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Rho guanine nucleotide exchange factors: regulators of Rho GTPase activity in development and disease

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Abstract

The aberrant activity of Ras homologous (Rho) family small GTPases (20 human members) has been implicated in cancer and other human diseases. However, in contrast to the direct mutational activation of Ras found in cancer and developmental disorders, Rho GTPases are activated most commonly by indirect mechanisms in disease. One prevalent mechanism involves aberrant Rho activation via the deregulated expression and/or activity of Rho family guanine nucleotide exchange factors (RhoGEFs). RhoGEFs promote formation of the active GTP-bound state of Rho GTPases. The largest family of RhoGEFs is comprised of the Dbl family RhoGEFs with 70 human members. The multitude of RhoGEFs that activate a single Rho GTPase reflect the very specific role of each RhoGEF in controlling distinct signaling mechanisms involved in Rho activation. In this review, we summarize the role of Dbl RhoGEFs in development and disease, with a focus on Ect2, Tiam1, Vav and P-Rex1/2.

Keywords

Rac1; RhoA; Cdc42; guanine nucleotide exchange factors; cancer; mouse models

Introduction

Ras homologous (Rho) family proteins (20 human members) comprise a major branch of the Ras superfamily of small GTPases,[1] with RhoA, Rac1 and Cdc42 the most extensively studied and characterized.[2] Rho GTPases specifically regulate actin organization, cell motility, polarity, growth, survival and gene transcription.[3] Rho GTPases are binary switches that cycle between an active GTP-bound and an inactive GDP-bound state (Figure 1).[4] Rho guanine nucleotide exchange factors (RhoGEFs) accelerate the intrinsic exchange activity of Rho GTPases to stimulate formation of Rho-GTP.[5] Rho GTPase activating proteins

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CONFLICT OF INTEREST

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(RhoGAPs) stimulate the intrinsic GTP hydrolysis activity of Rho GTPases, resulting in the inactive GDP-bound state. Rho guanine nucleotide-dissociation inhibitors (GDIs) comprise a third class of regulatory proteins.[6] RhoGDIs bind Rho GTPases in a GDP-bound state and also extract and sequester Rho GTPases from the cell membrane where Rho GTPases can be activated. Once activated, the GTP-bound GTPase then associates with effector targets that number over 100.[7–9] In this review, we focus on the classical Dbl family RhoGEFs and their role in development and disease. In particular, we focus on the RhoGEFs with the best validated roles in cancer (Vav1/2/3, Ect2, Tiam1/2, P-Rex1/2) and their analyses in cell culture, mouse models and human cancers.

The discovery of RhoGEFs: diverse in numbers and structure

The first RhoGEF was identified initially as an oncogene in mammalian cells (Table 1 and Figure 2). Using the same NIH 3T3 mouse fibroblast focus formation assay that led to the discovery of mutant Ras in human cancer,[10] analysis of genomic DNA isolated from a human diffuse B-cell lymphoma resulted in the discovery of the *DBL* oncogene that encoded an N-terminally truncated and activated protein.[11] Dbl was also detected earlier as an oncogene that caused the tumorigenic growth of NIH 3T3 cells after transfection with genomic DNA from the MCF-7 human breast carcinoma cell line (designated *mcf2*)[12], and only later was it determined to be identical to Dbl.[13] Dbl was subsequently shown to catalyze the GTP/GDP exchange activity of Cdc42.[14] Additional NIH 3T3 focus formation and related biological assays identified Dbl-related proteins, in particular Vav, Tiam1 and Ect2 (Table 1 and Figure 2). That RhoGEFs were discovered initially as oncoproteins provided the first suggestion that Rho GTPases may also have a function in oncogenesis.

Dbl and the Dbl-related proteins share a ~200 amino acid catalytic Dbl homology (DH; also called RhoGEF) domain and an immediately adjacent regulatory ~100 amino acid pleckstrin homology (PH) domain[5] (Figure 3). Additional RhoGEFs with this tandem DH-PH domain structure were identified by genetic and biochemical approaches and by in silico database searches. There are 70 human Dbl family RhoGEFs, many of which have conserved orthologs found in all vertebrate species and in invertebrates, including *Drosophila*, *C. elegans*, *S. cerevisiae* and *S. pombe*. The nomenclature for many Dbl RhoGEFs is complicated by the different names used in independent discoveries, by different names for orthologs of different species, by the existence of different gene products due to alternative RNA splicing and by establishment of the ARHGEF gene family nomenclature by the HUGO Gene Nomenclature Committee (http://www.genenames.org/genefamilies/ARHGEF). We have compiled a summary of Dbl RhoGEF nomenclature, based on the names most commonly used in the literature together with the additional names used for each RhoGEF (Supplementary Table 1).

In addition to their common structural elements that define them as RhoGEFs, there are some distinctive features that mark individual RhoGEF proteins. Two RhoGEFs possess tandem sets of DH-PH domains (Trio and Kalirin) and four RhoGEFs (Tuba1, 2, 3 and ARHGEF33) lack an apparent PH domain (Figure 3).[5] Dbl RhoGEFs also diverge significantly in the N- and C-terminal sequences flanking the DH/PH domains. These flanking sequences commonly contain a diversity of protein-protein or protein-lipid

interaction domains and motifs whose functions are to regulate intrinsic RhoGEF catalytic activity, determine subcellular localization and/or facilitate complex formation with other proteins (Figure 3).

This diversity in flanking non-RhoGEF sequences distinguishes the regulation and role of RhoGEFs that otherwise activate the same set of Rho GTPases. For example, extracellular stimuli that first activate RhoGEFs downstream of G-protein coupled receptors (GPCRs) and subsequently cause activation of G protein beta-gamma subunits (P-Rex), of Ras (Tiam1), or of Src family tyrosine kinases (Vav) can all converge on downstream Rac1 activation through distinct RhoGEFs (Figure 4). These flanking sequences may also regulate subcellular localization and activation of spatially distinct cellular pools of Rho GTPases, leading to their utilization of distinct effectors. Finally, these flanking sequences may facilitate scaffolding functions for RhoGEFs that can further influence the effectors activated. For example, Tiam1 possesses a PH-CC-Ex globular domain[15] that acts as a membrane targeting and protein-protein interaction domain.[16–19] This scaffolding function can then influence the effector utilization of the activated Rho GTPase.[20,21]

Humans also possess a second structurally and mechanistically distinct[22] class of RhoGEFs, the dedicator of cytokinesis or DOCK family (11 human members) that act as GEFs for Rac and/or Cdc42, but not RhoA.[23–25] DOCK RhoGEFs lack primary sequence homology with the DH domain and instead are characterized by a conserved Dock-homology region-2 (DHR-2; also called DOCKER, CZH2) that serves as the RhoGEF catalytic domain. DHR-2 domains exhibit no primary sequence homology to DH domains. Although plant species possess Rho-like GTPases (Rho of plants; Rop)[26], and are regulated by DOCK RhoGEFs, RhoGAPs and RhoGDIs homologous to mammalian RhoGAPs[27], they lack Dbl RhoGEFs. Instead, RopGEFs possess a structurally distinct plant-specific Rop nucleotide exchanger (PRONE) catalytic domain.[28,29] Finally, although there are no Rho orthologs in prokaryotes, pathogenic bacteria possess effector proteins that can regulate their mammalian host Rho GTPases in part by mimicking the function of mammalian RhoGEFs.[30,31]

RhoGEFs and development

The combined number of human Dbl and DOCK RhoGEFs greatly exceeds the number of Rho GTPases, suggesting apparent redundancy in RhoGEF function. This is particularly striking for RhoA, where at least 28 Dbl RhoGEFs can activate this single GTPase (Figure 3). With six Rho GTPases constitutively GTP-bound and active, and not believed to be regulated by RhoGEFs (Rnd1-3, RhoH/TTF, RhoBTB1/2)[32], the apparent redundancy in RhoGEFs is even more striking.

One approach to evaluate this apparent functional redundancy has been the generation of mice deficient in one or more RhoGEFs, which additionally addresses their involvement in development. The role of at least 26 Dbl family RhoGEFs in mouse development has been evaluated (Table 2), with only four (Sos1, Trio, AKAP13 and Ect2) found to be essential.[33–36] However, these proteins all possess other functions independent of their RhoGEF catalytic activities, and embryonic lethality may not be due specifically to loss of the RhoGEF function. For example, when only the AKAP13 RhoGEF domain was

disrupted, no lethality was seen, indicating that AKAP13 RhoGEF activity is not required for mouse development.[37] AKAP13 is also a scaffolding protein that associates with the regulatory subunit of protein kinase A to spatially regulate its substrate utilization. AKAP13 additionally interacts with protein kinase C and D isoforms and with heterotrimeric Gα subunits; whether any of these interactions are specifically required during development remains to be determined. Sos1 is also a RasGEF activated downstream of receptor tyrosine kinases. Trio contains a serine/threonine kinase domain in addition to its two distinct DH-PH RhoGEF domains. Although Ect2 has no other known catalytic function aside from its RhoGEF activity, there are Ect2 non-RhoGEF sequences shown to be important for regulation of cytokinesis in vitro.[38] Below we provide a summary of the developmental roles of RhoGEFs that have additional roles in cancer.

Ect2

Ect2 (Epithelial Cell Transforming Sequence 2) was originally discovered as an oncogene that transformed NIH 3T3 cells.[39] However, the Ect2 protein found to transform these fibroblasts was a truncated version of the full-length protein, formed during DNA manipulation in vitro (Figure 4). The N-terminus of Ect2 contains two regions that are homologous to XCCR1 and Clb6 domains that have functions in the DNA damage response and cell cycle regulation, respectively. However, while required for Ect2 support of cytokinesis,[38] no specific functions have been ascribed to these sequences. The N-terminus also contains two tandem BRCA1 C-terminal (BRCT) domains that have auto-inhibitory[40,41] and phosphoprotein binding functions[42,43] and two nuclear localization sequences (NLSs).[44] The central catalytic portion of Ect2 consists of the tandem DH-PH domains. Finally, the C-terminal sequence of full-length Ect2 has no known domains or motifs but is required for the transforming activity of N-terminally truncated Ect2, and was recently implicated in modulating the stability of full length Ect2 protein.[41,45,46] BRCT domains are not found in any other RhoGEFs and instead are found primarily in proteins involved in the DNA damage response network associated with cell cycle checkpoint functions.[47,48] Although analyses of full length recombinant Ect2 protein indicate that it is selective for RhoA and its related isoforms (RhoB and RhoC) in vitro, cell-based studies suggest that Ect2 can also regulate Rac and Cdc42 activity.[45]

Recently we showed that *Ect2*-deficient mice are not viable.[36] Whereas heterozygous $Ect2^{+/-}$ mice displayed normal development and lifespan, no $Ect2^{-/-}$ embryos were found at birth or as early as embryonic day 8.5. Our subsequent characterization of the defect in vitro demonstrated that isolated homozygous $Ect2^{-/-}$ blastocysts displayed abnormal outgrowth at day E3.5, indicating that Ect2 is required for peri-implantation development. The requirement for Ect2 at such an early stage of development suggests that it may play a key role in nearly all cells. Unlike a majority of Dbl RhoGEFs, there are no Ect2-related isoforms, which perhaps is a basis for its essential requirement in development.

The best-characterized normal function of Ect2 is its role in cytokinesis.[40] Ect2 has been shown to regulate RhoA during cytokinesis[49], the final step in both meiosis and mitosis. Therefore, it is likely that Ect2 is required for development because of its key role in normal cell division. Consistent with this possibility, a deficiency in *MgcRacGAP* (RacGAP1), a

RhoGAP that facilitates Ect2 localization to the central spindle during cytokinesis, also produced a similar embryonic lethality phenotype.[50] Thus, despite the existence of at least 27 other RhoGEFs that can activate RhoA (Figure 3), an Ect2 deficiency cannot be compensated for by other RhoGEFs to promote cytokinesis. However, since the Ect2 sequences flanking the DH-PH module can serve scaffolding functions and facilitate protein interactions, it is possible that non-GEF functions contribute to the critical role of Ect2 in development. A definitive resolution of this issue will require analyses of mice that harbor a RhoGEF-dead Ect2 allele.

Tiam1

Tiam1 (T-cell Lymphoma Invasion and Metastasis 1) was found by retroviral insertional mutagenesis of T lymphoma cells and then screened for invasiveness.[51] Tiam1 contains a PH domain followed by a coiled-coil motif and extra region (Ex) that together comprise a protein- and membrane-binding domain,[15] a Ras-binding domain (RBD),[52] a PSD-95/DlgA/ZO-1 (PDZ) domain, then the tandem DH-PH domains (Figure 4). Although the exact mechanism(s) of Tiam1 activation is not clear, it is apparent that it can be regulated through intramolecular inhibition (coiled-coil motif with the DH domain), protein-protein interactions (Ras binding), and cellular localization (phospholipids with the PH domain) (Figure 4). Tiam1 is a Rac-selective GEF and was also shown to function as an effector of Ras.[52] Although Rac1 deficiency causes severe embryonic lethality,[53] Tiam1 is non-essential for mouse development.[54] Thus, other GEFs that activate Rac can compensate for Tiam1 loss-of-function, for example the related Tiam2 protein (37% overall identity, 71% DH domain identity).[55,56]

P-Rex1/2

P-Rex1 (PtdIns(3,4,5)P3-dependent Rac Exchanger) was discovered by purifying proteins from the cytosol of neutrophils and assaying for proteins that activate Rac1 in the presence of phosphatidylinositol (3,4,5)-triphosphate (PtdIns(3,4,5)P3/PIP3).[57] Two different groups of researchers identified P-Rex2 by searching for proteins with sequence identity to P-Rex1.[58,59] P-Rex1 and P-Rex2 are very similar proteins (58% identity) that share amino-terminal DH-PH domains, followed by two Dishevelled/Egl-10/Pleckstrin (DEP) and two PDZ domains, and then a C-terminal sequence that displays homology to inositol polyphosphate-4-phosphatase (IP4P) (Figure 4). P-Rex1 and likely P-Rex2 are both regulated through GPCR activation and Gβγ activation and phosphoinositol 3-kinase (PI3K)-stimulated formation of PIP3. P-Rex1 function is attributed primarily to Rac activation, although P-Rex1 can also activate Cdc42 and other Rho family GTPases in biochemical assays.[57]

P-Rex1-deficient mice are healthy and viable but weigh less, with significantly smaller livers, than *P-Rex1*$^{+/+}$ mice[60]. It was also shown that *P-Rex1*$^{-/-}$ mice on a pure C57BL6 background displayed a “white belly”, white feet and tail, a phenotype with 100% penetrance.[61] This phenotype was caused by a defect in melanoblast migration. The belly, feet, and tail are the most distal points of melanoblast migration from the neural crest during mouse embryogenesis, leading to the anatomical pattern of this pigmentation defect.

P-Rex2 has two splice variants: a larger product that is very similar to P-Rex1 (P-Rex2a) and a smaller product (P-Rex2b) that does not contain the IP4P homology region. Whereas P-Rex1 is expressed in peripheral blood leukocytes, neither of the P-Rex2 variants is present there. Instead P-Rex2a is found predominantly in skeletal muscle, intestine, and brain, whereas P-Rex2b is found only in heart.[59]

P-Rex2-deficient mice are viable and fertile with normal appearance and weight. However, *P-Rex2*$^{-/-}$ mice displayed a mild defect with motor coordination that worsens with age.[62] Furthermore, the *P-Rex1*$^{-/-}$ *P-Rex2*$^{-/-}$ double knockout mice exhibited defects in motor activity, posture, and gait consistent with cerebellar dysfunction that are stronger compared to the *P-Rex1*$^{-/-}$ or *P-Rex2*$^{-/-}$ single knockout mice.[62]

Vav1/2/3

Vav1 (named after the sixth letter in the Hebrew alphabet) was also discovered initially as an oncogene that caused growth transformation of NIH 3T3 cells. [63,64] Vav1-containing DNA was isolated from a human esophageal carcinoma, and the transforming variant also contained an N-terminal truncation of sequences upstream of the DH-PH domains. Two highly related isoforms, Vav2 and Vav3, share significant sequence identity (51 and 58% identity, respectively) and domain structure with Vav1 (Figure 4).[65–68] Vav1/2/3 all contain the following domains: calponin homology (CH), an acidic (Ac) region, DH-PH, and a cysteine-rich zinc finger domain (C1) followed by a Src homology-2 and -3 (SH3-SH2-SH3) adaptor cassette[69] (Figure 4). The active site of the DH domain is autoinhibited by a helix in the Ac region of Vav. Src and Syk kinases relieve the autoinhibition by phosphorylation of tyrosine 174 within the helix. Furthermore, the inhibitory conformation of the Ac region is stabilized through interactions of the CH domain with the Ac region and the DH-PH domains. Thus, maximal GEF activity of Vav is achieved in a multi-step process wherein the initial Src-dependent phosphorylation events that disrupt CH domain interactions are followed by phosphorylation on tyrosine 174.[70,71] Although Vav function is attributed mainly to its ability to activate Rac, Vav proteins can also activate RhoA, Cdc42 and RhoG (Figure 4).

Vav2 and Vav3 are broadly expressed, whereas Vav1 is largely restricted to hematopoietic cells under normal conditions.[72] Vav proteins are critical for the regulation of hematopoietic cell signaling by linking intracellular signaling to multi-subunit immune-recognition receptors (MIRRs).[73] *Vav1*-deficient mice are viable but display a range of defects in the immune system, with the most severe defects found in T cell development and activation. By comparison, *Vav2* deficiency did not perturb T cell development, and double knockout *Vav1/2*$^{-/-}$ mice exhibited similar T cell defects as seen in *Vav1*-deficient mice, indicating that Vav2 has no major function in T cell development in the absence of Vav1. In contrast, although *Vav3*-deficient mice also showed normal T cell development, combined deficiency of both *Vav1/3* significantly exacerbated the T cell defect seen with *Vav1* deficiency alone, indicating partially redundant roles for these two isoforms in T cell function. Finally, while combined Vav2/3 deficiency also showed no T cell abnormalities, the combined Vav1/2/3 deficiency (viable, no gross organ abnormalities) showed a severe impairment in T cell

development, with no functional T cells, demonstrating that Vav2 can have a compensatory function in the absence of both Vav1 and Vav3.

Nonoverlapping B cell defects were observed in mice deficient in either *Vav1* (B1 cell reduction, partial decrease in B cell proliferation upon B cell receptor (BCR) cross-linking) or *Vav2* (reduced BCR responses). In contrast, combined *Vav1/2* deficiency showed severe B cell maturation and response defects, demonstrating their nonredundant roles in B cell biology.[74,75] Finally, mice deficient in all three *Vav* genes displayed additional B cell developmental defects absent in *Vav2/3*$^{-/-}$ double knockout mice.[76] Thus, the Vav isoforms support both distinct and redundant functions in T and B cell development and activities, several of which cannot be compensated by other RhoGEFs.

RhoGEFs and cancer

The three Ras proteins (H-Ras, K-Ras4A/B, and N-Ras) are the founding members of the Ras superfamily of small GTPases[1] and together they comprise the most frequently mutated oncoprotein family in human cancer (33%; http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/).[10,77] However, with the exception of the recently described mutational activation of Rac1 in melanoma[78,79], Rho family GTPases have not been reported to be mutated frequently in human cancer. Nevertheless, there is substantial experimental evidence that aberrant Rho GTPase function can contribute to cancer cell proliferation, invasion and metastasis.[80] Instead, Rho GTPases are aberrantly activated more commonly by indirect mechanisms in cancer. These mechanisms include altered Rho localization mediated by GDIs; increased RhoGEF and/or decreased RhoGAP activity; altered gene expression of Rho GTPases, RhoGEFs, RhoGAPs, and RhoGDIs (Figure 1), and alternative splicing to generate constitutively active Rho GTPase isoforms (e.g., Rac1b).[81,82]

As mentioned above, although Dbl, Ect2 and Vav were identified originally as oncoproteins due to activation by N-terminal truncation, these altered RhoGEFs arose as an artifact of the DNA isolation/transfection procedure.[83] Surprisingly, despite their potent transforming activities when assayed in NIH 3T3 mouse fibroblasts, such truncated, activated Dbl RhoGEFs have not been identified in human cancers. Instead, other mechanisms that lead to the deregulated expression and/or activation of full length RhoGEFs have been identified. Recent reviews have provided more comprehensive overviews of RhoGEF activation in cancer.[4,84,85] Therefore, we have focused on the four Dbl RhoGEFs with the strongest evidence for their involvement in cancer growth (Supplementary Table 2) and have summarized representative studies for each below.

Ect2

Ect2 gene and/or protein overexpression has been described in glioblastoma,[86–88] non-small cell lung cancer (NSCLC),[89,90] esophageal,[90] pancreatic,[91] oral squamous cell,[92] colorectal carcinomas,[93] and other cancers (Supplementary Table 2).

The *ECT2* gene is located in a region of chromosome 3q26 that is frequently altered in human tumors.[94] Ect2 overexpression was associated with gene amplification in NSCLC, and this also correlated with an increase in the copy number of the gene encoding protein

kinase C iota, PKCi (*PRKCI*).[89] Ect2 knockdown was associated with decreased Rac1 activation, and constitutively activated Rac1 could compensate for the loss of Ect2, implicating this Rho GTPase in Ect2-dependent NSCLC anchorage-independent growth and invasion in vitro. Interestingly, the role of Ect2 in NSCLC was independent of Ect2 normal function in cytokinesis. Depletion of Ect2 expression in NSCLC did not result in any proliferation or multi-nucleation defects, phenotypes associated with Ect2 loss in normal cells.[36]

One mechanism that may account for a non-cytokinesis role for Ect2 in driving cancer growth under some conditions involves altered subcellular localization. Ect2 is one of two Dbl RhoGEFs (the other is Net1) with a nuclear-restricted localization in interphase cells. Fields and colleagues described regulation of NSCLC tumor growth and invasion through mislocalization of Ect2 to the cytoplasm, where it binds to a PKCi-Par6 complex to activate Rac1.[89] They found that PKCi phosphorylated Ect2 at residue T328 adjacent to the Ect2 nuclear localization sequences (NLSs), and this was associated with Ect2 cytosolic mislocalization. Furthermore, a T328D phosphomimetic but not a T328A phosphodeficient Ect2 mutant could rescue loss of endogenous Ect2 to restore lung tumor cell growth and invasion in vitro. That cytoplasmic mislocalization is an important mechanism of Ect2 activation in cancer is consistent with the earlier observation that disruption of the Ect2 NLS motifs activated the transforming activity of full length Ect2 when evaluated in NIH 3T3 fibroblasts.[44] Cytoplasmic Ect2 may then cause spatially inappropriate Rho GTPase activation to drive cancer growth.

Two studies showed that Ect2 expression correlated with poor prognosis in glioma patients and that Ect2 was important for glioma cell proliferation, migration, and invasion in vitro.[87,88] However, acute depletion of Ect2 expression by siRNA in giloma tumor cell lines showed an increase in multinucleation, characteristic of a defect in cytokinesis. Therefore, the acute decrease in proliferation, migration and invasion may have been caused by the inability of cells to complete mitosis. In contrast, sustained shRNA depletion of Ect2 did not suppress proliferation in vitro, but orthotopic xenograft tumor growth was impaired and associated with increased mouse survival.[95] Thus, tumor cells may adapt to acute Ect2 loss, such that Ect2-dependent long-term tumor growth is independent of the role of Ect2 in cytokinesis.

In summary, future studies to determine whether conditional loss of Ect2 ablates tumor growth in mouse models will provide further assessment of the driver function of Ect2 overexpression in cancer. Also, whether the mislocalization of Ect2 to the cytoplasm seen in NSCLC is the basis for a non-cytokinesis function that supports cancer cell growth will be important to evaluate in other human cancers. The potent oncogenic activities of the originally discovered N-terminally truncated Ect2, lacking the nuclear localization sequences, provides strong evidence that simple mislocalization may be an important driver of Ect2 activation in cancer. However, despite the implication of Ect2 as a driver in a variety of human cancers, the absence of any detection of N-terminally truncated Ect2 in human cancers argues that this cytoplasmic mislocalization mechanism may not hold true in the patient. In support of this possibility, in our own studies where we have established driver functions of full length Ect2 in ovarian and colorectal cancers, we have not found evidence

for cytoplasmic mislocalization as the basis for the aberrant Ect2 function in these cancers (unpublished observations).

P-Rex

The first cancer-driving role of P-Rex1 was described in prostate cancer.[96] P-Rex1 protein expression was elevated in metastatic but not primary prostate tumor cell lines and tumor tissue. RNAi silencing of P-Rex1 expression reduced Rac activity, and migration and invasion in vitro.

PREX1 mRNA overexpression has also been associated with breast cancer, in particular the estrogen receptor-positive luminal subtype and, to a lesser degree, HER2-positive tumors.[97] Suppression of P-Rex1 expression was found to reduce HER2-stimulated Rac1 activation, motility, invasion, and tumorigenic growth.[97,98]

Increased P-Rex1 protein expression has also been seen in human melanoma tissue and cell lines relative to normal melanocytes,[61] and RNAi suppression reduced Matrigel invasion of human melanoma cell lines. High P-Rex1 protein expression was associated with increased metastatic potential in immunocompromised mice. To assess a role for P-Rex1 in melanoma progression, *P-Rex*1-deficient mice were crossed to a mouse model of primary and metastatic melanoma (Tyr:$NRas^{Q61K}$/*INK4a*−/−). Surprisingly, in contrast to the breast tumor studies, *P-Rex1* deficiency did not reduce the incidence or latency of primary tumor formation. However, a significant reduction in metastasis was seen. While a mechanistic basis for these distinct roles for P-Rex1 in breast versus skin cancer progression has not been determined, one logical speculation is that this may reflect tissue type or genetic context differences in P-Rex1 function. More provocatively, it may reflect the differential activation of other Rho GTPases, aside from Rac1 (e.g., Cdc42), that then contribute to aberrant P-Rex1-dependent functions in breast versus melanoma growth and tumor progression.

Recently, in a whole-genome sequence analysis of melanoma, non-synonymous mutations in *PREX2* were found with a 14% frequency in a cohort of 107 human melanomas.[99] The mutations occurred throughout the gene, including interchromosomal translocations and nonsense mutations, with some encoding C-terminally truncated protein products due to premature termination codons. To assess the biological relevance of these mutations, P-Rex2 truncation or missense mutants were ectopically expressed in TERT-immortalized, mutant *NRAS*-expressing human melanocytes and shown to accelerate tumor formation in nude mice.

At least one P-Rex2 splice variant can promote cancer growth through a mechanism independent of its RhoGEF function.[100] P-Rex2a can directly bind and inhibit the lipid phosphatase activity of the PTEN tumor suppressor, thereby causing activation of PI3K and Akt and subsequent stimulation of pro-growth and survival pathways. *P-REX2A* mRNA is preferentially overexpressed in *PTEN*-wildtype, *PIK3CA*-mutant breast tumors. Depleting P-Rex2a in *PTEN*-wildtype and *PIK3CA*-mutant MCF-7 breast cancer cells reduced phosphorylated AKT and cell growth in vitro.

The aberrant activation of both P-Rex1 and P-Rex2 in melanoma, together with the frequent mutational activation of their key substrate Rac1 in melanoma,[78,79] provides perhaps the most compelling evidence for a critical driver function of a Rho small GTPase in cancer. Determining which effector(s) may then drive Rac1-dependent melanoma growth will be an important next step in these studies.

Tiam1

Tiam1 mRNA and/or protein overexpression has been described in many cancers that include melanoma,[101] breast,[102,103] colon,[104,105] prostate,[106] and renal cell carcinoma[107] (Supplementary Table 2).

A role for Tiam1 in tumorigenesis has been evaluated in several mouse models of cancer. First, the consequence of *Tiam1* deficiency was evaluated using the classical two-stage chemical carcinogenesis model for the initiation and promotion of cutaneous tumors.[54] Tumor formation is induced using a single application of the mutagen 7,12-dimethylbenz[*a*]anthracene (DMBA), which is then followed by repeated applications of phorbol 12-myristate 13-acetate (PMA) tumor promoter. This treatment results in mutational activation of *HRAS*(Q61L) and the formation of benign papillomas that can progress to squamous cell carcinomas (SCC). Both the incidence of SCC formation and growth of SCC tumors were reduced in *Tiam1*−/− mice. Thus, TIam1 is an important contributor to *H-Ras*-induced SCC tumor growth. These results, together with the identification of Tiam1 as an effector of Ras[52] and findings that Rac1 deficiency reduced mutant K-Ras-induced lung and pancreatic tumor formation[56,108], all support Tiam1 as an important effector of Ras-driven cancer growth. Conversely, for those tumors that did arise, *Tiam1* deficiency enhanced metastatic growth. Thus, while Tiam1 was required for tumor initiation and progression, it was antagonistic for malignant progression in these models.

The role of Tiam1 has also been evaluated in a mouse model of colon cancer, the *Apc* Min (multiple intestinal neoplasia) mice that harbor a truncated and loss-of-function mutant of the *Apc* tumor suppressor. Absence of *Tiam1* resulted in significantly reduced formation and growth of polyps, but in enhanced invasion of malignant intestinal tumors.[109]

Finally, *Tiam1* deficiency reduced mammary tumor initiation and reduced metastasis in transgenic mice whose tumors are driven by mouse mammary tumor virus (MMTV) promoter-regulated mutant *ErbB2/Neu.* However, it had no effect on the growth of mammary tumors in MMTV-Myc transgenic mice.[110] Interestingly, although Tiam1 was originally discovered for its ability to promote T cell invasion in vitro[51], in some tissues it has also been shown to inhibit progression to malignant growth.

In patients, increased Tiam1 protein expression was found in a subset of colon tumors[104], and RNAi suppression reduced the growth of colon cancer cell lines in vitro and in vivo.[109,111] Tiam1 overexpression was found to be inversely proportional to the degree of promoter methylation.[105] In a small panel of breast cancer patient samples, Tiam1 protein expression levels were higher in grade III compared with grade II tumors[102] and Tiam1 mRNA levels were significantly higher in tumour tissue from breast cancer patients who died from their disease compared with those who survived.[112] However, in another study,

Tiam1 protein expression levels decreased with the progression of breast carcinomas.[103] The reasons for these opposing findings remain unclear but may be related to different patient populations whose diseases are driven by distinct mechanisms.

Much less is known regarding the involvement of Tiam2 in cancer. *TIAM2* encodes two isoforms, one similar in structure to Tiam1 (designated long; Tiam2L), and another lacking N-terminal sequences upstream of the DH domain (designated short; Tiam2S).[56] Interestingly, whereas mRNA for both isoforms could be detected in hepatocellular carcinoma (HCC), only the short isoform of Tiam2 protein was detected.[113] Finally, although neither Tiam2S mRNA nor protein was detected in normal liver, HCC tumors and cell lines expressed elevated levels of Tiam2S protein, and further ectopic expression of Tiam2S enhanced the tumorigenic growth of an HCC cell line.

In summary, while altered Tiam1/2 expression has been observed in a variety of human cancers, the most compelling evidence for a driver function in cancer comes largely from model studies, where Tiam1 can either promote tumor progression or conversely antagonize invasion. The basis for these seemingly opposing roles for Tiam1 is unclear, especially since, as described above, it is the gain-of-function of another Rac-selective GEF (P-Rex1) that is essential for metastatic but not primary melanoma tumor growth.[61] However, another possible explanation comes from the observation that Tiam1-Rac signaling restored E-cadherin-mediated adhesion in MDCK canine kidney epithelial cells, thereby reducing cell migration.[114] Further, since remodeling of cell-cell junctions is critical for tumor cell invasion, Tiam1 activity may have opposing consequences in cells invading individually versus collectively. Whether these in vitro findings provide the explanation for the antagonistic role of Tiam1 in tumor cell invasion and metastasis remains to be determined.

Vav1

Although the Vav1 isoform originally identified by its ability to transform NIH 3T3 cells was activated by N-terminal truncation, and equivalent truncations of Vav2 and Vav3 also convert them to transforming proteins (Figure 4),[67,115,116] no N-terminally truncated Vav proteins have been identified in cancer. Instead, Vav1 overexpression has been described in a spectrum of cancers, including neuroblastoma,[117] lung,[118] pancreatic,[119] metastatic melanoma[120] and B-cell chronic lymphocytic leukemia.[121] Additionally, persistent phosphorylation has also been seen in some cancers, consistent with the observation that N-terminal phosphorylation can reversibly disrupt the N-terminal autoinhibitory function to activate Vav1.

Although Vav1 expression is normally restricted to hematopoietic cells, overexpression in several non-hematologic cancers has been described (Supplementary Table 2). For example, Vav1 overexpression was found in pancreatic tumor tissue and cell lines compared to normal pancreatic epithelial cells.[119] Vav1 expression was inversely correlated with patient survival. RNAi silencing analyses found that Vav1 was necessary to support tumor growth in vivo and in vitro, and these pro-growth phenotypes were dependent on Vav1 RhoGEF activity. Vav1 support of tumor growth was associated with Rac1 activation of the PAK1 serine/threonine kinase, the NF-kappaB transcription factor, and the cyclin D1 regulator of G1 cell cycle

progression.[119] Similar expression of Vav1 has also been described in lung cancer and also shown to be required for tumor growth in vitro and in vivo.[118]

Vav1 is not the only family member to have a role in cancer: both Vav2 and Vav3 have been implicated. Vav2 is activated in metastatic melanoma through the chemokine receptor-ligand pair CXCR4-CXCL12 and promotes expression of metalloproteinases that lead to invasion.[120] Recently, *VAV3* gene transcription was found elevated primarily in estrogen and progesterone receptor-positive breast cancers.[122] RNAi suppression analyses demonstrated that Vav3 was required for mouse mammary tumorigenesis and metastasis and that Vav2 showed a synergistic role with Vav3 in breast cancer growth and metastasis. Vav2/3 controlled a transcriptional program that involved upregulation of gene targets important for lung-specific metastasis. Interestingly, the abundance of Vav2/3 transcripts was modulated through both Rac1-dependent and -independent pathways.

The development of androgen resistance in prostate cancer cells limits the effectiveness of androgen-deprivation therapy. Vav3 was found to be overexpressed in prostate tumors and its overexpression was associated with cell line progression to androgen-independence both in vitro and in vivo.[123–125] Vav3-driven androgen independence was RhoGEF-dependent, required nuclear localization,[126] and was promoted in part through activation of PI3K-Akt signaling.[127] Constitutively activated Rac1 alone could also drive androgen-independent growth in vitro and in vivo.[125] High Vav3 protein expression was associated with disease progression and reduced survival, whereas RNAi depletion reduced the tumorigenic and metastatic growth of androgen-independent prostate tumor cells.[128] Finally, suppression of Vav3 expression enhanced docetaxel-induced apoptosis in prostate cancer cells, further supporting a therapeutic value in blocking Vav3 function in prostate cancer.[129]

In summary, consistent with the mouse studies suggesting that the Vav isoforms exhibit both redundant and distinct functions in development, there appear to be Vav isoform-distinct functions in cancer. For example, Vav1 suppression alone was sufficient to impair growth of pancreatic cancer cells despite continued expression of Vav2, and Vav2 depletion did not alter growth.[119] Thus, Vav2 cannot substitute for Vav1 in pancreatic cancer. Similarly, suppression of either Vav2 or Vav3 alone greatly reduced the metastatic growth of mouse mammary tumor cells, and concurrent ablation of both isoforms further abolished metastatic growth.[122] The basis for why Vav2 and Vav3 may have distinct roles is not clear, since both activate RhoA, Rac1 and Cdc42. One possibility is that each activates spatially distinct subcellular pools of the Rho GTPases, leading to activation of distinct effector signaling networks. Another possibility is that the Vav isoforms activate an incompletely overlapping extended set of Rho GTPases. Analyses of RhoGEF specificity are often restricted to RhoA, Rac1 and Cdc42 but do not examine other family members. This tendency towards limited analysis of potential substrates remains an issue that needs to be addressed if the functions of specific RhoGEFs are to be fully understood.

RhoGEFs and other human diseases

FGD1, which encodes a Cdc42-specific RhoGEF, was originally identified as a mutated gene responsible for Aarskog-Scott Syndrome (ASS; also known as faciogenital

dysplasia).[130] ASS is an X-linked recessive developmental disorder characterized by short stature, by facial, skeletal, and urogenital anomalies, and by mild mental retardation. The ASS-associated *FGD1* mutations cause loss-of-function (deletion, nonsense mutations causing expression of truncated proteins, missense mutations in DH-PH domains) and account for ~20% of individuals with ASS.[130,131]

Loss-of-function mutations in the related *FGD4* gene are associated with one type (CMT4) of the Charcot-Marie-Tooth (CMT) disease group.[132,133] Like *FGD1*, *FGD4* encodes a Cdc42-specific GEF (Fgd4/Frabin). This heterogeneous disease group is characterized by damage to peripheral nerves that can result in loss of sensation and atrophy of muscles in the feet, legs, and hands. It is one of nine genes with causal association with CMT4, an autosomal recessive demyelinating form of CMT.

Mutations in *OBSCN*, the gene encoding the RhoGEF Obscurin, have been identified in melanoma and glioblastoma[134] and may predispose towards these cancers and towards hereditary myopathies.[135] *OBSCN* mutations have also been identified by exome sequencing of breast and colon cancers, supporting a possible driver function in these two cancers.[136] Obscurin is a very large protein with GEF activity for both RhoA and TC10.

Alsin, a GEF for Rac and for the Rab5 small GTPase involved in vesicular transport, is encoded by the gene *ALS2*.[137–139] Loss-of-function mutations in *ALS2* have been found in a subset of individuals with amyotrophic lateral sclerosis (ALS),[140–143] a disease characterized by progressive movement problems and muscle wasting caused by motor neuron death. Accordingly, Alsin expression is highest in motor neurons of the brain. *ALS2* mutations have been associated with autosomal recessive juvenile onset ALS and the related conditions of infantile-onset ascending hereditary spastic paraplegia and of juvenile-onset primary lateral sclerosis. The exact contribution of Alsin, Rac1 and/or Rab5 to ALS diseases remains to be determined.

Germline gain-of-function mutations in *SOS1* have been found in ~20% of individuals with Noonan syndrome.[144,145] This syndrome is characterized by a range of distinctive facial characteristics, short stature, cardiac defects, bleeding disorders, skeletal malformations, and a diversity of other signs and symptoms. Sos1 has dual functions and can act as either a RacGEF or a RasGEF. However, the frequent findings in this disorder of gain-of-function mutations in *RAS* and in genes encoding its Raf effectors (*BRAF* and *RAF1*) argue that enhanced activity of Sos1 on Ras rather than on Rac contributes to the role of *SOS1* mutations in Noonan syndrome.[143,146,147]

Loss-of-function mutations in Collybistin/ARHGEF9/hPEM2, a Cdc42-specific Dbl RhoGEF, have been identified in patients with X-linked mental retardation associated with epilepsy.[148–152] Collybistin expression is neuronally restricted; Collybistin is found in neurons throughout the adult brain and spinal cord.

Additional Dbl RhoGEFs have also been associated with biology and disease.[153] Altered expression and mouse model knockout studies implicate Kalirin, with an N- and C-terminal DH domain that activate Rac1 and RhoA respectively (Figure 3), in a number of

neurological and cardiovascular diseases.[154] These include Huntington's Disease, Alzheimer's, schizophrenia, depression, cocaine addiction and ischemic stroke.

Finally, many pathogenic bacteria employ a virulence strategy to subvert and hijack the function of Rho GTPases in their eukaryotic hosts. This strategy is mediated by a type III secretion system to inject virulence factors, or effectors, to facilitate cell invasion, intracellular survival, and modulation of the host immune responses.[155] Among the effectors that target Rho GTPases are those that either mimic or antagonize the function of eukaryotic RhoGEFs.[31] The first discovered was the *Salmonella enterica* serovar typhimurium protein SopE,[156] with related RhoGEFs (SopE2, BopE2, CopE) later identified in other bacteria. Members of a second class of bacterial proteins that share a WxxxE sequence were first thought to act as straightforward Rho GTPase mimics,[157] but were later found to also mimic RhoGEF activity. Examples include the Map protein from enteropathogenic *E. coli*, and IpgB1 and IpgB2 from *Shigella flexneri*, that mimic Cdc42, Rac1 and RhoA, respectively. These bacterial RhoGEFs lack sequence homology to the DH domain but nevertheless utilize a biochemical mechanism similar to that of Dbl family RhoGEFs.[158] In contrast, another class of type III effectors antagonizes eukaryotic RhoGEF function.[159] For example, it was determined that EspH binds directly to the DH-PH domain of multiple RhoGEFs, preventing their binding to Rho substrates. While the full impact of these bacterial Rho GTPase regulators remains to be determined, they provide yet another example of how aberrant RhoGEF function may contribute to human disease. Understanding the pressures that led to their convergent evolution may help to further define the roles of RhoGEFs in cancer and other diseases.

Perspectives

Given the essential involvement of Rho GTPases in virtually every aspect of normal cell physiology, it is not surprising that the aberrant activities of Rho GTPases are associated with cancer and other diseases. Since RhoGEFs are key mediators of Rho GTPase activation, it is also not surprising that aberrant RhoGEF activation is a major mechanism driving aberrant activation of their Rho GTPase targets. Finally, given the very divergent sequence and domain structures beyond their shared DH-PH domains, the diversity of mechanisms deregulating RhoGEF functions is also to be expected. Large challenges remain in unraveling the precise mechanisms and contributions of RhoGEFs to specific cancers and other diseases, but certainly improved understanding will help to promote therapeutic interference in their activities.

The possibility that RhoGEFs may be directly targeted is supported by the mechanism of the natural product brefeldin A, an ArfGEF inhibitor that binds to the interface between the Arf small GTPase and the catalytic domain of ArfGEF, freezing the complex in an abortive conformation that cannot complete nucleotide exchange.[160–162] However, the significant conformational change seen in Arf upon association with its GEF and upon switching from the GDP- to GTP-bound states makes this interaction distinct from that of Rho GTPases. To date, there has been very limited success in developing pharmacologic inhibitors of RhoGEF function.[4] Thus, whether potent and selective RhoGEF inhibitors can be developed remains to be determined. An alternative strategy for blocking RhoGEF activity has been the

identification of small molecules that bind the GTPase and prevent GEF stimulation. Antagonists of GEF activation of Rho [163–165] and Ras [166–168] have been described. Whether such small molecule inhibitors of protein-protein interactions can achieve the potency and selectivity needed for clinical efficacy remains to be determined, but it is clear that we are at last on the way to finding out.

Finally, with the recent successful clinical development of numerous protein kinase inhibitors for cancer treatment (e.g., imatinib, erlotinib, vemurafenib), perhaps the most promising avenues for short term success in developing RhoGEF inhibitors may be blockade of the protein kinases that drive RhoGEF activation or that are activated by their Rho GTPase substrates. These may include inhibitors of the Src family protein tyrosine kinases[169] that activate Vav family proteins; of the PKCi serine/threonine kinase[170] responsible for Ect2 mislocalization to the cytoplasm; of PI3K[171] to impair PIP3 production and activation of P-Rex; or of the PAK[172] or ROCK[173] serine/threonine kinases activated downstream of Rac1 or RhoA, respectively. Whether RhoGEFs themselves turn out to be druggable or not, their key roles in cancer warrant continued close attention to this large and interesting family of Rho GTPase regulators.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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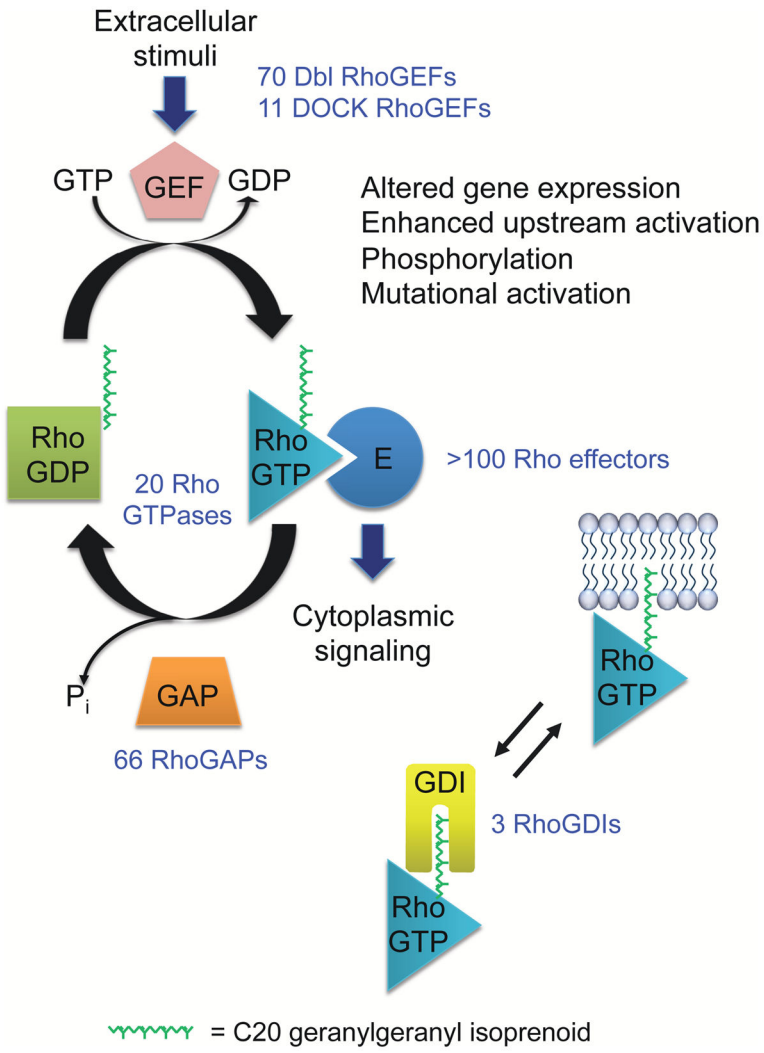

Figure 1. Rho GTPase regulation

The human Rho GTPase family is comprised of 20 members. The majority cycle between GDP-bound inactive and GTP-bound active states. Unlike RhoGEFs and RhoGAPs that possess shared domains and sequence identity, allowing precise determination of the number encoded in the human genome, the majority of Rho GTPase effectors lack a common well-defined recognition domain/motif. The information here applies mainly to RhoA, Rac1, Cdc42 and related isoforms. Not all Rho GTPases are regulated by GEFs, GAPs and/or GDIs, not all are posttranslationally modified by a geranylgeranyl isoprenoid lipid, and some do not have known membrane targeting elements.

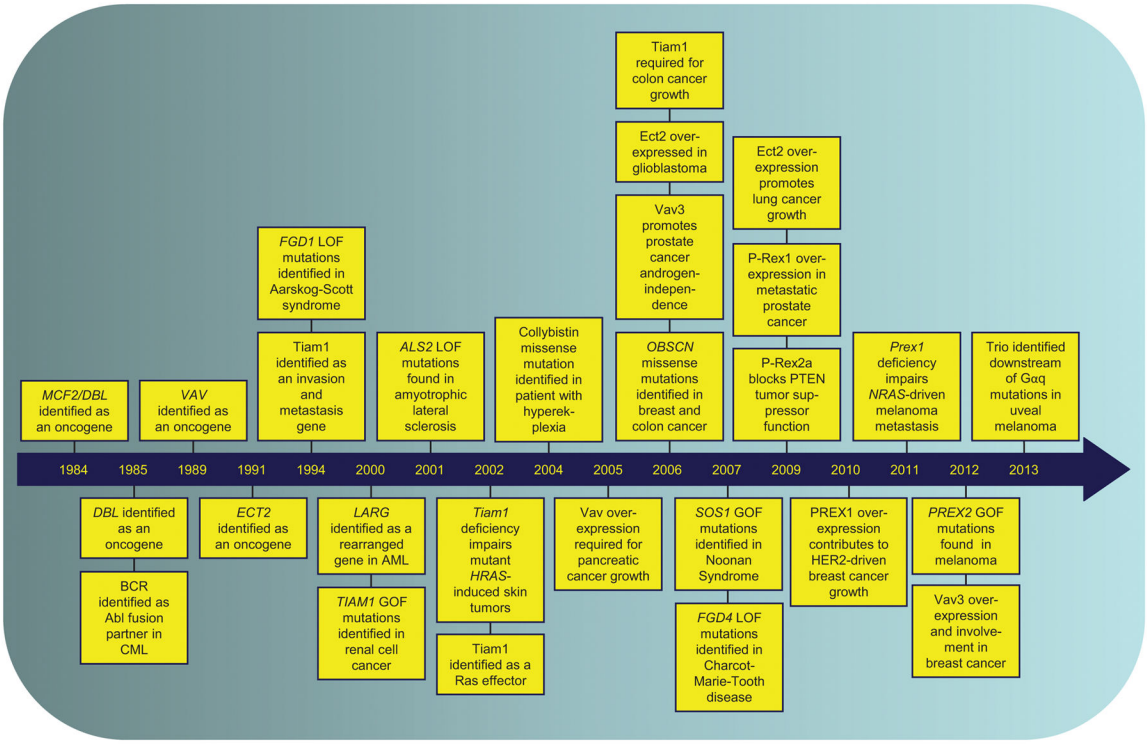

Figure 2. Key discoveries linking Dbl RhoGEFs to human disease

We highlight representative discoveries in the study of RhoGEFs that link their aberrant function to human disease. Gain-of-function, GOF; loss-of-function, LOF.

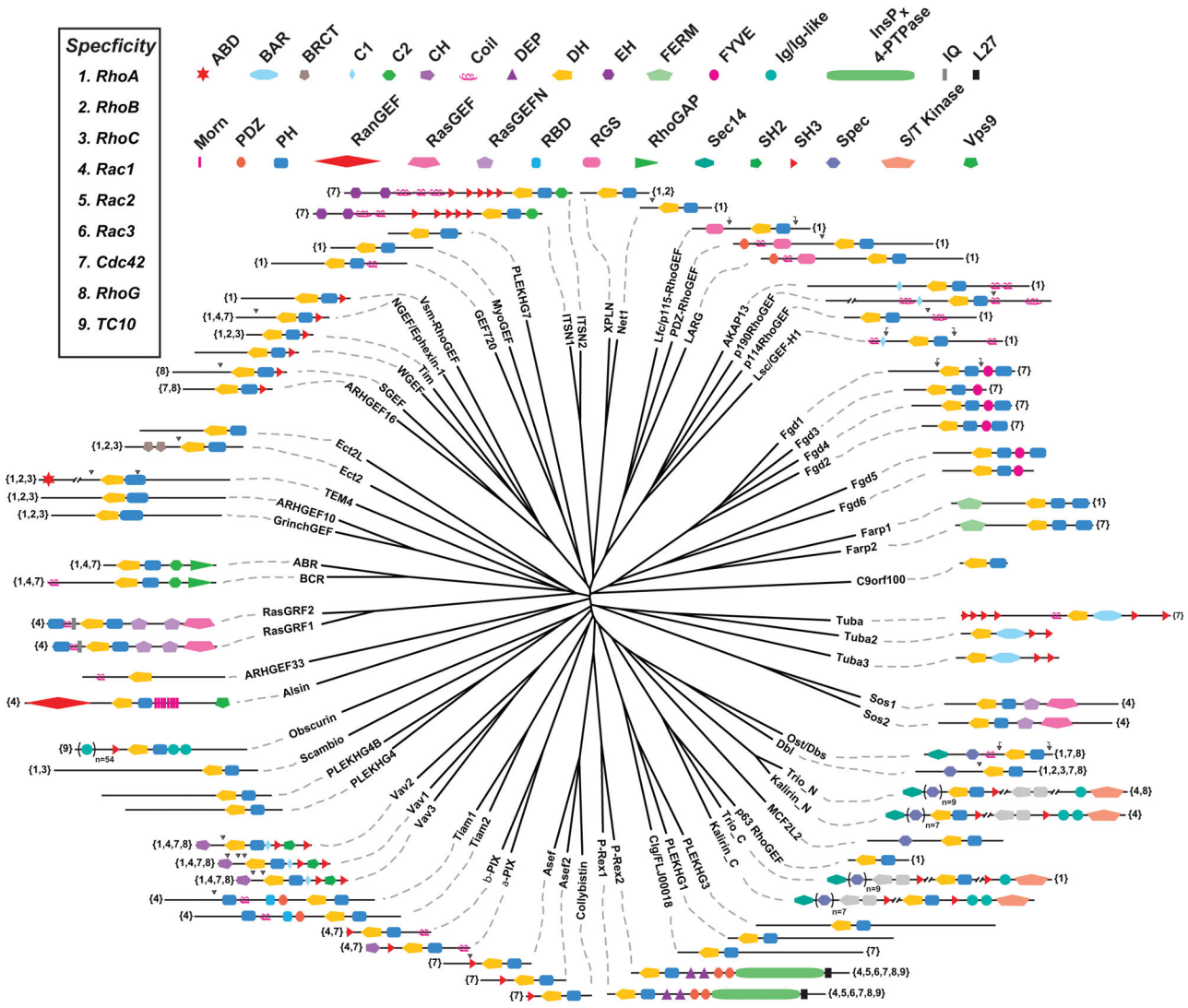

Figure 3. The human Dbl RhoGEF family

The 72 DH domains found within the 70 members of human Dbl family RhoGEFs were aligned using ClustalX[174] and a phylogenetic tree was subsequently drawn using the program FigTree (http://tree.bio.ed.ac.uk/software/figtree/). The protein domain architecture for each full-length Dbl protein is shown as annotated by SMART (http://smart.embl.de/) or described in the published literature. Black arrowheads indicate sites of genetic truncation known to activate RhoGEF function, while bracketed arrowheads designate biologically or catalytically active fragments of a GEF. Numbers within braces next to a Dbl protein indicate the reported Rho GTPase specificity of the DH domain. Please refer to Supplementary Table 1 for other names utilized for these RhoGEFs.

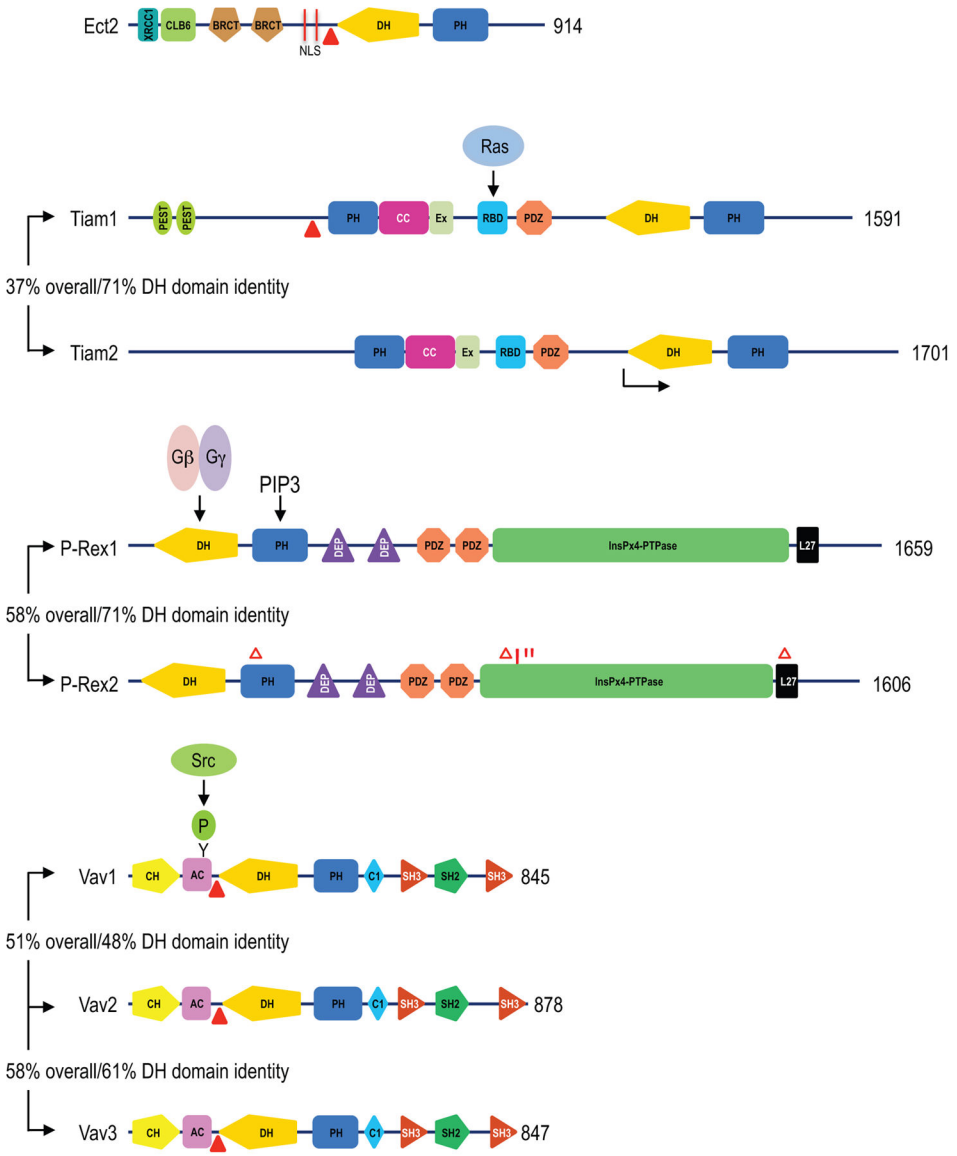

Figure 4. Mechanisms of RhoGEF activation

Shown here are the “canonical” isoforms as identified in UniProt (http://www.uniprot.org/). Domain structures were determined, in part, by SMART (http://smart.embl-heidelberg.de/). Additional domains not identified in SMART were added based on previous sequence analyses of Ect2[38], Tiam1[16], P-Rex[57] and Vav proteins.[175] The number at the right end of each protein indicates the number of amino acids in the full length protein. Solid triangles indicate the position of N-terminal truncations that result in the formation of constitutively activated and transforming variants of Ect2, Tiam1, and Vav1-3. The arrow indicates the site of initiation of the shorter Tiam2S splice variant. The % values refer to amino acid sequence identity for the full length protein (overall) or the isolated DH domain, when compared to the first isoform shown. Gain-of-function P-Rex2a truncation (Δ) or missense (*) transforming mutations.[99] Abbreviations: Ac, acidic domain; CC, coiled-coil; CH, calponin homology; Clb6 region, homology to yeast Clb6 B-type cyclin; Ex, extra region; NLS,

nuclear localization sequence; PIP3, phosphatidylinositol (3,4,5)-triphosphate; XRCC1, homology to human repair protein XRCC1.

Table 1

Discovery of Dbl RhoGEFs as oncogenes

Gene	Description	References
Dbl/ Mcf2	Identified as an oncogene that caused NIH 3T3 nude mouse tumorigenic growth after transfection of genomic DNA from the MCF-7 human breast carcinoma cell line (designated *mcf2*); oncogene in an NIH 3T3 focus formation assay using genomic DNA from a primary human diffuse B-cell leukemia; identified in an NIH 3T3 focus formation screen using genomic DNA from a human nodular poorly differentiated lymphoma	11, 12, 83
Vav	Identified as an oncogene that caused NIH 3T3 nude mouse tumorigenic growth after transfection by genomic DNA from a human esophageal carcinoma. Since this represented the sixth oncogene identified from this group, it was given the name of the sixth letter of the Hebrew alphabet.	63
Ect2	Identified as an oncogene in an NIH 3T3 focus formation assay using a cDNA expression library from the Balb/MK mouse keratinocyte cell line; designated epithelial cell transforming cDNA 2	39, 176
Tim	Identified as an oncogene in an NIH 3T3 focus formation assay using a cDNA expression library from a human mammary epithelial cell line; designated transforming immortalized mammary oncogene	177
Net1	Identified as an oncogene in an NIH 3T3 focus formation assay using a cDNA expression library from a human neuroepithelioma cell line; designed neuroepithelial cell transforming gene 1	178
Lbc	Identified as an oncogene that caused NIH 3T3 nude mouse tumorigenic growth after transfection by genomic DNA from an acute phase CML; designated lymphoid blast crisis oncogene	179
Lfc	Identified as a transforming gene in an NIH 3T3 focus formation assay in cDNA expression library from the 32D mouse hematopoietic cell line; designated Lbc's first cousin	180
Tiam1	Identified by retrovirus insertion for genes that induced T cell lymphoma in vitro; designated T cell lymphoma invasion and metastasis 1	51
Ost/ Dbs	Identified as a transforming gene in an NIH 3T3 focus formation assay using a cDNA expression library generated from the rat osteosarcoma cell line ROS; identified as a transforming gene in an NIH 3T3 focus formation assay in cDNA expression library from the mouse hematopoietic cell line 32D; designated Dbl's big sister	181, 182
Lsc/ Lip	Identified as a transforming gene in an NIH 3T3 focus formation assay using a cDNA expression library generated from the murine myeloid progenitor cell line B6SUtA$_1$; designated Lbc's second cousin; identified as a transforming gene in an NIH 3T3 focus formation assay in genomic DNA from a pleiomorphic liposarcoma	177, 183
Clg	Identified as a common-site lymphoma/leukemia guanine nucleotide exchange factor proviral integration site in mouse leukemias	184

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Table 2

Roles of Dbl RhoGEFs in mouse development and tissue function

Dbl Protein	Other Functions[a]	Mammalian Phenotype Associated with Deficiency	References
Abr	RhoGAP	No abnormal phenotype detected Together with Bcr deficiency caused partial neonatal lethality, but viable mice have cerebellar and vestibular defects	185, 186
AKAP13	Protein kinase A binding	Embryonic lethal at E10.5–11; cardiac developmental defect	35, 187
Alsin	RanGEF, Rab5GEF	Viable and fertile, no obvious developmental abnormalities	188–192
Asef1	APC[b] binding	Viable and fertile, normal morphology and lifespan; impaired tumor angiogenesis and adenoma formation in *Apc*$^{min/+}$ mice	193, 194
Asef2	APC[b] binding	Viable and fertile, normal morphology and lifespan; impaired intestinal adenoma formation in *Apc*$^{min/+}$ mice and reduced further when combined with *Asef1* deficiency	194
Bcr	RhoGAP	Viable and fertile, but mice display abnormal neutrophil physiology	195
Collybistin		Viable and fertile, but increased levels of anxiety; abnormal spatial learning and synaptic plasticity	196
Dbl		Abnormal dendrite morphology	197
Ect2		Embryonic lethal, perimplantation defect	36
LARG	GAP for $G\alpha_{12/13}$	Reduced birthrate, but adult mice are viable with no defects in anatomy, survival or behavior; resistant to salt-induced hypertension When combined with p115-RhoGEF deficiency, causes embryonic lethality during midgestation, with multiple vascular defects	198, 199
p115-RhoGEF	GAP for $G\alpha_{12/13}$	Viable, normal life-span; altered marginal zone B cell and other hematopoietic cell functions; defect in activated neutrophil polarization, migration, and adhesion, peripheral leukocytosis with splenomegaly and extramedullary hematopoiesis; impaired neuronal innervation and motor dysfunction	200–204
Kalirin	2nd DH-PH tandem domain; serine/threonine kinase[c]	Kalirin-7-specific knockout are viable and fertile, but display defects in long-term potentiation (LTP), behavioral alterations; DH1 knockout deficient in the three major isoforms (7,9 and 12) viable and fertile, with cortical morphology alterations, reduced hippocampus; LTP and memory defects	205, 206
Obscurin[d]	Two serine/threonine kinase domains	Viable and fertile, no obvious physiological or pathological abnormalities; generally appear healthy but display mild myopathy and abnormal sarcoplasmic reticulum morphology	207
α–PIX		Viable, no gross changes in brain morphology; reduced numbers of mature lymphocytes and defective immune responses; abnormal spatial learning and dendrite morphology	208, 209
P-Rex1		Viable and healthy, slightly smaller size, with mild neutrophilia and a small liver Impaired melanoblast migration from the neural crest during embryogenesis	60, 61
P-Rex2		Viable and fertile, abnormal Purkinje cell dendrite morphology and impaired coordination; viable and fertile when combined with *Prex1 −/−*, but coordination is further impaired	62
RasGRF1	RasGAP	Viable and healthy; reduced long term potentiation, abnormal spatial learning, decreased body weight, impaired glucose tolerance and abnormal DNA methylation	210–215
RasGRF2	RasGAP	Viable and fertile, but abnormal T cell physiology	216, 217
Sos1	RasGEF	Embryonic lethal between E15.5 and birth, abnormal extraembryonic tissue morphology	33, 218
Sos2	RasGEF	No abnormal phenotype detected	219
Tiam1		Viable, but impaired oncogenesis; defect in embryonic brain development and decreased brain size	54, 220

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Dbl Protein	Other Functions[a]	Mammalian Phenotype Associated with Deficiency	References
ARHGEF5		Viable; dendritic cell migration defect	221
Trio	Serine/threonine kinase	Embryonic lethality in late gestation, abnormal development of skeletal muscle and neural tissue	34, 222
Vav1		Partial defect in T-cell development and function, normal B-lymphocyte development and function	223–225
Vav2		Normal T- and B-cell development and function, but B-cell defect in combination with Vav1 deficiency	74, 75
Vav3		Normal, but increased hematopoietic cell defects when combined with loss of Vav1 and Vav2	76

[a] Based on sequence homology; not all have been validated to be catalytically active;

[b] Adenomatous polyposis coli;

[c] Kalirin-12 isoform, absent in Kalirin-7 isoform;

[d] Knockout retains expression of the splice variant that contains only the kinase domain.