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Role of Rho GTPases and their regulators in cancer progression

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1. ABSTRACT

Rho family of GTPases is an ubiquitiously expressed and evolutionarily conserved family of GTP binding proteins that regulate actin dynamics and intracellular signaling. Among the Rho family GTPases, three members RhoA, Rac1 and CDC42 have been well characterized. They each play pivotal roles in gene expression, cell proliferation, apoptosis and various cellular functions. They are driven by signaling from RhoGDIs, RhoGEFs, RhoGAPs and cell surface receptors. Abnormalities in Rho GTPase function have major consequences on cell behavior. Over expression of Rho GTPases is associated with reorganization of actin cytoskeleton, an increase in cell migration, invasion and metastasis which are important aspects of cancer progression. This review will explore these Rho GTPases and the function of their associated signaling pathways in different types of cancers.

2. INTRODUCTION

Rho family-GTPases are a subgroup of the Ras superfamily of GTPases and it consists of 23 members that can be subdivided into six groups; Rho proteins (RhoA, Rho B, RhoC), Rac proteins (Rac1, Rac2, Rac3, Rho G), CDC42 proteins (CDC42, TC10, TCL, Wrch1, Chp), Rnd proteins (Rnd1, Rnd2, Rnd3/Rho E), Rho BTB proteins (Rho BTB1, Rho BTB2, Rho BTB3) and Miro proteins (Miro1, Miro2). Among the 23, three best studied members are Rac1, RhoA and CDC42. These three Rho members share significant homology in amino acid sequence. Each member has a different biological consequence on the actin cytoskeleton (1, 2). Rho GTPases bind to either GDP or GTP, are active when bound to GTP, and GTP binding is induced by guanine nucleotide exchange factors (GEFs), and GTP hydrolysis resulting in GDP binding is controlled by GTPase activating proteins (GAPs) (3). Rho GTPases regulate the assembly and organization of actin cytoskeleton in all cells. They have been implicated in

normal cell behavior and in cancer. Activation of Rho, Rac and CDC42 leads to the assembly of stress fibers, lamellipodia and filopodia respectively. These effects allow for the dynamic organization of actin. Also, Rho GTPases regulate several biological processes relevant to cancer including cell cycle control, epithelial cell polarity, cell migration, cell survival and angiogenesis. Rho GTPases stimulate cell proliferation by promoting G1-S transition or through cyclin D1 and/or by negatively regulating cell cycle inhibition. Activation state of Rho, Rac and CDC42 depends upon balance with their regulators (GEFs, GAPs, GDIs) (4-6).

More than 70 effector proteins for Rho GTPases have been described. One of the best characterized effector proteins for Rac1 and CDC42 is p21 activated kinase (PAK). Another well characterized effector protein for RhoA is RhoA associated coiled coil containing protein kinase (ROCK). Actin dynamics are regulated by orchestration of different signaling pathways downstream of the small GTPases. RhoA interacts with Rock, which in turn activates MLCK leading to activation of myosin, increased contractility and formation of stress fibers. Also, RhoA stimulates actin polymerization through mDia1 and mDia2. CDC42 binds to N-Wasp and signals to Arp2/3 complex leading to formation of filopodia and actin polymerization. Rac1 activates PAK and WAVE, which results in altered actin nucleation activity of Arp2/3 complex. In addition, RacGEF Tiam1 can bind IRSp53 and Arp2/3 protein to regulate Tiam/Rac mediated actin polymerization.

Rho family of small GTPases has been reported to involve in tumorigenesis and metastasis. Depletion of Rac1 strongly inhibits lamellipoida formation, cell migration and invasion in glioblastoma and breast cancer cells. Rho GTPases are critical for tumor progression in glioma and breast cancer cells and play important roles in tumor growth, progression, metastasis (7,8). The recently identified GEF Asef2, increases the expression of Rac and CDC42, reduces RhoA and promotes cancer cell migration and metastasis (9). Understanding the functions of these GTPases will provide strategies to predict tumor progression and therapeutic outcomes.

3. REGULATION OF RHO, RAC AND CDC42 IN CANCER

Rho GTPases regulate several biological processes relevant to cancer including cell cycle control, epithelial cell polarity, cell migration, cell survival and angiogenesis. Dysregulation of cell polarity is a common phenomenon of carcinoma cells and CDC42 is implicated in regulation of cell polarity. Cell migration is a major event in invasion of malignant tumor cells into neighboring tissues and developing metastases. As discussed above Rho GTPases induce filpodial (CDC42) or lamellipodial (Rac) protrusions. Whereas, RhoA induces formation of stress fibers and focal adhesions, cell contraction and detachment at the rear of the cells. Rho GTPases control the directionality of cell migration and CDC42 plays an important role in directionality. Many tumors exhibit enhanced expression of Rho GTPase genes and increases in Rho GTPase activities suggesting that RhoGTPases are important regulators of tumor progression.

3.1. Rac1 in cancer

The GTPase Rac is important in lamellipodia formation during migration of cells in response to various signaling events. Rac1 expression and activation are important for tumor cell diapedesis and reduction of RhoA expression increases invasion and diapedesis (10). We will briefly discuss how Rac1 regulates tumor cell function through some important proteins including JNK, D4GDI, Mellitin, PhospholipaseCγ1 (PLCγ1), and cAMP.

JNK signaling has been implicated in signaling cascades that regulate tumorigenesis. Environmental stresses trigger JNK signaling (11) and JNK downstream pathways control cell differentiation, proliferation and apoptosis. Rac is an important positive regulator of JNK activation in these cascades (12). In MDA-MB-231 cells cultured on soft agar, targeted disruption of the Rho-GDP dissociation inhibitor, D4-GDI ablates growth and metastasis. Absence of D4-GDI leads to a reduction of Rac1 mediated apoptosis. Rac1 normally activates p38/JNK kinase to induce apoptosis and inhibit tumor growth (Figure 1) (12). Motility, invasion and metastasis of renal, breast and liver carcinomas are regulated by proteases, growth factors and Rac1 mediated JNK signaling. In support of this notion, pharmacological suppression by Mellitin (a toxic peptide derived from bee venom) of Rac1 activity inhibits metastasis of liver cancer cells in nude mice (13).

PLCγ1 is involved in cell motility and invasion which contribute to tumor progression and metastasis. Down regulation of phospholipase Cγ1 in breast cancer cells impairs Rac1 activity (Figure 1) (14). PLCγ1 is expressed in metastatic breast and colon carcinomas and is active in response to integrin receptor engagement or growth factor receptor signaling, and induces hydrolysis of phosphatidylinositol-4,5-bisphosphate to form the second messengers diacyl glycerol and inositol-1,4,5-triphosphate. Recent studies reveal that invasion and metastasis of breast cancer cells requires PLCγ1 activation of Rac1 (14).

Lamellipodia formation is one of the important events required at the leading edge during migration. The second messenger, inositol-1,4,5-triphosphate is reported to inhibit migration of embryonic fibroblasts and breast cancer cells and it acts downstream of the Rho GTPase, Rac (15). Migration of mouse embryonic fibroblast cells and 4T1 breast tumor cells is inhibited by cAMP downstream of Rac signaling (Figure 1). Elevated levels of cAMP disrupt cell migration by interfering with lamellipodia formation, phosphorylation of MLC and promotion of VASP phosphorylation (15).

Increased expression of Rac1 is observed in numerous cancers including testicular cancer, gastric and breast cancer. In an experimental model, it was shown that Rac1 deficient mice exhibited reduced tumor growth and prolonged survival. Also *in vitro* data indicate Rac1

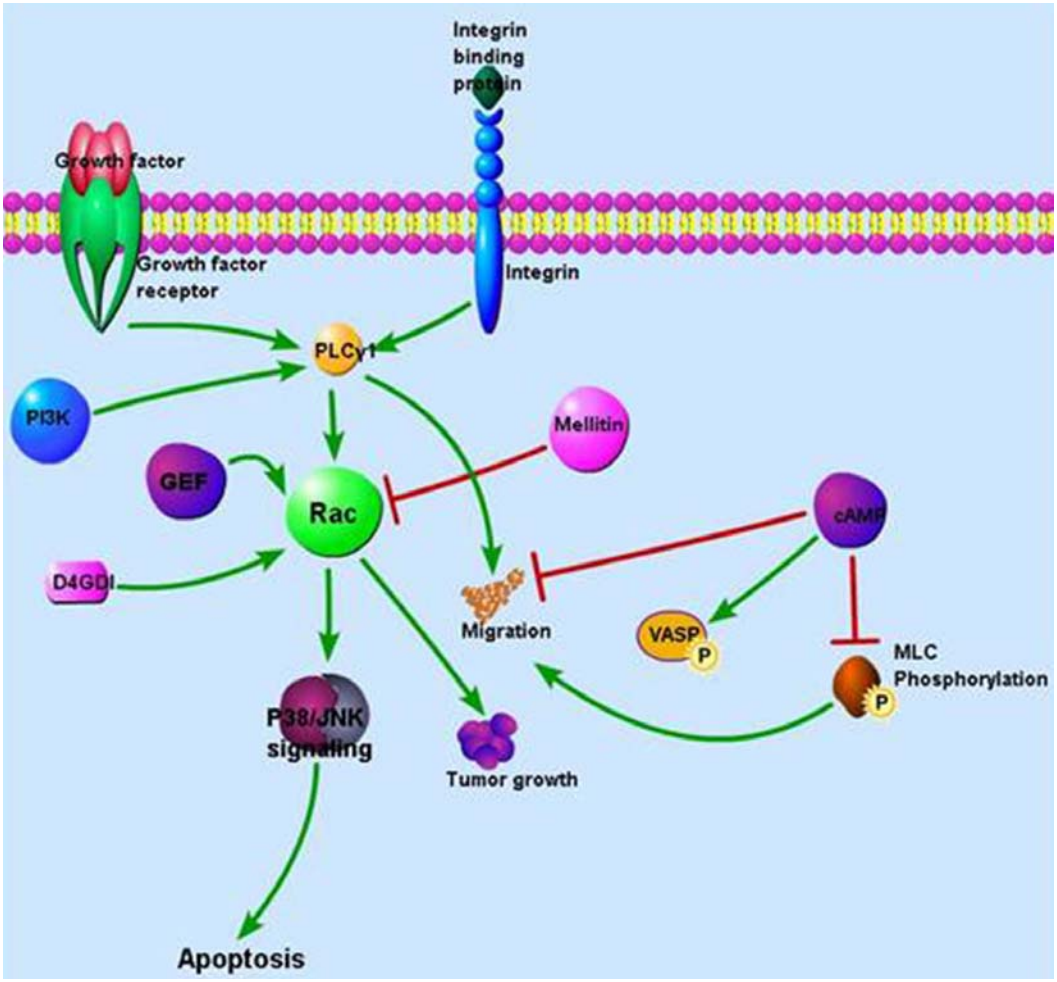

Figure 1. Rac signaling; Integrin and growth factor mediated signal activates PLCγ1; the activated PLCγ1, GEF and D4GDI induce Rac activity in turn activates P38/JNK signaling to affect apoptosis. Also Rac induces tumor growth. Mellitin inhibits Rac function; cAMP induces VASP phosphorylation and it inhibits migration directly or through inhibition of MLC phosphorylation; PLCγ1 also affects migration.

promotes transformation of primary epithelial cells. In chronic myolegenous leukemia (CML) elevated Rac1 activation is observed. Furthermore, the oncogene BCR-ABL is capable of direct activation of Rho GTPases. Rac1 is important for formation of lamellipodia and migration in primary cells and invasion in tumor cells in many different types of cancers. Rac1 is up-regulated in several cancers and plays a pivotal role in tumorigenesis.

3.2. RhoA in cancer

Over expression of RhoA promotes invasion both *in vitro* and *in vivo*. RhoA regulates actomyosin-based transmigration of tumor cells. Recently, activation of RhoA has been observed upon autocrine motility factor-induced motility in HCC. Myosin-interacting guanine nucleotide exchange factor (Myo-GEF) regulates cell polarity, RhoA (Figure 2) and RhoC activities in MDA-MB-231 breast cancer cells. Myo-GEF mediates actin polymerization through RhoA and RhoC but not through Rac1 and CDC42 (16). In this section we will discuss about pathways connecting RhoA to cAMP, E-cadherin, p120-catenin, LC3 and PI-3K.

A methyl xanthine derivative, Pentoxifylline (PTX) increases the levels of cAMP and protein kinase A. Also, PTX inhibits Rac1 and RhoA induced stress fibers and lamellipodial protrusions (17). As a result of this regulation of cAMP, PTX inhibits invasion, MMP secretion and cell adhesion in B16F10 melanoma line. During epithelial tumor progression tumor cells acquire the ability to invade surrounding tissues and metastasize. E-cadherin is the main epithelial receptor for cell-cell adhesion and loss of this receptor function coincides with an epithelial to mesenchymal transition, associated with transition of a benign adenoma into carcinoma. P120 catenin promotes invasive capability of E-cadherin deficient cancer cells through p120 mediated reduction of RhoA activity (Figure 2). The N-terminal domain of p120 is essential to promote invasion and the N-terminal domain of P120 binds to RhoA to inhibit its activity (18).

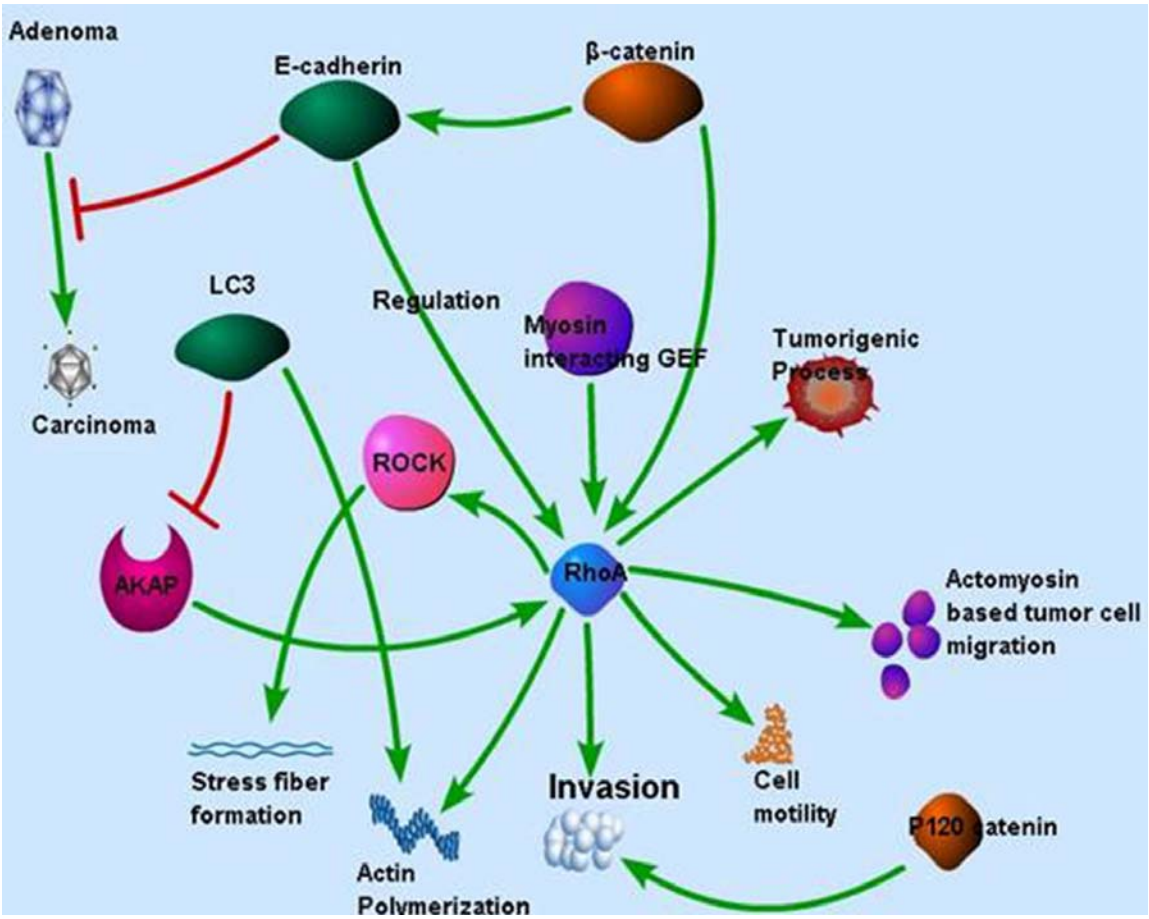

Figure 2. RhoA signaling; β-catenin upregulates function of E-cadherin which inhibits the process of conversion of Adenoma into carcinoma and E-cadherin regulates RhoA. Function of RhoA is induced by various factors including myosin interacting GEF, β-catenin and AKAP. RhoA regulates tumorigenesis (cell migration, invasion) through its actions on actin cytoskeleton reorganization process. Stress fiber formation is regulated by ROCK which is a downstream effector of RhoA. P120catenin involved in the process of invasion. LC3 inhibits AKAP.

During tumor cell migration, RhoA mediates membrane retraction at rear of the cell. Inhibition of Rho or ROCK enhances protrusion and significantly reduces cell motility due to this loss of retraction leading to stable anchoring that impedes forward motion. Inhibition of RhoA also leads to activation of Rac1 at the edge of the cell (19). A consequence of mutation in E-cadherin is enhanced motility and invasion resulting from alterations of the expression of Rac1 and RhoA and reductions in membrane localization of p120-catenin. E-cadherin is proposed to normally function as an endogenous inhibitor of Rac1 that increases RhoA activity to maintain cell-cell contacts and impede migration (20). p120-catenin controls the expression of RhoA, CDC42 and Rac1 in lung cancer. The reduced expression of p120-catenin correlates with reductions in E-cadherin level. This reduction of E-cadherin and elevation of Rho GTPases by p120catenin can lead to malignancy in lung cancer (21).

The hepatocellular carcinoma (HCC) is a very aggressive cancer with low patient survival rates. In progression of HCC, alteration of Rho GTPase pathways plays an important role. Changes in the Rho pathways in HCC are detected with the GTPases, the inhibitors upstream and downstream effectors. Tumorigenesis is initiated by over expression of RhoA and RhoC or the prevention of Rho GAP (1). The A-kinase anchoring protein (AKAP) member AKAP-Lbc has been implicated in tumor development and it activates RhoA as a GEF (Figure 2). LC3 is a novel interacting protein of AKAP-Lbc that inhibits AKAP-Lbc function by disruption in its interaction with RhoA. Consistent with a regulation of Rho, LC3 promotes cytoskeletal rearrangements and actin fiber assembly (22). RhoA expression is high in liver, skin, and colon cancers. An increase in RhoA expression is observed in conjunction with elevated RhoA activity, poor prognosis and increased frequency of recurrence in liver cancer. Furthermore, increased RhoA levels were reported in ovarian (23), bladder (24), gastric (25) and breast (26) and testicular (27). These data indicate that RhoA plays an important role in cancer progression.

3.3. CDC42 in cancer

ErbB2 is an important factor in breast cancer. ErbB2 regulates Ras/Raf-1/MAPK/ERK pathway leading to tumor growth and migration and this signaling pathway is influenced by PAK, Rac and CDC42 (28). CDC42 controls formation of filopodia which are thin protrusions containing actin bundles through, in part, the ability of CDC42 to bind the Wiskott-Aldrich syndrome protein (WASP) during actin polymerization. It is speculated that CDC42 signals for reduction of cofilin phosphorylation and this induces formation of filopodium (29). In this section we will discuss the involvement of CDC42 in tumor morphology and factors known to regulate CDC42 dependent functions such as DOCK10, Nucleophosmin, P38, SAPK, JNK, SRF and Nd1-L.

Tumor cell motility in a 3D matrix tends to frequently exhibit two modes of migration mesenchymal and amoeboid. Rac induced protrusions are formed at the leading edge with mesenchymal movement. In contrast, during amoeboid tumor cell motility, CDC42 is activated and the CDC42 effectors N-WASP (Figure 3) and PAK2 are important for maintenance of amoeboid movement (30). Arp2/3-dependent actin assembly requires CDC42 activity at the leading edge of lamellipodia. CDC42 regulates directionality in mesenchymal movement and CDC42 is controlled by DOCK10 in melanoma cells. DOCK10 expression induces epithelial to mesenchymal transition and is implicated in tumor progression. DOCK10 controls CDC42 and amoeboid movement in coordination with integrin signaling. The DOCK10-CDC42-PAK2 pathway leads to the phosphorylation of MLC2 (Figure 3) (30). Further investigation is required about the relationship between CDC42 and DOCK10 to understand how CDC42 and DOCK10 pathways control tumor progression.

In lymphomas, nucleophosmin (NPM)-anaplastic lymphoma kinase (ALK) activates RhoGEF VAV1 and downstream signaling to Rho-family GTPases. NPM-ALK regulation of CDC42 controls lymphoma cell migration, proliferation and survival. Absence of CDC42 in anaplastic large cells lymphoma (ALCL) impairs proliferation and survival. Vav1 increases CDC42 activity in ALCL cells and this regulates tumor cell shape and migration. The inhibition of either Vav1 or CDC42 is associated with cell cycle arrest and apoptosis. So CDC42 is an important regulator of lymphoma cell survival, migration, and morphology (31).

H-Ras induced transformation of rodent fibroblasts requires CDC42 and Rac1. Rac1 and CDC42 are upstream signaling proteins for several downstream effectors such as PAK, P38/SAPK, JNK and serum responsive factor (SRF). However, activated Rac1 or CDC42 alone are not sufficient for the malignant transformation of human fibroblasts (32). Nd1-L is an actin cytoskeleton stabilizing protein and is expressed ubiquitously in mouse tissues. The over expression of Nd1-L in NIH3T3 cells leads to inhibition of Rho, Rac and CDC42 (Figure 3) (33). The over expression of Nd1-L in colon carcinoma and melanoma cell lines prevents metastasis and ND1-L knockdown increases metastasis perhaps due to this regulation of RhoGTPases. CDC42 is highly expressed in breast cancer (34) and testicular cancer (27) and CDC42 expression levels correlate with tumor progression. Animal models failed to reveal this tumor promoting function due to over expression of CDC42. In contrast, loss of CDC42 resulted in neoplasia (35). In summary, these reports indicate that CDC42 may function as both a tumor promoter as well as tumor suppressor.

4. GUANINE NUCLEOTIDE EXCHANGE FACTORS (GEFs)

Rho GTPases regulate diverse cell functions such as cell motility, proliferation and apoptosis through their molecular switch properties. This regulatory function is controlled by different protein families which include negative regulators GAPs and positive regulators GEFs. GTP hydrolysis is induced by GAPs to reduce Rho binding to GTP and GEFs enhance the exchange of GDP for GTP to increase RhoGTP binding. Most GEFs belong to the Dbl-RhoGEF family, they are characterized by Dbl-homology and pleckstrin-homology (PH) domains (36). Tumor cell invasion and migration are regulated by Rho, Rac and CDC42 and these specifically function in actin cytoskeletal organization. Rho GTPases are regulated by the integration of signals from both RhoGEFs and RhoGAPs (37) however we will focus our discussion on the function of a selection of RhoGEFs that will elucidate their roles in cancer.

4.1. Rac1 GEFs in cancer

Vav3 is a Rho-GEF that regulates vascular smooth muscle cell proliferation and migration by activating PAK signaling and is reported to be a point of convergence in Rac1 activation. Of the over 60 RhoGEFs, only Vav3 regulates vascular smooth muscle cell proliferation and migration. Thus, targeting Vav3 provides a therapeutic approach to reduce vascular proliferation during tumor-associated angiogenesis (38).

Tiam-1 is a Rac GEF that associates with adapter protein IRSp53 to link Rac and Wave2 in a complex and promotes Arp2/3 dependent actin polymerization (6). P-Rex1 is a Rac-GEF that enhances cell migration following Rac activation in neuronal cells. P-Rex1 is important for localization and regulation of actin cytoskeletal dynamics in the axonal growth cone that controls neurite differentiation (39). Tiam1 protein levels are high in prostate carcinomas and correlate positively with poor disease prognosis. A similar correlation was found in breast cancer; where in high expression of Tiam-1 is linked to invasion of breast cancer cells.

Rac influences the canonical Wnt pathway through regulation of β-catenin. DOCK4 is a Rac-GEF that regulates β-catenin levels in response to Wnt signaling. Phosphorylation of DOCK4 is enhanced by GSK3-β, and DOCK4 phosphorylation enhances Wnt-induced Rac activation supporting notion that DOCK4 is a scaffold protein in Wnt/β-catenin signaling (40). Asef2 promoted cell migration requires Rac1 and the signaling pathway is dependent on phosphotidyl inositol-3kinase (PI3-K), Akt

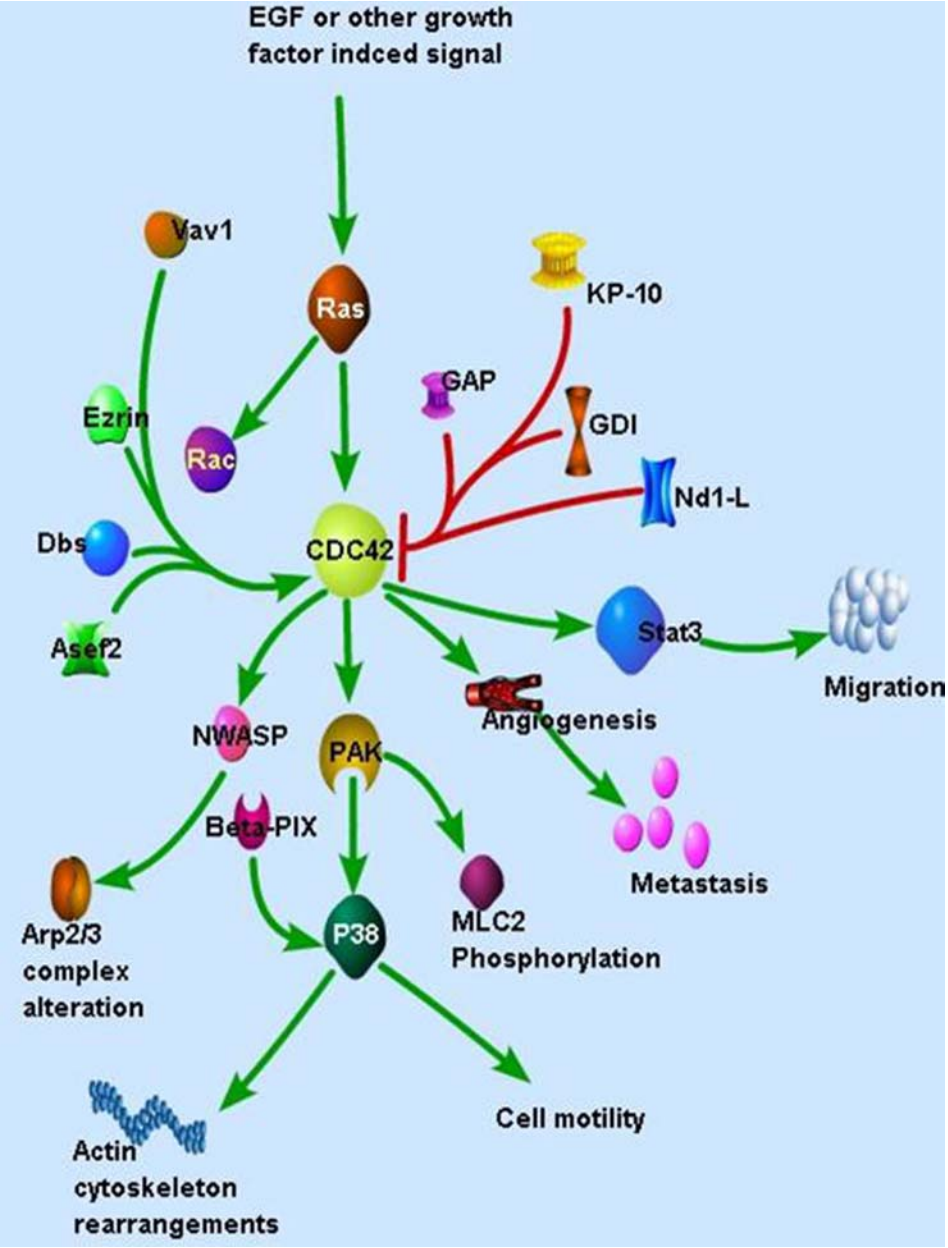

Figure 3. CDC42 signaling; Epidermal growth factor or other growth factor mediated Ras activity leads to CDC42 function. CDC42 activity is upregulated by RhoGEFs including Vav1, Ezrin, Dbs and Asef2, and is inhibited by GAP, KP-10, GDI and ND1-L. CDC42 is involved in migration, metastasis through alteration actin reorganization by upregulating stat3 or PAK/P38 signaling. PAK induces MLC2 phosphorylation and beta-PIX induces P38.

and Rho (9). Protein kinase Ciota (PKCiota) expression is elevated in lung cancer, and activates Rac1-PAK-MEK1,2-Erk1,2 signaling. RhoGEF epithelial cell transforming sequence 2 (Ect2) is amplified coordinately with PKCiota. Depletion of Ect2 leads to down-regulation of Rac1 and constitutively active Rac1 restores the transformed phenotype of Ect2-deficient cells (41).

RacGEF DOCK180 activates Rac in a process dependent on ELMO1. In glioma cells, DOCK180 expression is elevated. Over expression of the proteins in the DOCK 180-ELMO1 complex enhances cell migration and invasion (42). These reports clearly indicate that several Rac GEFs may function as oncogenes.

4.2. RhoA GEF in cancer

RhoA is localized to the leading edge of migrating cells and functions to induce protrusion and ruffling. RhoA activates assembly of contractile actomyosin filaments and FAK complexes. However, lower levels of active RhoA are found in PDGF-induced membrane protrusions (43). In breast cancer, RhoA-

GEFs were investigated and MyoGEF was identified as a positive regulator of RhoA activity in the breast cancer line MDA-MB-231. In the leading edge of migrating cells, MyoGEF colocalizes with non-muscle myosinIIA and depletion of MyoGEF impairs invasion and cell polarity. MyoGEF is also expressed in invasive breast cancer cells resulting in high levels of RhoA activation. GEF H1, a RhoA specific GEF, increases tumor cell proliferation and it is hypothesized that this increased activation of RhoA enhances invasion and metastasis (16).

4.3. CDC42 GEF in cancer

CDC42 enhances filopodia formation and is involved in cell polarity. Breast epithelial cell motility is regulated by a signaling complex that contains CDC42. Dbs is a Rho-GEF that activates CDC42 and Rac1 in breast cancer cells during cell motility. Transient inhibition of CDC42 using siRNAs attenuates Dbs-induced motility (44). ECM protein Ezrin controls breast cancer cell migration and promotes metastasis in osteosarcoma and rhabdomyosarcoma. Ezrin may do so through regulation of Rho GTPases through protein interactions with Dbl that promotes binding to and activation of CDC42. Onco-Dbl stimulated changes in morphology are dependent on CDC42 and ERM proteins. In absence of ERM binding, CDC42 activity is reduced (45). These studies indicate CDC42 GEFs regulate cell polarity, migration and metastasis.

4.4. Other regulators of Rho GTPases

Activation of Rho GTPases is regulated by guanine nucleotide exchange factors (GEFs), while inactivation is mediated by GTPase activating proteins (GAPs). Rho guanine nucleotide dissociation inhibitors (GDIs) regulate GTPase function by preventing cycling of the GDP bound form and by suppressing the interaction of GTP bound Rho proteins with their effectors.

Over 60 GAPs have been discovered that hydrolyze GTP to GDP. A well characterized example of a Rho GAP is p190 Rho GAP that down-regulates RhoA (46). Suppression of GAP function leads to high levels of GTP bound RhoA causing an elevation of GTPase activity and sustained downstream signaling. In support of important role of RhoA activities in carcinomas, tumorigenic Rho GAP for RhoA and CDC42, DLC2 (Deleted in Liver Cancer), is down regulated in HCC. Furthermore, DLC1 is frequently deleted in breast tumors (reviewed in (42)).

GDIs interact with GTP bound GTPases and prevent activation of downstream effectors. Rho GDI binds to Rho, Rac and CDC42 proteins and is over expressed in ovarian cancers (47, 48). An increase in Rho GDI expression positively correlates with invasion of breast cancer cells (49, 50). In contrast, Rho GDI down regulation stimulates development of metastatic bladder cancer (51). These observations suggest that dysregulation of Rho signaling may either promote or inhibit tumor progression.

5. RHO GTPase MEDIATED SIGNALING PATHWAYS

Cellular activities observed during tumor progression are cell migration, invasion, survival and metastasis. It is clear that actin cytoskeleton dynamics during cell migration are mediated by Rho/ROCK/LIMK and/or Rac/PAK/LIMK pathway (52). Cofilin prevents actin polymerization by converting F-actin into G-actin. Cofilin is inactivated by LIMK phosphorylation (53) resulting in an increase of actin polymerization (52, 54). LIMK activity is regulated by the downstream effectors of Rho, namely Rho kinase (ROCK) and PAK (55, 56). Downstream of Rac and CDC42 (57) ROCK activates LIM kinase, cofilin phosphorylation and this enhances actin polymerization (58). PAK regulates the filament branch nucleator Arp2/3, cortactin and WAVE (59, 60).

5.1. Rac signaling

MCF7 cells are highly proliferative in response to estrogen if Rac1 is activated by adhesion to collagen I. In contrast, proliferation and Rac1 activity are reduced by adhesion to laminin. The activities of JNK and c-Jun parallel Rac1 and are elevated on collagen I and reduced on laminin suggesting a novel role for ECM and integrin function in the regulation of Rac1-JNK and JNK/c-Jun/cyclin D1 pathways of proliferation induced by estrogen in breast cancer (61).

Cell motility is regulated by LOX induced p130 (Cas) phosphorylation that leads to an increase in Rac-GTP through p130Cas/Crk/DOCK180 (62). Patients receiving treatment for ErbB2 positive breast cancers with trastuzumab frequently acquire drug resistance within a year. The levels of Rac1 increase and EGFR and ErbB2 decrease in trastuzumab resistance. Inhibition of Rac1 activity is sufficient to disrupt resistance through blocking the trastuzumab-mediated endocytic down-regulation of ErbB2 and triggering a reduction in the levels of ERK (63).

C-Src is a proto-oncogene involved in the progression of cancers in many tissues including breast and colon. Activated Src enhances cell transformation via Ras-ERK pathways and Rac1 is strongly activated and is required for transformation by Src. Src induces phosphorylation of the RhoGEF Tiam1 that targets Vav2 and Rac1 (64). Thus, Rac signaling is important in proliferation, migration, transformation and resistance to trastuzumab.

5.2. CDC42 Signaling

Betapix is a PAK binding exchange protein and it is highly expressed in human breast cancers. Betapix has been shown to be involved in the regulation of CDC42/Rac GTPases and actin cytoskeletal organization. Over expression of betaPix increases activation of p38 (Figure 3), an important downstream effector of CDC42/Rac GTPases (65). Transforming growth factor β (TGFβ) receptor is a TGFβ superfamily co-receptor with β-glycan. Levels of this receptor are significantly reduced in most cancers. β-glycan activates CDC42 interaction with β-arrestin2, and this leads to rearrangements of the actin cytoskeleton (66).

Kisseptins were originally identified as metastasis suppressors in melanomas. KP-10 is a decapeptide derived from the primary translation product of the Kisseptin KISS1. KP-10 recently was demonstrated to prevent angiogenesis by inhibiting the activation of c-Src/FAK and Rac/CDC42 pathways (Figure 3) and suppressing the transcription of VEGF (67). Thus, KP-10 inhibits tumor metastasis through disrupting the ability of tumors to initiate angiogenesis. Signal transducer and activator of transcription-3 (STAT3) is activated by a number of receptor and non-receptor tyrosine kinases and STAT3 expression is associated with neoplastic transformation. Rac1 and CDC42 play important roles in activation of STAT3 (68). Constitutively activated Rac1 enhances IL-6 expression and STAT3 activation and endogenous Rac leading to cell migration and proliferation (68). Important actin cytoskeletal regulators are P21-activated kinases (PAKs) which are effector proteins for the Rho-GTPases Rac and CDC42 (69). PAKs have been to shown to have pro-tumorigenic function in various cancers (70). Thus, Rac and CDC42 cooperatively regulate various signals during cancer progression (Figure 3).

5.3. RhoA signaling

P27 is a RhoA binding protein important for controlling cell proliferation. P27 regulates cell cycle progression and has an essential role in cell motility and migration. p27 binding to RhoA modulates activation of RhoA/ROCK pathway (Figure 2) (71). Membrane type-1 matrix metalloproteinase (MT1-MMP) and p27RF-RhoA are essential to tumor cell invasion. p27RF-Rho promotes RhoA activation and triggers formation of punctate actin structures called invadopodia that are involved in tumor cell invasion. In invasive tumor cells Rho induces invadopodia with localized concentrations of matrix protease activity that colocalize with MT1-MMP, cortactin and actin (72). p90 ribosomal S6 kinase (RSK1) is an effector of Ras-MAPK cascade and it enhances RhoA inhibition to promote cell motility by loss of actomyosin stability. Thus, RhoA regulates cell migration through several signaling mechanisms.

5.4 Integrins and Rho GTPase signaling

Integrins are an important group of cell surface receptors that regulate survival, proliferation, gene transcription and migration (73). Cytoplasmic tails of integrins recruit signaling proteins that control adhesion-dependent processes such as cell migration and cell growth (74). Extracellular faces of integrins bind with ligands in extracellular matrix. Integrin receptor engagement with ligands regulates activity of then signaling pathways through function of protein complexes at the cytoplasmic face of integrin receptor. Here we discuss the function of the integrin ligand laminin and factors recruited to integrins, namely syndecan 1 and caveolin in cancers.

The transmembrane heparin sulfate proteoglycan, syndecan1, may regulate the pathways connecting integrins to actin remodeling and tumor cell motility. Syndecan1 is expressed in numerous carcinomas. Syndecan1 depletion ablates adhesion induced RhoA activation, but strongly activates Rac1 signaling. A consequence of syndecan overexpression is inhibition of invasion. In Squamous cell carcinoma, syndecans promote adhesion to collagen through α2β1 integrin. In breast carcinoma lines, vitronectin binding by integrins αvβ1 and αvβ5 also recruits syndecan (75). Syndecan pathways that cooperatively regulate integrin functions and adhesion-dependent processes need to be further studied in cancer.

Cellular responses to growth factors are controlled by cell adhesion to ECM. Presence of growth factors in the ECM is sensed by growth factor receptors in cooperation with integrins and controls intracellular signaling linked to proliferation and motility. Rho GTPases are integrators of integrin and growth factor receptor signals. Alteration of signaling components, downstream of integrins or growth factor receptors can promote tumor invasion and metastasis. Rac1 and RhoA drive transcription of cyclinD1 in response to integrins and growth factors through activating JNK, PI3K, NF-kB or MAPK signaling. Integrin uncoupling through detachment from ECM leads to internalization of cholesterol enriched membrane micro-domains in which caveolin 1 (Cav1) proteins are localized and cav1 plays an important role in limiting the ECM-associated signals. Cav1 null mouse embryonic fibroblasts have increased Rac-PAK signaling and deregulation of Cav1 correlated with increased cell proliferation in some cancers (76). Caveolin-1 induces membrane targeting of Rac, concentrating Rac with its effectors to induce signaling for growth, independent of cell adhesion or anchorage. In conclusion, cav 1 is frequently down-regulated in cancer and cav-1 regulates integrin downstream signaling for growth (76). Integrins play critical roles in regulation of Rho GTPases and their downstream effectors. Integrin function is regulated in a cooperative manner with growth factor and other cell surface receptors.

6. PERSPECTIVE

The Rho GTPases Rac1, CDC42 and RhoA are critical regulators of cell-cycle progression, proliferation and tumor progression through control of cell signaling. But further investigation is required to understand the Rho GTPase functions and signaling pathways that differ in normal and cancer cells. Further understanding of RhoGTPase signaling pathways will yield therapeutic insights into approaches to selectively disrupt tumor proliferation and tumor progression.

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