**Rho GTPases in cancer pathophysiology and treatment**

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**ABSTRACT:** Rho GTPases, a prominent member of the RAS proteins family, function as molecular switches regulating fundamental biological processes in all eukaryotic cells, by modifying cell response on different molecular signalling pathways. Unlike the majority of RAS oncogenes, mutant forms of these proteins are rarely found. Nonetheless switching between the active GTP-bound forms to the inactive GDP forms, are additionally facilitated by a different set of molecules known as GEFs, GAPs and GDIs, whose deregulation is widely associated with the cancer phenotype. Principally, early research involved them in actions pertinent to cytoskeletal dynamics, cell polarity, focal adhesion and vesicle trafficking. However, recent evidence underpins the contribution of their aberrant expression in acquiring distinct hallmarks governing tumorigenesis, on top of premature assumptions on metastatic potential, like replicating immortality, apoptotic resistance and angiogenesis.

**Keywords:** Rho GTPases, cancer biology, metastasis, cell polarity

**INTRODUCTION**

RHO proteins were initially identified upon completion of the Human Genome Project, based on their sequential similarity to Ras oncogenes. So far, 20 members have been successfully cloned and categorized in accordance with their functional and biochemical similarities, besides homologous nucleotide regions. Guanine nucleotide binding properties along with intrinsic GTPase activity stand as fundamental functional hallmarks in the majority of all isoforms. Pertinent research has effectively widened their contribution to biological processes from regulating cytoskeletal dynamics through actin remodeling, cell adhesion and migration. Documented evidence suggests that their wide range of associated intracellular signaling molecules involves core biological procedures such as cell polarity, vesicle trafficking, apoptosis, chromosome segregation and downstream regulation of cyclin levels to induce cell cycle progression and proliferation.

Similar to Ras proteins, regulation of RHO proteins is achieved through adopting the “on and off” conformation by hydrolyzing GTP to GDP and cycling between the active and inactive state. The limited effectiveness of RHO-GTPases activity has also led to the discovery of three distinct molecular agents governing their kinetics. RHO guanine nucleotide exchange factors (RHO-GEFs) promote formation of the GTP-bound active state, RHO-GTPase-activating proteins (RHO-GAPs) catalyze GTP hydrolysis to achieve the GDP-inactive state and RHO-GDP dissociation inhibitors (RHO-GDIs) sequester GDP-bound RHO proteins from the GDP-GTP cycle back in the cytoplasm and inhibit GDP nucleotide dissociation. Newly identified sequences within the human genome suggest that crucial housekeeping proteins carry domains, homologous to RHO-GEFs and RHO-GAPs, perplexing their partial role in a systemic level.

Well-researched members of RHO protein family include RHOA, RAC1 and CDC42, with extensively studied aspects in physiological and developmental processes. A wide variety of candidate effectors also interact with the activated form of most RHO proteins. Their multi-faceted role in malignant neoplasms is associated with an increasing population of mediators, encompassing the majority of the hallmarks of cancer, through their involvement in multiple signaling cascades that modify cell response. Unlike Ras mutants (such as K-Ras, N-Ras, and H-Ras), where activating muta-
RHOA in metastasis, CELL cycle progression, metabolism and endocytosis

Modern research has consistently accentuated aberrant expression of RHOs as the predominant oncogenic factor, contrary to an abundant set of point mutations found in Ras. An increasing number of diseases associate with overexpression and underexpression of different RHO members. Orgaz et al. has reviewed over 15 cancer types with differential RHOA, RHOB, RHOC, RAC1 and CDC42 expression levels. RHOA is found overexpressed in most primarily developed malignant neoplasms, linking prognosis by grading in a dose-dependent manner, in correlation to its concentration levels. The majority of findings revolve around their role in stress fiber arrangement and actin crosslinking. RHOA has an important role in all protrusive events involved in cellular movements, by interacting with cytoskeletal and cell membrane proteins at the leading and trailing edge, which drive cell motility. RHOA, RHOB, RHOC share various classes of overlapping effectors comprising lipid kinases, scaffold proteins, and serine/threonine kinases. Among those, thoroughly studied targets within RHOA pathways include ROCK I and II, PRK1/PKN, Citron, NgR1, LINGO1, p75, TROY, CSPG and DIAPH1 or mDia.

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regulatory networks include: 1) ROCK-mediated phosphorylation of LIM kinase (LIMK) that inactivates coflin (also known as ADF, actin-binding agent that disassembles actin filaments), stabilizing actin filaments and facilitating in situ polymerization and 2) phosphorylation and activation of adducins (ADD1, ADD2 and ADD3) that control ruffling motility through spectrin recruitment. Contraction on the actin filaments affects cell shape as progressive bundling, clusters and pulls along membrane integrins that bind to free actomyosin ends, distorting the cell’s microscopic outline. Evidence suggests that inhibited effectors, despite RHOA excessive activity, can explain molecular events conducive to positive cell migration signals like lowered density in actomyosin bundles, reduced F-actin polymerization and unstable focal adhesions. Therefore, amoeboid movement of tumor cells in the extracellular matrix and transmigration below the layer of the basic membrane coincide with systematic changes in contractile force.

Altering the ratio between DIAPH1 and ROCK seemed to be the main factor affecting the balance between membrane ruffles with distinct protrusions (including filopodia and lamellae) and stress fiber formation. Those functions constitute a molecular oscillation between actomyosin contractility and actin polymerization. Expression of integrin α6β4 has been linked with early stages of developing membrane protrusions. LPA stimulation promotes RHOA-dependent lamellipodia formation, enhancing considerably cell migration and invasive potential.

Bioenergetics were chosen as an emerging hallmark of cancer, after seminal observation on tumor cells exhibiting increased glycolytic activity, a phenomenon known as the Warburg effect. Recently, it was discovered that mutant members of the tumor suppressor protein p53 stimulate aerobic glycolysis by promoting GLUT1 translocation to the plasma membrane, a mechanism called gain of function (GOF). The procedure is mediated by active RHOA and ROCK.

Uncontrolled proliferation and growth transformation involves both Ras and RHO pathways. RHOA stimulates the cyclin D1 promoter, inducing upregulation of cyclin D1, while causing downregulation of critical cyclin-dependent kinase inhibitors like p21 (WAF1/CIP1) and p27 (KIP1). A synergetic mechanism involving Ras, allows cell cycle entry, as RHOA can repress p21 (CIP1) activation by oncogenic Ras, which plays a pivotal role in G1 cell cycle progression. Downregulation on p27 (KIP1) is hypothetically attributed to elevated cyclin E-CDK2 complex or sustained ERK-signaling. Skp2-mediated degradation, involving both DIAPH1 and ROCK to promote G1 progression has also been proposed. RHOA, presumably along with CDC42 or RAC1, also increase MYC levels, leading to increased cyclin E expression, which in turn stimulates cyclin-E–CDK2 activity and activates the epithelial mesenchymal transition route (EMT). Many growth factor pathways including EGF, HGF, LPA, PDGF indicate RHOA activation, the WNT-adenomatous polyposis coli (APC)-β-catenin oncogenic signaling route is perhaps among the most described. WNT1 activates RHOA and the less known RHO family member, WRCH1. RHOA, in conjunction with RAC1, regulates the function of ERM member ezrin (protein family of ezrin, moesin and radixin) taking part in cell motility and proliferation, by crosslinking actin filaments to the plasma membrane using membrane-spanning ECM receptor CD44.

A recent molecular switch has been proposed in cytoskeletal reorganization, involving Rhotekin and S100A4, by applying measurements in activity and membrane anchoring of RHOA in breast cancer cells. According to the proposed model, S100A4 binds to the myosin IIA heavy chain, preventing oligomerization and coarsening contractility. Parallel expression of rhotekin and S100A4, followed by growth factor induced RHOA activation, forms a macromolecular complex that restricts myosin II oligomerization, pushing the cytoskeletal balance towards the membrane protrusion end, away from stress fiber formation. siRNA murine models have underlined the importance of simultaneous expression between rhotekin and S100A4, as well as the existence of downstream regulators like ROCK, myosin light-chain kinase (MLCK), heavy chain of non-muscle myosin IIA (MHC-IIA) and phosphorylated myosin light chain (pMLC).

Extracellular matrix (ECM) degradation and remodeling is indirectly mediated by RHOA through regulating the levels of matrix metalloproteinases (MMPs), known for their hydrolytic activity on stable ECM protein complexes or by regulating the levels of pertinent antagonists, like tissue inhibitors of metalloproteinases (TIMPs). Metastasizing cells from distant sites to enter either the blood or the lymphatic vasculature as well as oxygen and nutrient supply, is facilitated by the acquisition of a different cancer hallmark, angiogenesis. Activated RHOA-ROCK signaling...
pathway in tumor endothelial cells, shifts a strictly homeostatic balance towards increased pro-angiogenic factors like vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin-6 (IL-6), interleukin-8 (IL-8) and regulates hypoxia-triggered induction of HIF1α. Roles of less known members like RHOC and RHOJ are barely understood, while partial contribution from CDC42 and RAC1 has also been described. 25 Yeast models also revealed the existence of a clathrin-independent endocytic pathway involving the GTPase RHO1, a RHO member whose mammalian homolog is RHOA. 26

RHOB in thyroid and pancreatic malignancies
RHOB localizes in endocytic vesicles and regulates intracellular trafficking through vesicle formation. Normally, they can alter epidermal growth factor receptor (EGFR) localization by inducing endocytic trafficking and transcytosis of endosomes from basolateral to apical membranes in polarized epithelial cells.27

RHOB is successfully identified as a direct target of miR-19a, which precipitates tumor growth, and is downregulated in human pancreatic cancer samples. Putative treatments aim towards decreasing miR-19a levels and restoring RHOB concentration by inducing apoptosis, inhibiting cell cycle progression and metastasis.28

Similar experiments were performed in thyroid cell lines, which were infected by recombinant adenovirus vector, carrying oncogenic forms of thyroid hormone receptor β (TRβ). Decreased expression or inactivating somatic mutations is usually associated with reduced dissociation of HDAC1 and HDAC3 that catalyze histone deacetylation from the RHOB promoter. Acetylated nucleotides vitiate DNA helix binding with histone proteins due to rearrangements in opposite charges between the two molecules, leading to enhanced RHOB expression. Normally, adequate expression leads to p21-associated cell-cycle arrest in the G0/G1 phase inhibiting proliferation.29

RHOH alterations in tumors of myeloid origin
Among the 20 members of the RHO family, only RAC2 and RHOH demonstrate expression activity restricted to the haematopoietic cell lineage in normal bone marrow. Oncogenic RHOHs include aberrant somatic hypermutation and genetic translocations in order to produce fusion transcripts. RHOH, initially named trans-
lation three four (TTF), due to the discovered chimeric transcript, has a regulatory role in T-cell receptor expression in normal thymocytes and RAC1 antagonist properties, contributing to apoptosis and cell division control. Examples of identified diseases involving aberrant expression of RHOH include multiple non-Hodgkin’s lymphoma types, B-cell post-transplant lymphoproliferative disorder (PTLD), follicular lymphoma (FL), multiple myeloma (plasma cell neoplasm) and hairy-cell leukaemia.  

**CDC42 in proliferative signaling, cell motility and gene expression**

Cell division control protein 42, known as CDC42, consists of two distinct subunits; Cdc42Hs and G25K. Initially discovered in Saccharomyces cerevisiae, experimentation confirmed its role as a mediator of cell division and involvement polarity establishment.  

Extracellular stimuli such as epidermal growth factor receptor (EGFR) ligands affect intracellular concentration and activity of CDC42. Overexpressed or mutant EGFR-related downstream effectors, as well as the receptor itself, also take part in many types of human cancer. Nevertheless, mitogenic signaling in terms of molecular pathology also involves EGFR endocytosis and degradation, by E3 ubiquitin ligase Cbl (casitas b-lineage lymphoma) adaptor proteins. Proposed models involve the formation of an inhibitory complex between CDC42 effector Cool-1/ß-pix and c-Cbl ubiquitin ligase, in order for increased CDC42 levels to affect EGFR abundance.  

Metabolic abnormalities in a cellular level have emerged as a putative hallmark of cancer development, based on mutagenic properties in a hypothetical array of metabolic byproducts with genotoxic potential, as well as deregulated catabolic pathways, which provide an endless supply of energy substrates to cancer cells. Experiments on alterations in metabolic features have focused in high glutamine consumption, which in turn, fosters proliferation by replenishing TCA (tricarboxylic acid cycle, or Krebs) intermediates utilized for nucleic acid and glucose biosynthesis. Cdc42F28L mutant-transformed fibroblasts exhibited elevated mitochondrial glutaminase activity, sustaining glutamine metabolism. Excessive glutamine production has been proposed as a typical finding in tumor cells. These cancers enhance glutaminolysis and a-ketoglutarate production through increased levels of glutamate dehydrogenase (GDH), which converts glutamate to aKG. GTP loading of a different set of small RHO GTPases like RagA/B, RagB/C and RheB is modified by aKG levels facilitating stimulation of lysosomal translocation and subsequent activation of mammalian target of rapamycin C1 (mTORC1), also inducing cell growth signals and inhibiting autophagy pathways.  

Transendothelial migration and endothelial cell-cell interaction depends on β1 integrin expression levels, modified via the transcription factor serum response factor (SRF), which is strictly regulated by CDC42. Integrins define interactions between cell surface and underlying extracellular matrix. Deregulation can lead to cell spreading and protrusion extension along blood vessels, facilitating the establishment of metastatic colonies. IQGAP1 was originally discovered as an effector of both CDC42 and RAC1, taking part in cadherin-mediated cell adhesion through β-catenin, regulated by calmodulin. Its GRD domain interacts with small GTPases CDC42, RAC1, and TC10. Recent evidence has shed light on its multiple targets and the transient spatiotemporal organization of the signaling events involved, regulating cytoskeletal rearrangement, MAPK activation and β-catenin-mediated gene transcription.  

CDC42 regulates the formation of actin contacting protrusions that extend into the extracellular matrix (ECM), known as invadopodia. Experiments on breast cancer cell lines have shown that CDC42-interacting protein 4 (CIP4) interacts with actin remodeling proteins, promoting endocytosis and internalization of transmembrane type I matrix metalloprotease (MT1-MMP), responsible for protein degradation within the ECM that allows, often metastatic, cell migration. Overexpression of CDC42 also induces formation of fingerlike protrusions, which come out from the cell periphery in an actin polymerization-dependent manner, known as filopodia. Despite filopodia being extensively researched over their role in the leading edge formation of migrating cells, recent works underpin filopodia’s significance in blood vessel lumen morphogenesis for cancer tissues. Arp2/3 is a protein complex associated with proteins N-WASP, WAVE1, cortactin, and Cdc42 that regulates three-dimensional cell migration in pathophysiological states such as cancer. Fibrosarcoma cell line findings support that nucleating the actin filament assembly, is deregulated by forming secondary protrusions, branching off from the primary protrusions, thanks to activated complexes by unregulated CDC42 activity.
Similar to RHOA, cell polarity acts as a homeostatic factor in maintaining exposure to strictly-regulated mitogenic or apoptotic signals, creating apico-basal special membrane domains, as well as limiting invasion and disruption of cell-ECM architecture. CDC42 orchestrates major molecular events of this machinery by spatial regulation of its Golgi pool. Golgi matrix protein 130 (GM130) was found to reduce the activity of CDC42 at the Golgi by activating RasGRF which sequesters CDC42 back to the apparatus, without affecting cell membrane CDC42 in the same cell. Depletion of GM130 disrupts polarity, by regulating active Golgi-localized CDC42 that supplies the leading edge, while no alteration in ER-Golgi structure and trafficking was observed. Actin-binding protein IQGAP1 is identified as an effector of RAC and CDC42. Extensively researched, IQGAP1 is the founding member of the IQ-GAP family, consisting of three isoforms with particular expression patterns. IQGAP1 controls actin-crosslinking in focal adhesion and lamellipodia formation by interacting with active forms of CDC42/RAC, determining subcellular localization. GTP-bound CDC42 also relieves N-WASP auto-inhibition and activates actin nucleation points in conjunction with the Arp2/3 complex, whose binding regions recognize IQGAP1 and the VCA domain in N-WASP.

RAC1 in pancreatic cancer development

RAC proteins take part in lamellipodium extension and stimulate membrane-ruffle formation. Three isoforms have been described (RAC1, RAC2, RAC3) with high sequence similarity but various expression patterns among human tissues. RAC1, is the most researched member of the family, playing key roles in embryonic development through germ layer formation. Ubiquitously expressed, this isoprenylated membrane-bound protein takes part in many types of neoplasms. Pancreatic mutations in mediators of pancreatic ductal adenocarcinoma (PDA) seem to activate Ras pathways involving RAC1 signaling. Long-time research has rendered pharmacological inhibition of mutated KRAS - commonly expressed among PDAs from oncogenic al-
leles, ostensibly ineffective. Current data support targeting of downstream regulators like RAC1, which interacts with p110α/p85 complexes, localized at the cell membrane in order to promote cytoskeletal rearrangement and transformation. Increased levels of second messengers like phosphatidylinositol facilitated by that complex stimulate secondary molecular pathways and might require complementary targeting to inverse selective cellular functions pertaining to oncogenesis. 42

RHO-guanine nucleotide exchange factors (GEFS) in tumorigenesis

RHO-GEFs play a key role in different aspects of tumorigenesis, according to the nature of the underlying mutation. Generally, their potential oncogene capabilities in cancer progression have been linked with their physiological action in activating GTPases. Genetically engineered deletion of coding sequences in experimental cell lines and mRNA libraries have proposed, however, a minority of tumor-suppressor effects mainly attributed to discrepancies among downstream effector targeting. 43 RHO GTPase hyperactivation can mainly occur through RHO-GEF overexpression and stands as a dominant cause of aberrant RHO GTPase signaling, in contrast to elevated enzyme levels observed in Ras GTPases.

Chromosome rearrangements can produce structurally mutated GEFs and form chimeric fusion proteins, whose constitutual activation is connected with human cancers. The breakpoint cluster region (BCR)–Abelson 1 (ABL1) fusion protein encoded by the chimeric gene, stems from a balanced translocation found in 90% of chronic myelogenous leukemias. Translocation between those genetic regions residing in chromosomes 22 and 9 is responsible for the cytogenetic trademark of the disease, known as Philadelphia chromosome. BCR was initially recorded as a gene of unknown function. Nevertheless, recent findings support its RHO regulating role, as RHO-GEF and RHO-GAP domains have been found within its structure. In the resulting BCR-ABL1 only the integrity of the RHO-GEF is selectively retained excluding RHO-GAP properties. Hence oncogenesis is mediated by excessive RHO-GEF activation along with constitutively activated ABL1 tyrosine kinase. 44

T cell lymphoma invasion and metastasis 1 (TIAM1) factor, also acting as a downstream effector of Ras serves as guanine nucleotide exchange factor in multiple cancers. As its name suggests elevated levels in aggressive forms of T-cell lymphoma, facilitated premature association with malignant potential. 45 Two decades ago, it was identified as a putative Rac1-specific GEF. However, correlations in a lesser extent with Cdc42 and RhoA widened putative targets, combining different protein interaction domains found in its structure that affect vesicle trafficking, cell adhesion, microtubule dynamics and migration.

Dedicator of cytokinesis 1 (known as DOCK1 and Dock180) along with engulfment and cell motility 1 (ELMO1) factor are evidently associated with invasive forms of glioblastoma. Both in vitro and in vivo model studies have demonstrated that overexpressed DOCK1, a bipartite guanine nucleotide exchange factor (GEF), endows glial cells with the ability to disperse throughout the brain, thus, promoting recurrence and resistance to established therapeutic schemes. 46

RHO-GTPASE-activating proteins (GAPS) in malignant transformation

GAPs promote the opposite act of GEF signaling nodes and contribute to malignant transformation by curtailing GTPase activity, modifying the amplitude and duration of a particular RHO pathway. In contrary to many Ras GAPs less is known about their population and binding sites, although the majority of them have distinct tumor-suppressor properties. 47

Members of the ‘Deleted in Liver Cancer’ RhoGAP protein family (abbreviated as DLC), predominantly DLC1 GAP, have been found under-expressed in tumor specimens of human hepatocellular carcinoma (HCC). Three DLC genes are encoded in the human genome, sharing much of their genetic sequence and protein structure, but differ in their individual subcellular localizations. DLC1 has been discovered in 1998 in homozygously deleted HCC-derived cell lines. Nevertheless, comprehensive research supports its role in a multitude of cancers such as breast, colon, lung, prostate, ovarian and pancreatic types. Structurally pertinent, DLC2 is significantly downregulated in a wide group of human malignant neoplasms in humans and DLC3, originally isolated from human myeloid cell line libraries is also associated with a variety of primarily developed tumors inflicting kidney, lung, ovarian, uterine and breast tissues. Uncontrolled cell proliferation, independence to growth signals and metastatic motility are the dominant pathophysiological procedures. 48

Agaps and Asaps, members of the ArfGAP subfamily are also implicated in oncogenesis, by acting on different biological responses. Missense mutations of AGAP2, also known as centaurin-γ1, PIKe and G-
GAP2, have been closely related with heterogeneous diagnostic profiles in prostate cancer initiation and progression by acting as a putative ligand for different mediators that amplify activating signaling via both the AKT and NFkB pathways. On top of that, phosphorylated GGAP2 affects NF-kB transcriptional activity by binding the p50 subunit of the cytoplasmic form. 49

ASAP1 (also known as centaurin-β4, AMAP1 and DDEF1) overexpression is associated with invasive phenotypes in head and neck squamous cell carcinoma. Expression of epidermal growth factor receptor (EGFR) and cortactin has been highly correlated with cancer phenotypes in various forms of HNSCC. Two discrete AMAP1 functions have been described; physical association with cortactin as a ligand, inhibiting metastatic properties and interaction with Arf6 as a downstream regulator, which employs GEP100 to enable invasive capabilities. 50

**RHO-GDP dissociation inhibitors (GDIS) expressed in tumors**

Variations in RHOGDI1 and RHOGDI2 expression levels have been discovered in an inexhaustible array of organ-specific cancers types and every tissue has found to carry a typical gene expression pattern, rendering the differential comparison of mRNA levels useful in extracting information with therapeutic value. RHOGDI1 expression is upregulated in ovarian and colorectal cancer, while a dose-dependent model has been proposed to link metastatic potential and resistance to tubulin-oriented chemotherapy (eg. Paclitaxel). 51 On the other hand, RHOGDI1 levels are decreased in human brain tumors, deregulating RAC1 balance between active and inactive state, although several main RHO units like RHOA and RHOB seem to be downregulated. 53 Inverse correlation among mRNA concentrations tied with quantifiable degrees of malignancy and RHOGDI expression reflect different groups of candidate effectors, in RHO subfamilies, conflicting results or lacking data tend to widen that particular knowledge gap. Decreased RHOGDI1 transcripts in human breast cancers are strongly associated with significantly higher levels of Rho-C, Rho-6, and Rho-G among other Rho members. 54 In hepatocellular carcinoma, amplified miR-151 exerts this function by directly targeting RHOGDI1 and lowered levels bear higher invasive status. Current evidence suggests that RHOGDI1 acts as a putative metastasis suppressor, so its downregulation by miRNA’s inhibitory effect on ribosomal translation contributes to intrahepatic metastatic phenotypes, with subsequent RAC1, CDC42 and RHO activation. 55 miR-151 is localized in an intron of its hosting gene, encoding focal adhesion kinase (FAK). Increased levels of both miR-151 and FAK transcripts promote RHOGDI1 miRNA degradation by forming complementary base pair connections.

Deregulation of RHOGDI2 expression also points to certain cancer types. Exacerbated pathological profiles in pancreatic carcinoma, linked with increased invasiveness, were found along with increased RHOGDI2 levels, modulating the expression of matrix metalloproteinase-2 (MMP-2). 56 Uncontrolled proteolysis affecting the extracellular matrix by different metalloproteinase enzymes, undermines stable cellular adhesion and polarity in terms of tissue architecture, facilitating metastatic motility. Despite the abundant expression of RHOGDI2 in hematopoietic cell neoplasms and non-Hodgkin lymphomas, it has been discovered that the same protein was downregulated in Hodgkin's typical Reed-Sternberg cells. 57 Besides, apart from the aforementioned role of genetically altered RHOH in tumours of myeloid origin, intensive research needs to be undertaken in order to extrapolate their relative contribution to the critical stages of survival and growth. Decreased RHOGDI2 expression has also been found in transitional cell carcinoma of the bladder, related with proportionally unpropitious patient survival and underlying tissue-specific pathophysiological events behind the stages of tumorigenesis. 58 Evidence suggests both elevated and lower levels of RHOGDI2 expression in different cell lines of human breast cancer, as those inhibitors seem to interact with the estrogen receptor (ER) and modify its transcriptional activity. Long-term research has proven the role of ER expression on affecting migration, encompassing key elements of exocrine secretion from cells stemming from the surface epithelium in gland chambers. Invasive ductal carcinoma types are mostly associated with that mechanism. 59

There’s a barely profound reasoning justifying the inability to come up with holistic models explaining RHOGDI increased or decreased expression levels and correlation with uncontrolled proliferation and migration capabilities. Because RHOGDI’s actions are manifested through a variety of effector proteins, whose transcription activity ostensibly differs among several types of normal and malignant tissues, evidence suggests distinguished motifs of mediator expression among particular cancer types in order to explain diversity between protein concentration and cellular re-
response. That doesn’t come as a surprise, given that phenotypical traits are mainly attributed to tissue-specific genes, whose products are being modified by phosphorylation mediated through downstream effector kinases that interact with multiple RHO GTPases.

FUTURE DIRECTIONS

Aberrant expression of RHO family proteins, along with their set of regulatory agents-GAPs, GEFs and GDIs, is involved in nearly all stages of tumorigenesis, as contemporary research underpins an increasing amount of molecular mediators promoting cell cycle progression, anti-apoptotic signaling, neoplasm angiogenesis, inflammation and altered metabolic profile. A plethora of experimental data from gene knockout mice and interfering RNA (iRNA) is being constantly added to the current body of knowledge. Ultimately, efforts are coordinated to solve an array of controversies surrounding molecular understanding of the implicated functions and facilitate comparisons and strict distinctions among closely related isoforms within the RHO archetype. More sophisticated in vitro studies should be carried out to fully elucidate the complete network of mediators and effectors that constitute cellular responses responsible for distinguished phenotypes in malignant transformation. Coordinated attempts could establish reliable therapeutic ventures in vivo to extend chemotherapy treatment schemes.

ABBREVIATIONS

ABL1 – Abelson 1 factor
ADD1/2/3 - Alpha adducin 1/2/3
ADF - Actin depolymerizing factor
AGAPs - Arf GTPase-activating proteins
aK - α-ketoglutarate
APC - Adenomatous polyposis coli
Arp2/3 - Actin-Related Proteins 2/3 complex
ASAPs - Artery-specific antigenic proteins
BCR - Breakpoint cluster region
bFGF - Basic fibroblast growth factor
CBL - Casitas B-lineage Lymphoma
CD44 - Cluster of differentiation or Classification determinant 44
CDC42 - Cell division control protein 42 homolog
CDK2 - Cyclin-dependent kinase 2
CSPG - Chondroitin sulfate proteoglycan
DLC - Deleted in Liver Cancer
DOCK1 - Dedicator of cytokinesis 1
ECM - Extracellular matrix
EGF - Epidermal growth factor
ELMO1 - Engulfment and cell motility protein 1
EMT - Epithelial-mesenchymal transition
ER - Estrogen receptor
ERM - Ezrin, radixin and moesin
FAK - Focal adhesion kinase
FL - Follicular lymphoma
GAP - GTPase activating protein
GDI - GDP dissociation inhibitor
GDP - Guanosine diphosphate
GEF - Guanine nucleotide exchange factor
GLDH - Glutamate dehydrogenase
GM130 - Golgi matrix protein 130
GTP - Guanosine triphosphate
HDAC - Histone deacetylase
HGF - Hepatocyte growth factor
HIF1A - Hypoxia-inducible factor 1-alpha
HRAS - Transforming protein p21
IQGAP1 - Ras GTPase-activating-like protein
IL-6 - Interleukin-6
IL-8 - Interleukin-8
KRAS - Transforming protein p21
LIMK1 - LIM domain kinase 1
LINGO1 - Leucine rich repeat and immunoglobulin-like domain-containing protein 1
LPA - Lyso phosphaticid acid
DIAPH1/mDia1 - Diaphanous-related formin 1
mDia2 - Diaphanous-related formin 2
MHC-IIA - Non-muscle Myosin Heavy Chain II-A
MLC - Myosin light chain
MLCK - Myosin light-chain kinase
MMPs - Matrix metalloproteinases
MT1 - Membrane type 1-matrix metalloproteinase 1
mTORC1 - Mammalian target of rapamycin C1
NgR1 - Negative growth regulatory protein 1
NRAS - Neuroblastoma RAS viral oncogene homolog
p21/Waf1/Cip1 - CDK-interacting protein 1
p27Kip1 - Cyclin-dependent kinase inhibitor 1B
PDA - Pancreatic ductal adenocarcinoma
PDGF - Platelet-derived growth factor
PRK1 - Protein kinase C-related kinase 1
PRN1 - Protein kinase N1
PTLD - Post-transplant lymphoproliferative disorder
RAC1 - Ras-related C3 botulinum toxin substrate 1
ROCK - Rho-associated protein kinase
S100A4 - S100 calcium-binding protein A4
siRNA - Small interfering RNA
SKP2 - S-phase kinase-associated protein 2
SRF – Serum Response Factor
TCA - Tricarboxylic acid cycle
TIA1 - T-cell lymphoma invasion and metastasis 1
TIMPs - Tissue inhibitors of metalloproteinase
TRβ - Thyroid hormone receptor beta
TROY/ TNFRSF19 - Tumor necrosis factor receptor superfamily, member 19
VEGF - Vascular endothelial growth factor
WASP - Wiskott–Aldrich Syndrome protein
Oι RHO GTPάσες στην παθοφυσιολογία και θεραπεία του καρκίνου

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**REFERENCES**

13. Bishop A, Hall A. Rho GTPases and their effector pro-
34. Groenewoud M, Zwartkruis F. Rheb and Rags come together at the lysosome to activate mTORC1: Figure 1. Biochm Soc Trans. 2013;41(4):951-955.