. *Cancer Research*, 64(20), 7237–7240.
- Rodrigues, P., Macaya, I., Bazzocco, S., Mazzolini, R., Andretta, E., Dopeso, H., et al. (2014). RHOA inactivation enhances wnt signalling and promotes colorectal cancer. *Nature Communications*, 5, 5458.
- Rodriguez-Hernandez, I., Cantelli, G., Bruce, F., & Sanz-Moreno, V. (2016). Rho, ROCK and actomyosin contractility in metastasis as drug targets. *V1000Research*, 5. <http://dx.doi.org/10.12688/f1000research.7909.1> (eCollection 2016).
- Romain, B., Hachet-Haas, M., Rohr, S., Brigand, C., Galzi, J. L., Gaub, M. P., et al. (2014). Hypoxia differentially regulated CXCR4 and CXCR7 signaling in colon cancer. *Molecular Cancer*, 13 (58-4598-13-58).
- Rosenbluh, J., Nijhawan, D., Cox, A. G., Li, X., Neal, J. T., Schafer, E. J., et al. (2012). Beta-catenin-driven cancers require a YAP1 transcriptional complex for survival and tumorigenesis. *Cell*, 151(7), 1457–1473.
- Rosenfeldt, H., Castellone, M. D., Randazzo, P. A., & Gutkind, J. S. (2006). Rac inhibits thrombin-induced rho activation: Evidence of a pak-dependent GTPase crosstalk. *Journal of Molecular Signaling*, 1, 8.
- Rossman, K. L., Der, C. J., & Sondek, J. (2005). GEF means go: Turning on RHO GTPases with guanine nucleotide-exchange factors. *Nature Reviews. Molecular Cell Biology*, 6(2), 167–180.
- Rudolph, J., Murray, L. J., Ndubaku, C. O., O'Brien, T., Blackwood, E., Wang, W., et al. (2016). Chemically diverse group I p21-activated kinase (PAK) inhibitors impart acute cardiovascular toxicity with a narrow therapeutic window. *Journal of Medicinal Chemistry*, 59(11), 5520–5541.
- Sabeh, F., Shimizu-Hirota, R., & Weiss, S. J. (2009). Protease-dependent versus -independent cancer cell invasion programs: Three-dimensional amoeboid movement revisited. *The Journal of Cell Biology*, 185(1), 11–19.
- Sadok, A., McCarthy, A., Caldwell, J., Collins, I., Garrett, M. D., Yeo, M., et al. (2015). Rho kinase inhibitors block melanoma cell migration and inhibit metastasis. *Cancer Research*, 75(11), 2272–2284.
- Sahai, E. (2007). Illuminating the metastatic process. *Nature Reviews. Cancer*, 7(10), 737–749.
- Sahai, E., & Marshall, C. J. (2003). Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. *Nature Cell Biology*, 5(8), 711–719.
- Sahlgren, C., Gustafsson, M. V., Jin, S., Poellinger, L., & Lendahl, U. (2008). Notch signaling mediates hypoxia-induced tumor cell migration and invasion. *Proceedings of the National Academy of Sciences of the United States of America*, 105(17), 6392–6397.
- Saito, K., Ozawa, Y., Hibino, K., & Ohta, Y. (2012). FilGAP, a Rho/Rho-associated protein kinase-regulated GTPase-activating protein for rac, controls tumor cell migration.

- Molecular Biology of the Cell*, 23(24), 4739–4750.
- Sakurai-Yageta, M., Recchi, C., Le Dez, G., Sibarita, J. B., Daviet, L., Camonis, J., et al. (2008). The interaction of IQGAP1 with the exocyst complex is required for tumor cell invasion downstream of Cdc42 and RhoA. *The Journal of Cell Biology*, 181(6), 985–998.
- Santoyo-Ramos, P., Likhatcheva, M., Garcia-Zepeda, E. A., Castaneda-Patlan, M. C., & Robles-Flores, M. (2014). Hypoxia-inducible factors modulate the stemness and malignancy of colon cancer cells by playing opposite roles in canonical wnt signaling. *PLoS One*, 9(11), e112580.
- Sanz-Moreno, V., Gadea, G., Ahn, J., Paterson, H., Marra, P., Pinner, S., et al. (2008). Rac activation and inactivation control plasticity of tumor cell movement. *Cell*, 135(3), 510–523.
- Schmidt, S., & Debant, A. (2014). Function and regulation of the rho guanine nucleotide exchange factor trio. *Small GTPases*, 5, e29769.
- Schmidt, M., Dekker, F. J., & Maarsingh, H. (2013). Exchange protein directly activated by cAMP (epac): A multidomain cAMP mediator in the regulation of diverse biological functions. *Pharmacological Reviews*, 65(2), 670–709.
- Semprucci, E., Tocci, P., Cianfrocca, R., Sestito, R., Caprara, V., Vegliione, M., et al. (2015). Endothelin A receptor drives invadopodia function and cell motility through the beta-arrestin/PDZ-RhoGEF pathway in ovarian carcinoma. *Oncogene*, 35(26), 3432–3442.
- Shang, X., Marchionni, F., Evelyn, C. R., Sipes, N., Zhou, X., Seibel, W., et al. (2013). Small-molecule inhibitors targeting G-protein-coupled rho guanine nucleotide exchange factors. *Proceedings of the National Academy of Sciences of the United States of America*, 110(8), 3155–3160.
- Shao, J., Jung, C., Liu, C., & Sheng, H. (2005). Prostaglandin E2 stimulates the beta-catenin/T cell factor-dependent transcription in colon cancer. *The Journal of Biological Chemistry*, 280(28), 26565–26572.
- Shen, Y., Hirsch, D. S., Sasiela, C. A., & Wu, W. J. (2008). Cdc42 regulates E-cadherin ubiquitination and degradation through an epidermal growth factor receptor to src-mediated pathway. *The Journal of Biological Chemistry*, 283(8), 5127–5137.
- Shutes, A., Onesto, C., Picard, V., Leblond, B., Schweighoffer, F., & Der, C. J. (2007). Specificity and mechanism of action of EHT 1864, a novel small molecule inhibitor of rac family small GTPases. *The Journal of Biological Chemistry*, 282(49), 35666–35678.
- Soto, E., Yanagisawa, M., Marlow, L. A., Copland, J. A., Perez, E. A., & Anastasiadis, P. Z. (2008). p120 catenin induces opposing effects on tumor cell growth depending on E-cadherin expression. *The Journal of Cell Biology*, 183(4), 737–749.
- Spaul, P., Ciufici, P., Veillat, V., Leclercq, A., Daubon, T., Kramer, I. J., et al. (2014). Importance of RhoGTPases in formation, characteristics, and functions of invadosomes. *Small GTPases*, 5, e28195.
- Staller, P., Sulitkova, J., Lisztwan, J., Moch, H., Oakeley, E. J., & Krek, W. (2003). Chemokine receptor CXCR4 downregulated by von hippel-lindau tumour suppressor pVHL. *Nature*, 425(6955), 307–311.
- Stallings-Mann, M. L., Waldmann, J., Zhang, Y., Miller, E., Gauthier, M. L., Visscher, D. W., et al. (2012). Matrix metalloproteinase induction of Rac1b, a key effector of lung cancer progression. *Science Translational Medicine*, 4(142), 142ra95.
- Steege, P. S. (2016). Targeting metastasis. *Nature Reviews Cancer*, 16(4), 201–218.
- Stengel, K., & Zheng, Y. (2011). Cdc42 in oncogenic transformation, invasion, and tumorigenesis. *Cellular Signalling*, 23(9), 1415–1423.
- Stofega, M. R., Sanders, L. C., Gardiner, E. M., & Bokoch, G. M. (2004). Constitutive p21-activated kinase (PAK) activation in breast cancer cells as a result of mislocalization of PAK to focal adhesions. *Molecular Biology of the Cell*, 15(6), 2965–2977.
- Struckhoff, A. P., Rana, M. K., Kher, S. S., Burrow, M. E., Hagan, J. L., Del Valle, L., et al. (2013). PDZ-RhoGEF is essential for CXCR4-driven breast tumor cell motility through spatial regulation of RhoA. *Journal of Cell Science*, 126(Pt 19), 4514–4526.
- Tay, H. G., Ng, Y. W., & Manser, E. (2010). A vertebrate-specific chp-PAK-PIX pathway maintains E-cadherin at adherens junctions during zebrafish epiboly. *PLoS One*, 5(4), e10125.
- Teicher, B. A., & Fricker, S. P. (2010). CXCL12 (SDF-1)/CXCR4 pathway in cancer. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, 16(11), 2927–2931.
- ten Klooster, J. P., Jaffer, Z. M., Chernoff, J., & Hordijk, P. L. (2006). Targeting and activation of Rac1 are mediated by the exchange factor beta-pix. *The Journal of Cell Biology*, 172(5), 759–769.
- Thiery, J. P., Acloque, H., Huang, R. Y., & Nieto, M. A. (2009). Epithelial-mesenchymal transitions in development and disease. *Cell*, 139(5), 871–890.
- Thomas, E. K., Cancelas, J. A., Chae, H. D., Cox, A. D., Keller, P. J., Perrotti, D., et al. (2007). Rac guanosine triphosphatases represent integrating molecular therapeutic targets for BCR-ABL-induced myeloproliferative disease. *Cancer Cell*, 12(5), 467–478.
- Thompson, J. M., Landman, J., & Razorenova, O. V. (2017). Targeting the RhoGTPase/ROCK pathway for the treatment of VHL/HIF pathway-driven cancers. *Small GTPases*, 0.
- Thompson, J. M., Nguyen, Q. H., Singh, M., Pavesic, M. W., Nesterenko, I., Nelson, L. J., et al. (2017). Rho-associated kinase 1 inhibition is synthetically lethal with von hippel-lindau deficiency in clear cell renal cell carcinoma. *Oncogene*, 36(8), 1080–1089.
- Thurnher, M., Nussbaumer, O., & Gruenbacher, G. (2012). Novel aspects of mevalonate pathway inhibitors as antitumor agents. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, 18(13), 3524–3531.
- To, S. K., Zeng, W. J., Zeng, J. Z., & Wong, A. S. (2014). Hypoxia triggers a Nur77-beta-catenin feed-forward loop to promote the invasive growth of colon cancer cells. *British Journal of Cancer*, 110(4), 935–945.
- Tsai, C. C., Liu, H. F., Hsu, K. C., Yang, J. M., Chen, C., Liu, K. K., et al. (2011). 7-chloro-6-piperidin-1-yl-quinoline-5,8-dione (PT-262), a novel ROCK inhibitor blocks cytoskeleton function and cell migration. *Biochemical Pharmacology*, 81(7), 856–865.
- Tsalkova, T., Mei, F. C., & Cheng, X. (2012). A fluorescence-based high-throughput assay for the discovery of exchange protein directly activated by cyclic AMP (EPAC) antagonists. *PLoS One*, 7(1), e30441.
- Tsalkova, T., Mei, F. C., Li, S., Chepurny, O. G., Leech, C. A., Liu, T., et al. (2012). Isoform-specific antagonists of exchange proteins directly activated by cAMP. *Proceedings of the National Academy of Sciences of the United States of America*, 109(45), 18613–18618.
- Turcotte, S., Desrosiers, R. R., & Beliveau, R. (2004). Hypoxia upregulates von hippel-lindau tumor-suppressor protein through RhoA-dependent activity in renal cell carcinoma. *American Journal of Physiology. Renal Physiology*, 286(2), F338–48.
- Valastyan, S., & Weinberg, R. A. (2011). Tumor metastasis: Molecular insights and evolving paradigms. *Cell*, 147(2), 275–292.
- Valentijn, L. J., Koppen, A., van Asperen, R., Root, H. A., Haneveld, F., & Versteeg, R. (2005). Inhibition of a new differentiation pathway in neuroblastoma by copy number defects of N-myc, Cdc42, and nm23 genes. *Cancer Research*, 65(8), 3136–3145.
- Valon, L., Marin-Llaurado, A., Wyatt, T., Charras, G., & Treppe, X. (2017). Optogenetic control of cellular forces and mechanotransduction. *Nature Communications*, 8, 14396.
- van Golen, K. L., Bao, L., DiVito, M. M., Wu, Z., Prendergast, G. C., & Merajver, S. D. (2002). Reversion of RhoC GTPase-induced inflammatory breast cancer phenotype by treatment with a farnesyl transferase inhibitor. *Molecular Cancer Therapeutics*, 1(8), 575–583.
- van Golen, K. L., Wu, Z. F., Qiao, X. T., Bao, L., & Merajver, S. D. (2000a). RhoC GTPase overexpression modulates induction of angiogenic factors in breast cells. *Neoplasia (New York, N.Y.)*, 2(5), 418–425.
- van Golen, K. L., Wu, Z. F., Qiao, X. T., Bao, L. W., & Merajver, S. D. (2000b). RhoC GTPase, a novel transforming oncogene for human mammary epithelial cells that partially recapitulates the inflammatory breast cancer phenotype. *Cancer Research*, 60(20), 5832–5838.
- van Leeuwen, F. N., van Delft, S., Kain, H. E., van der Kammen, R. A., & Collard, J. G. (1999). Rac regulates phosphorylation of the myosin-II heavy chain, actinomyosin disassembly and cell spreading. *Nature Cell Biology*, 1(4), 242–248.
- Vandenbroucke, R. E., & Libert, C. (2014). Is there new hope for therapeutic matrix metalloproteinase inhibition? *Nature Reviews Drug Discovery*, 13(12), 904–927.
- Varelas, X., Miller, B. W., Sopko, R., Song, S., Gregoriou, A., Fellous, F. A., et al. (2010). The hippo pathway regulates Wnt/beta-catenin signaling. *Developmental Cell*, 18(4), 579–591.
- Vartiainen, M. K., Guettler, S., Larjani, B., & Treisman, R. (2007). Nuclear actin regulates dynamic subcellular localization and activity of the SR3 cofactor MAL. *Science (New York, N.Y.)*, 316(5832), 1749–1752.
- Vega, F. M., Colomba, A., Reymond, N., Thomas, M., & Ridley, A. J. (2012). RhoB regulates cell migration through altered focal adhesion dynamics. *Open Biology*, 2(5), 120076.
- Vega, F. M., Fruhwirth, G., Ng, T., & Ridley, A. J. (2011). RhoA and RhoC have distinct roles in migration and invasion by acting through different targets. *The Journal of Cell Biology*, 193(4), 655–665.
- Vega, F. M., & Ridley, A. J. (2008). Rho GTPases in cancer cell biology. *FEBS Letters*, 582(14), 2093–2101.
- Vigil, D., Kim, T. Y., Plachco, A., Garton, A. J., Castaldo, L., Pachter, J. A., et al. (2012). ROCK1 and ROCK2 are required for non-small cell lung cancer anchorage-independent growth and invasion. *Cancer Research*, 72(20), 5338–5347.
- Vignal, E., De Toledo, M., Comunale, F., Ladopoulos, A., Gauthier-Rouviere, C., Blangy, A., et al. (2000). Characterization of TCL, a new GTPase of the rho family related to TC10 and Cdc42. *The Journal of Biological Chemistry*, 275(46), 36457–36464.
- Vogelsgesang, M., Pautsch, A., & Aktories, K. (2007). C3 exoenzymes, novel insights into structure and action of rho-ADP-ribosylating toxins. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 374(5–6), 347–360.
- Waalder, J., Machon, O., Tumova, L., Dinh, H., Korinek, V., Wilson, S. R., et al. (2012). A novel tankyrase inhibitor decreases canonical wnt signaling in colon carcinoma cells and reduces tumor growth in conditional APC mutant mice. *Cancer Research*, 72(11), 2822–2832.
- Wang, D., & Dubois, R. N. (2006). Prostaglandins and cancer. *Gut*, 55(1), 115–122.
- Wang, J. R., Gan, W. J., Li, X. M., Zhao, Y. Y., Li, Y., Lu, X. X., et al. (2014). Orphan nuclear receptor Nur77 promotes colorectal cancer invasion and metastasis by regulating MMP-9 and E-cadherin. *Carcinogenesis*, 35(11), 2474–2484.
- Wang, W., Li, N., Li, X., Tran, M. K., Han, X., & Chen, J. (2015). Tankyrase inhibitors target YAP by stabilizing angiotensin family proteins. *Cell Reports*, 13(3), 524–532.
- Wang, P., Liu, Z., Chen, H., Ye, N., Cheng, X., & Zhou, J. (2017). Exchange proteins directly activated by cAMP (EPACs): Emerging therapeutic targets. *Bioorganic & Medicinal Chemistry Letters*, 27(8), 1633–1639.
- Wang, S., Watanabe, T., Matsuzawa, K., Katsumi, A., Kakeno, M., Matsui, T., et al. (2012). Tiam1 interaction with the PAR complex promotes talin-mediated Rac1 activation during polarized cell migration. *The Journal of Cell Biology*, 199(2), 331–345.
- Wang, H. R., Zhang, Y., Ozdamar, B., Ogunjimi, A. A., Alexandrova, E., Thomsen, G. H., et al. (2003). Regulation of cell polarity and protrusion formation by targeting RhoA for degradation. *Science (New York, N.Y.)*, 302(5651), 1775–1779.
- Weis, S. M., & Cheresh, D. A. (2011). Tumor angiogenesis: Molecular pathways and therapeutic targets. *Nature Medicine*, 17(11), 1359–1370.
- Wennerberg, K., Forget, M. A., Ellerbroek, S. M., Arthur, W. T., Burridge, K., Settleman, J., et al. (2003). Rnd proteins function as RhoA antagonists by activating p190 RhoGAP. *Current Biology: CB*, 13(13), 1106–1115.
- Wildenberg, G. A., Dohn, M. R., Carnahan, R. H., Davis, M. A., Lobdell, N. A., Settleman, J., et al. (2006). p120-catenin and p190RhoGAP regulate cell-cell adhesion by coordinating antagonism between rac and rho. *Cell*, 127(5), 1027–1039.
- Wilkinson, S., Paterson, H. F., & Marshall, C. J. (2005). Cdc42-MRCK and rho-ROCK signalling cooperate in myosin phosphorylation and cell invasion. *Nature Cell Biology*, 7(3), 255–261.
- Wilson, E., Leszczynska, K., Poulter, N. S., Edelman, F., Salisbury, V. A., Noy, P. J., et al. (2014). RhoJ interacts with the GIT-PIX complex and regulates focal adhesion disassembly. *Journal of Cell Science*, 127(Pt 14), 3039–3051.
- Wolf, K., Mazo, I., Leung, H., Engelke, K., von Andrian, U. H., Deryugina, E. I., et al. (2003). Compensation mechanism in tumor cell migration: Mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *The Journal of Cell Biology*, 160(2), 267–277.
- Wong, L. L., Lam, I. P., Wong, T. Y., Lai, W. L., Liu, H. F., Yeung, L. L., et al. (2013). IPA-3 inhibits the growth of liver cancer cells by suppressing PAK1 and NF-kappaB

- activation. *PLoS One*, 8(7), e68843.
- Wong, C. M., Yam, J. W., Ching, Y. P., Yau, T. O., Leung, T. H., Jin, D. Y., et al. (2005). Rho GTPase-activating protein deleted in liver cancer suppresses cell proliferation and invasion in hepatocellular carcinoma. *Cancer Research*, 65(19), 8861–8868.
- Woodcock, S. A., Rooney, C., Lontos, M., Connolly, Y., Zoumpourlis, V., Whetton, A. D., et al. (2009). SRC-induced disassembly of adherens junctions requires localized phosphorylation and degradation of the rac activator tiam1. *Molecular Cell*, 33(5), 639–653.
- Wu, X., Tu, X., Joeng, K. S., Hilton, M. J., Williams, D. A., & Long, F. (2008). Rac1 activation controls nuclear localization of beta-catenin during canonical wnt signaling. *Cell*, 133(2), 340–353.
- Wu, M., Wu, Z. F., Kumar-Sinha, C., Chinnaiyan, A., & Merajver, S. D. (2004). RhoC induces differential expression of genes involved in invasion and metastasis in MCF10A breast cells. *Breast Cancer Research and Treatment*, 84(1), 3–12.
- Wyckoff, J. B., Pinner, S. E., Gschmeissner, S., Condeelis, J. S., & Sahai, E. (2006). ROCK and myosin-dependent matrix deformation enables protease-independent tumor-cell invasion in vivo. *Current Biology: CB*, 16(15), 1515–1523.
- Xi, Y., Wei, Y., Sennino, B., Ulsamer, A., Kwan, I., Brumwell, A. N., et al. (2013). Identification of pY654-beta-catenin as a critical co-factor in hypoxia-inducible factor-1alpha signaling and tumor responses to hypoxia. *Oncogene*, 32(42), 5048–5057.
- Xing, F., Sharma, S., Liu, Y., Mo, Y. Y., Wu, K., Zhang, Y. Y., et al. (2015). miR-509 suppresses brain metastasis of breast cancer cells by modulating RhoC and TNF-alpha. *Oncogene*, 34(37), 4890–4900.
- Xue, Y., Bi, F., Zhang, X., Zhang, S., Pan, Y., Liu, N., et al. (2006). Role of Rac1 and Cdc42 in hypoxia induced p53 and von hippel-lindau suppression and HIF1alpha activation. *International Journal of Cancer*, 118(12), 2965–2972.
- Yamaguchi, H., Lorenz, M., Kempia, S., Sarmiento, C., Coniglio, S., Symons, M., et al. (2005). Molecular mechanisms of invadopodium formation: The role of the N-WASP-Arp2/3 complex pathway and cofilin. *The Journal of Cell Biology*, 168(3), 441–452.
- Yamazaki, D., Kuriu, S., & Takenawa, T. (2009). Involvement of rac and rho signaling in cancer cell motility in 3D substrates. *Oncogene*, 28(13), 1570–1583.
- Yan, J., Mei, F. C., Cheng, H., Lao, D. H., Hu, Y., Wei, J., et al. (2013). Enhanced leptin sensitivity, reduced adiposity, and improved glucose homeostasis in mice lacking exchange protein directly activated by cyclic AMP isoform 1. *Molecular and Cellular Biology*, 33(5), 918–926.
- Yang, N., Higuchi, O., Ohashi, K., Nagata, K., Wada, A., Kangawa, K., et al. (1998). Cofilin phosphorylation by LIM-kinase 1 and its role in rac-mediated actin reorganization. *Nature*, 393(6687), 809–812.
- Yang, Z., Rayala, S., Nguyen, D., Vadlamudi, R. K., Chen, S., & Kumar, R. (2005). Pak1 phosphorylation of snail, a master regulator of epithelial-to-mesenchyme transition, modulates snail's subcellular localization and functions. *Cancer Research*, 65(8), 3179–3184.
- Yang, L., Wang, L., & Zheng, Y. (2006). Gene targeting of Cdc42 and Cdc42GAP affirms the critical involvement of Cdc42 in filopodia induction, directed migration, and proliferation in primary mouse embryonic fibroblasts. *Molecular Biology of the Cell*, 17(11), 4675–4685.
- Yang, M. H., Wu, M. Z., Chiou, S. H., Chen, P. M., Chang, S. Y., Liu, C. J., et al. (2008). Direct regulation of TWIST by HIF-1alpha promotes metastasis. *Nature Cell Biology*, 10(3), 295–305.
- Yates, L. L., Schnatwinkel, C., Hazelwood, L., Chessum, L., Paudyal, A., Hilton, H., et al. (2013). Scribble is required for normal epithelial cell-cell contacts and lumen morphogenesis in the mammalian lung. *Developmental Biology*, 373(2), 267–280.
- Yeganeh, B., Wiechec, E., Ande, S. R., Sharma, P., Moghadam, A. R., Post, M., et al. (2014). Targeting the mevalonate cascade as a new therapeutic approach in heart disease, cancer and pulmonary disease. *Pharmacology & Therapeutics*, 143(1), 87–110.
- Yeo, D., He, H., Patel, O., Lowy, A. M., Baldwin, G. S., & Nikfarjam, M. (2016). FRAX597, a PAK1 inhibitor, synergistically reduces pancreatic cancer growth when combined with gemcitabine. *BMC Cancer*, 16 (24-016-2057-z).
- Yoon, C., Cho, S. J., Aksoy, B. A., Park do, J., Schultz, N., Ryeom, S. W., et al. (2016). Chemotherapy resistance in diffuse-type gastric adenocarcinoma is mediated by RhoA activation in cancer stem-like cells. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, 22(4), 971–983.
- Yu, H. W., Chen, Y. Q., Huang, C. M., Liu, C. Y., Chiou, A., Wang, Y. K., et al. (2015). Beta-PIX controls intracellular viscoelasticity to regulate lung cancer cell migration. *Journal of Cellular and Molecular Medicine*, 19(5), 934–947.
- Yu, F. X., Luo, J., Mo, J. S., Liu, G., Kim, Y. C., Meng, Z., et al. (2014). Mutant Gq/11 promote uveal melanoma tumorigenesis by activating YAP. *Cancer Cell*, 25(6), 822–830.
- Yu, F. X., Mo, J. S., & Guan, K. L. (2012). Upstream regulators of the hippo pathway. *Cell Cycle (Georgetown, Texas)*, 11(22), 4097–4098.
- Yu, H. G., Nam, J. O., Miller, N. L., Tanjoni, I., Walsh, C., Shi, L., et al. (2011). p190RhoGEF (rgnf) promotes colon carcinoma tumor progression via interaction with focal adhesion kinase. *Cancer Research*, 71(2), 360–370.
- Zagzag, D., Krishnamachary, B., Yee, H., Okuyama, H., Chiriboga, L., Ali, M. A., et al. (2005). Stromal cell-derived factor-1alpha and CXCR4 expression in hemangioblastoma and clear cell-renal cell carcinoma: Von hippel-lindau loss-of-function induces expression of a ligand and its receptor. *Cancer Research*, 65(14), 6178–6188.
- Zanconato, F., Cordenonsi, M., & Piccolo, S. (2016). YAP/TAZ at the roots of cancer. *Cancer Cell*, 29(6), 783–803.
- Zaritsky, A., Tseng, Y. Y., Rabadan, M. A., Krishna, S., Overholtzer, M., Danuser, G., et al. (2004). p21-activated kinase 1 phosphorylates and regulates 14-3-3 binding to GEF-H1, a microtubule-localized rho exchange factor. *The Journal of Biological Chemistry*, 279(18), 18392–18400.
- Zhang, Q., Bai, X., Chen, W., Ma, T., Hu, Q., Liang, C., et al. (2013). Wnt/beta-catenin signaling enhances hypoxia-induced epithelial-mesenchymal transition in hepatocellular carcinoma via crosstalk with hif-1alpha signaling. *Carcinogenesis*, 34(5), 962–973.
- Zhang, L., Valdez, J. M., Zhang, B., Wei, L., Chang, J., & Xin, L. (2011). ROCK inhibitor Y-27632 suppresses dissociation-induced apoptosis of murine prostate stem/progenitor cells and increases their cloning efficiency. *PLoS One*, 6(3), e18271.
- Zhao, X. H., Laschinger, C., Arora, P., Szaszi, K., Kapus, A., & McCulloch, C. A. (2007). Force activates smooth muscle alpha-actin promoter activity through the rho signaling pathway. *Journal of Cell Science*, 120(Pt 10), 1801–1809.
- Zhao, B., Lei, Q. Y., & Guan, K. L. (2008). The hippo-YAP pathway: New connections between regulation of organ size and cancer. *Current Opinion in Cell Biology*, 20(6), 638–646.
- Zhao, B., Ye, X., Yu, J., Li, L., Li, W., Li, S., et al. (2008). TEAD mediates YAP-dependent gene induction and growth control. *Genes & Development*, 22(14), 1962–1971.
- Zhou, C., Licciulli, S., Avila, J. L., Cho, M., Troutman, S., Jiang, P., et al. (2013). The Rac1 splice form Rac1b promotes K-ras-induced lung tumorigenesis. *Oncogene*, 32(7), 903–909.
- Zhou, X., Thorgeirsson, S. S., & Popescu, N. C. (2004). Restoration of DLC-1 gene expression induces apoptosis and inhibits both cell growth and tumorigenicity in human hepatocellular carcinoma cells. *Oncogene*, 23(6), 1308–1313.
- Zhu, Y., Chen, H., Boulton, S., Mei, F., Ye, N., Melacini, G., et al. (2015). Biochemical and pharmacological characterizations of ESI-09 based EPAC inhibitors: Defining the ESI-09 “therapeutic window”. *Scientific Reports*, 5, 9344.
- Zhu, G., Wang, Y., Huang, B., Liang, J., Ding, Y., Xu, A., et al. (2012). A Rac1/PAK1 cascade controls beta-catenin activation in colon cancer cells. *Oncogene*, 31(8), 1001–1012.
- Zhu, F., Zhang, Z., Wu, G., Li, Z., Zhang, R., Ren, J., et al. (2011). Rho kinase inhibitor fasudil suppresses migration and invasion though down-regulating the expression of VEGF in lung cancer cell line A549. *Medical Oncology (Northwood, London, England)*, 28(2), 565–571.
- Zins, K., Gunawardhana, S., Lucas, T., Abraham, D., & Aharinejad, S. (2013). Targeting Cdc42 with the small molecule drug AZA197 suppresses primary colon cancer growth and prolongs survival in a preclinical mouse xenograft model by downregulation of PAK1 activity. *Journal of Translational Medicine*, 11 (295-5876-11-295).
- Zins, K., Lucas, T., Reichl, P., Abraham, D., & Aharinejad, S. (2013). A Rac1/Cdc42 GTPase-specific small molecule inhibitor suppresses growth of primary human prostate cancer xenografts and prolongs survival in mice. *PLoS One*, 8(9), e74924.