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Nonredundant Functions for Ras GTPase-Activating Proteins in Tissue Homeostasis

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Abstract

Inactivation of the small guanosine triphosphate-binding protein Ras during receptor signal transduction is mediated by Ras guanosine triphosphatase (GTPase)-activating proteins (RasGAPs). Ten different RasGAPs have been identified and have overlapping patterns of tissue distribution. However, genetic analyses are revealing critical nonredundant functions for each RasGAP in tissue homeostasis and as regulators of disease processes in mouse and man. Here, we discuss advances in understanding the role of RasGAPs in the maintenance of tissue integrity.

Introduction

Ras proteins constitute one subset of a large family of evolutionarily conserved monomeric small guanosine triphosphate (GTP)-binding proteins involved in intracellular signal transduction in eukaryotes (1). They are activated by various cell surface receptors in response to ligand binding, whereupon they convey signals that regulate cell proliferation, survival, and differentiation in essentially all cell types. There are three different classical Ras isoforms (H-Ras, K-Ras, and N-Ras), each of which is tethered to the inner leaflet of the cell membrane through prenylation at the C-terminal end. Ras proteins, like all members of the Ras superfamily, function as molecular switches that cycle between inactive guanosine diphosphate (GDP)-bound and active GTP-bound states. During the course of receptor signal transduction, Ras is converted to its active form when one or more Ras guanine nucleotide exchange factor proteins (RasGEFs) is recruited to the membrane (2). RasGEFs dissociate GDP from the Ras guanine nucleotide-binding pocket, which allows Ras to bind GTP, which is present at higher intracellular concentrations than GDP (Fig. 1). In its GTP-bound form, Ras engages Ras effector molecules, which results in the activation of downstream signaling pathways, such as the mitogen activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and protein kinase B (PKB) pathways that drive cellular responses in part through modulation of the activity of transcription factors (3, 4).

Ras has only weak intrinsic guanosine triphosphatase (GTPase) activity. Therefore efficient conversion of Ras back to its inactive state is dependent upon Ras GTPase-activating proteins (RasGAPs) (2, 5). Through physical interaction with Ras, RasGAPs increase the ability of Ras to hydrolyze GTP by several orders of magnitude. The GAP domain of

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RasGAPs extends a critical arginine side chain into the Ras active site, which allows the glutamine residue in position 61 of human H-Ras (Gln⁶¹) to participate in catalysis (6–8). Mutations at Gln⁶¹ or at residues 12 or 13, which alter the orientation of Gln⁶¹, prevent GTP hydrolysis, which locks Ras in a permanently “on” state (9, 10). These mutations are found in up to 30% of all human cancers and occur in a much higher percentage of pancreatic cancer and colon cancer (9, 10), which underscores the importance of this cooperative Ras-RasGAP mechanism.

The RasGAP Family: Structure and Signaling-Dependent Membrane Localization

RASA1 and NF1

The first RasGAP to be characterized was p120 RasGAP or Ras p21 protein activator 1 (RASA1) (11, 12). Subsequently, the protein product of the gene mutated in the human disease neurofibromatosis type 1 (NF1) was identified to contain a region of homology to RASA1 and to have RasGAP activity (13–17). In addition to a GAP domain, RASA1 contains Src homology 2 (SH2) and SH3 domains, which recognize phosphotyrosine- and proline-rich sequences in proteins, respectively, as well as pleckstrin homology (PH) and protein kinase C2 homology domains that bind membrane phospholipids (Fig. 2A) (18). One important function of these four domains is thought to be in the targeting of RASA1 to membranes and, hence, Ras. For example, SH2 domains enable binding of RASA1 to phosphorylated receptor tyrosine kinases (RTKs) (Fig. 2B), such as the epidermal growth factor receptor, platelet-derived growth factor receptor, and insulin receptors, which promotes inactivation of Ras in the respective signaling pathways (19–24). Similarly, PH and C2 domains may also promote membrane targeting through direct binding to the lipid bilayer or indirect binding mediated through adaptor proteins, such as annexin A6 (25, 26). However, of the large number of RASA1 binding proteins that have been identified [reviewed in (27)], some may not have a role in targeting RASA1 to Ras but, instead, may function with RASA1 in Ras-independent mechanisms of signal transduction. One example of a Ras-independent function is the interaction between RASA1 and p190 RhoGAP, which acts as a GAP for the Ras superfamily protein Rho (28, 29).

NF1 contains just one recognized modular binding domain, a SEC14-PH domain, in addition to a GAP domain (Fig. 2A) (30). The SEC14-PH domain of NF1 binds glycerophospholipids and, thus, could potentially target NF1 to membranes (31). Sprouty-related protein with an Ena and vasodilator-stimulated phosphoprotein (VASP) homology (EVH) domain 1 (SPRED1) recruits NF1 to membranes and is required for NF1 to inactivate Ras in cell lines (32). The EVH domain of SPRED1 engages NF1 and an N-terminal Sprouty (SPR) domain allows membrane localization of the SPRED1-NF1 complex, most likely through interaction with phospholipids and caveolin 1 (32–34) (Fig. 2B). Which NF1 protein region is recognized by the EVH domain and whether or not the SEC14-PH domain of NF1 cooperates with SPRED1 in membrane targeting is not known.

The GAP1^m family

The GAP1 family of mammalian RasGAPs was identified on the basis of homology to the *Drosophila* RasGAP, Gap1 (35, 36). This family comprises mammalian GAP (GAP1^m or RASA2); inositol 1,3,4,5 tetrakisphosphate-binding GAP1 (GAP1^{IP4BP} or RASA3); calcium-promoted Ras inactivator (CAPRI or RASA4); and RASA like-1 (RASAL1) (37–40). Each member of the GAP1 family contains two N-terminal C2 domains followed by a GAP domain and a PH domain with a Bruton tyrosine kinase (BTK) motif (Fig. 2A). The PH domains of RASA2 and RASA3 bind to phosphatidylinositol (3,4,5)-trisphosphate (PIP₃), and the PH domain of RASA3 additionally recognizes phosphatidylinositol (4,5)-bisphosphate (PIP₂), phospholipids that are enriched at the plasma membrane (41–44) (Fig. 2B). This difference in PIP₂ or PIP₃ recognition means that RASA2 is recruited to membranes upon activation of PI3K, which converts PIP₂ to PIP₃, whereas RASA3 is found constitutively at membranes. The C2 domains of RASA4 and RASAL1 (Fig. 2B) mediate membrane association in response to increases in intracellular calcium concentration (39, 45, 46). RASA4 associates stably with membranes in response to calcium. By contrast, RASAL1 senses oscillations in intracellular calcium and shuttles between the membrane and cytoplasm.

RASA3, RASA4, and RASAL1 also show GAP activity toward the Ras-related small GTPase, Rap (47). RapGAP activity of these GAP1 family members is also dependent on an arginine finger in its GAP domain, even though Rap lacks the equivalent of Ras Gln⁶¹ (48, 49). Furthermore, isolated soluble RASA4 and RASAL1 are devoid of RasGAP activity that requires conformational changes in the GAP domain triggered by membrane localization. For RASAL1, calcium-dependent C2 domain interaction with membrane phosphatidylserine and phosphatidylinositol lipids is the critical event that triggers its RasGAP activity (50).

The SynGAP family

Synaptic GAP (SynGAP) was the first described member of another family of RasGAPs that contain a PH domain and a single C2 domain both N-terminal to the GAP domain (51, 52) (Fig. 2A). Ligands of the SynGAP PH and C2 domains remain to be defined. SynGAP, similar to RASA3, RASA4, and RASAL1, has RapGAP as well as RasGAP activity (53). RapGAP activity is dependent on the SynGAP C2 domain. In neurons, SynGAP is concentrated within the postsynaptic density (PSD) structure located at the cytoplasmic side of glutamatergic excitatory synapses. Incorporation into the PSD is mediated through physical interaction with PSD proteins PSD-95 and SAP102. Both proteins contain PDZ (PSD95-DLG-ZO1) domains that bind to a C-terminal peptide sequence of SynGAP (51).

Other members of the SynGAP family include disabled-2 (DAB2)–interacting protein [DAB2ip, also known as apoptosis signal-regulating kinase 1 (ASK1)–h-interacting protein (AIP1)], nematode-like Ras-GAP (nGAP or RASAL2), and RASAL3 (54–58). The DAB2ip PH and C2 domains promote constitutive plasma membrane association, and the C2 domain additionally mediates interaction with ASK1 (55, 59). In blood vessel endothelial cells (ECs), DAB2ip interacts constitutively with tumor necrosis factor receptor type 1 (TNFR1) and, in response to vascular endothelial growth factor A (VEGF-A), with VEGF receptor 2 (VEGFR2) (59, 60). Interaction with these receptors would allow additional means of

membrane recruitment, although no evidence currently exists to indicate that the purpose of binding is to regulate the activation of Ras in the respective receptor-induced signaling pathways. Association of RASAL2 with membranes at the leading edge of migrating astrocytes is mediated through physical interaction with epithelial cell transforming factor 2 (ECT2), which acts as a RhoGEF (61). The regions of RASAL2 that bind ECT2 and the effect of the interaction upon Ras are unknown.

Tissue Distribution of RasGAPs

Several of the RasGAPs show broad patterns of tissue distribution, whereas others are found in a restricted number of tissues. Ubiquitously distributed RasGAPs include RASA1, NF1, RASA2, DAB2ip, and RASAL2 (37, 54, 56, 62–66). Of the other Ras-GAPs, RASA3 is most abundant in the brain and spleen, RASA4 is found mostly in the spleen and the lymph nodes, RASAL1 is largely confined to the thyroid and the adrenal glands, and SynGAP is present specifically in neurons (36, 38, 40, 51, 52, 65–67). The tissue distribution of RASAL3 has not been reported, but information from public databases indicates that this RasGAP may be confined to the hematopoietic system. In any one cell type, therefore, multiple RasGAPs are likely present. Despite this, loss of expression or genetic alteration of a single RasGAP-encoding gene is often sufficient to perturb tissue homeostasis in mouse and human.

Physiological Roles of RASA1

Genetically RASA1-deficient mice die at day 10 of embryonic development (E10) (Table 1). In yolk sacs, ECs fail to organize into a honeycombed network, and in the embryo, blood vessel growth abnormalities and ruptures are observed (68). Therefore, RASA1 is essential for normal development of the blood vascular system. Specific deletion of RASA1 within vascular ECs, achieved with the use of a conditional RASA1-deficient mouse strain, results in the same phenotype, which shows that RASA1 performs an EC-intrinsic role as a regulator of blood vessel growth (64, 69). Curiously, de novo deletion of RASA1 in adult mice does not cause spontaneous blood vessel abnormalities. Instead, massive overgrowth of the lymphatic vascular system results, associated with lymphatic vessel leakage and death from chylothorax (leakage of lymph fluid into the pleural cavity) (69). Specific deletion of RASA1 within lymphatic vessel ECs showed that RASA1 performs an EC-intrinsic role as a regulator of lymphatic vessel growth and function (69). In lymphatic vessel ECs, RASA1 is necessary to suppress Ras activation induced by VEGFR3 in response to its ligand, VEGF-C. It is postulated that, in the absence of RASA1, VEGF-C, which is present at low concentrations in the extravascular space in resting (unstimulated) animals, binds VEGFR3 and triggers chronic unopposed activation of the Ras pathway, which leads to lymphatic vessel hyperplasia and dysfunction.

The molecular basis for abnormal blood vessel development in RASA1-deficient embryonic mice is unknown. However, relevant to this phenotype is the finding that RASA1 is necessary for directed cell movement in vitro (28). Therefore, impaired directed movement of ECs could cause blood vessel abnormalities, because they fail to position themselves correctly during embryonic development. This role for RASA1 in directed movement is

mediated through its interaction with p190 RhoGAP and is apparently independent of its ability to regulate Ras (28, 29). The notion that RASA1 performs a Ras-independent function specifically in blood vessel development is consistent with the finding that transgenic overexpression of Ras in ECs has no influence on the blood vascular system but induces lymphatic vascular abnormalities similar to those reported in induced RASA1-deficient mice (70).

Although RASA1 does not regulate blood vessel growth in the steady state in adults, clear evidence has emerged of its function as an inhibitor of pathological angiogenesis (71) (Tables 1 and 2). Blood vessel growth in response to proangiogenic growth factors, such as fibroblast growth factor (FGF), is augmented in induced RASA1 conditional-knockout mice. Moreover, blood vessel ECs from human tumors and hemangiomas, but not normal vessels, express large amounts of microRNA-132 (miR-132) that causes degradation of *RASA1* mRNA. The importance of miR-132-induced loss of RASA1 in tumor angiogenesis is illustrated by the finding that antagonism of miR-132 inhibits angiogenesis and reduces tumor burden in a mouse model of human breast carcinoma.

The influence of RASA1 loss in mice is mostly restricted to ECs. In nonconditionally RASA1-deficient embryos, increased neuronal apoptosis is also observed, although it is unclear if this is secondary to abnormal vascular development (68). RASA1 also inhibits Ras during positive selection of T cells in the thymus and promotes naïve T cell survival in the peripheral immune system (72). However, RASA1 plays a relatively minor role in T cell survival, and its function in positive selection is revealed only in T cell receptor transgenic mice or in competitive bone marrow transfers (72, 73). In relation to cancer, mice in which RASA1 deficiency is induced in adults show no increased susceptibility to tumor development during their life span (64, 69).

RASA1 is a critical regulator of blood vessel development in humans. Germline-inactivating mutations of the *RASA1* gene cause the blood vascular disorder: capillary malformation–arteriovenous malformation (CM-AVM) (74, 75) (Table 2). The defining characteristic of CM-AVM is the presence of small multifocal cutaneous CMs. In addition, one-third of patients develop arteriovenous fistulas, Parkes-Weber syndrome, and life-threatening AVM in which there is hypertrophy of an affected limb. The disease is inherited in an autosomal dominant pattern, and in affected families 95% of individuals with a mutated *RASA1* allele develop CM-AVM, which attests to the high penetrance of the mutations. A random somatic second-hit mutation of the intact *RASA1* allele during development is considered responsible for manifestation of disease, which would be consistent with the focal nature of lesions. Lymphatic vessel abnormalities in the form of lymphedema and chylothorax have also been observed in some CM-AVM patients, which indicates that RASA1 also promotes proper maintenance and function of lymphatic vasculature in humans as it does in mice (75, 76).

Physiological Roles of NF1

Germline mutations of the *NF1* gene are the cause of the autosomal dominant disease neurofibromatosis, one of the most common human genetic disorders (77–81).

Neurofibromatosis is characterized by the development of benign cutaneous or plexiform neurofibromas that are composed of multiple cell types normally found in peripheral nerves. In some cases, plexiform neurofibromas develop into malignant peripheral nerve sheath tumors. Neurofibromatosis patients show increased susceptibility to a number of other neoplasms, such as gliomas, pheochromocytomas, and juvenile chronic myelogenous leukemia (JCML). Other noncancerous manifestations of neurofibromatosis include abnormal skin pigmentation, skeletal malformations, and learning disabilities. Inherited *NF1* mutations associated with neurofibromatosis are inactivating mutations, and in samples from both tumors and noncancerous lesions, second-hit mutations of the intact *NF1* allele have been identified (82–86). Together, the findings suggest NF1 is a tumor suppressor and a regulator of tissue homeostasis in humans.

Mouse genetic studies have provided additional insight into the mechanisms by which NF1 regulates cell function. Genetically NF1-deficient mice develop cardiovascular abnormalities and hyperplasia of sympathetic ganglia and die in utero at E13.5 (87, 88). In mice, conditional deletion of NF1 in the ECs results in the same cardiovascular phenotype and death, which reveals an EC-intrinsic function for NF1 in cardiovascular development (89). Expression of the isolated GAP domain of NF1 in NF1-deficient mice rescues the cardiovascular phenotype, but hyperplasia of neural crest–derived tissues is still observed, and mice die shortly after birth (90). One interpretation of these findings is that NF1 performs functions in neural crest–derived tissue that are unrelated to its ability to inactivate Ras. However, in this case, the isolated GAP domain lacks the SEC14-PH domain and other regions that may be necessary for membrane targeting and juxtaposition to Ras. Furthermore, the notion that the loss of a Ras-independent NF1 function in neural crest–derived cells is responsible for abnormal neurological development in mice is inconsistent with the finding that these abnormalities can be reversed by inhibition of Ras signaling (91).

Specific biallelic deletion of NF1 in Schwann cells in mice results in the development of plexiform neurofibromas but only in an NF1 heterozygous-null background (92). The NF1 heterozygous-null, non-Schwann cell type that is necessary for tumor development is the bone marrow–derived mast cell, which is abundant in plexiform neurofibromas (93). NF1 heterozygous-null mast cells show increased proliferation and motility in response to the c-kit RTK ligand, stem cell factor (SCF), which is produced in large quantities by NF1-deficient Schwann cells. Resulting mast cell infiltration into peripheral nerves promotes a tumor-permissive environment through production of mitogens and angiogenic growth factors (91). Benign cutaneous neurofibromas are not observed in mice with Schwann cell–specific NF1 deletion, which indicates a different cell of origin for these lesions. Indeed, deletion of NF1 in skin-derived neural progenitors transplanted into naïve recipients induces cutaneous neurofibromas, which indicates that biallelic loss of NF1 in skin-derived neural progenitors is responsible for the development of these benign lesions (94).

Constitutive hematopoietic-specific deletion of NF1, transfer of NF1-deficient hematopoietic cells into wild-type recipient mice, or induced global deletion of NF1 in adult mice all result in the development of myeloid proliferative disease that parallels the increased susceptibility to JCML observed in neurofibromatosis patients (95–98). NF1-deficient hematopoietic cells and leukemic cells from neurofibromatosis patients with JCML show increased Ras

activation and cell growth in response to granulocyte-macrophage colony-stimulating factor (GM-CSF) (95, 96). In addition, NF1-deficient hematopoietic cells show increased sensitivity to interleukin 3 (IL-3) and SCF (99). Therefore, uncontrolled Ras activation downstream of multiple cytokine receptors may explain susceptibility to myeloid proliferative disorders in NF1-deficient mice and humans.

Legius syndrome is a mild form of neurofibromatosis that is associated with skin pigmentation abnormalities, macrocephaly, facial dysmorphism, and increased susceptibility to some types of tumors, although not specifically neurofibromas (100–105). It is caused by germline mutations of the *SPRED1* gene that act in a dominant fashion. The majority of SPRED1 mutations are nonsense mutations that lack the SPR domain, which results in the loss of membrane localization of NF1 in cultured cells. The phenotypic similarity between Legius syndrome and neurofibromatosis suggests that targeting of NF1 to membranes and, therefore, its targeting to Ras is mediated by SPRED1 in some cell types. In other cell types, such as Schwann cells and skin-derived neural progenitors, NF1 must use alternative mechanisms to localize to the membranes. It is possible that NF1 binds membranes in these cell types through recognition of SPRED2 or SPRED3, proteins that are homologous to SPRED1 (106, 107). In cell lines, SPRED2 and SPRED3 mediate the recruitment of NF1 to membranes in the absence of SPRED1 (32).

Physiological Roles of the GAP1^m Family

RASA3

Mice with a mutant form of RASA3 that lacks catalytic activity, but in which all other domains are intact, die in utero at E12.5 to E13.5 from massive hemorrhaging (108), probably resulting from the underdeveloped nature of adherens junctions between capillary ECs in these mice. Therefore, similar to RASA1 and NF1, RASA3 has a nonredundant function in the regulation of blood vessel development in mice. Furthermore, this function of RASA3 is relevant to its ability to inactivate Ras (or, theoretically, Rap). A rare, spontaneous G125V point mutation between the two C2 domains in the *RASA3* gene causes the autosomal recessive disorder severe combined anemia and thrombocytopenia (SCAT) in BALB/cBy mice (109, 110). Anemia and thrombocytopenia in this model are a consequence of blocked erythropoiesis and megakaryopoiesis and are characterized by alternating cycles of crisis and remission with high mortality (109). The block in erythropoiesis appears to be at a terminal stage in which reticulocytes give rise to erythrocytes. In wild-type reticulocytes, RASA3 is membrane-localized. However, in Scat reticulocytes, RASA3 is cytosolic. Failure to target RASA3 to Ras is consistent with both the increased amount of Ras-GTP found in Scat erythrocytes and the Scat phenotype, because excessive Ras activation is known to block erythropoiesis in mice (111). Why vascular function is not also impaired in Scat mice is unclear. Potentially, this could be explained by the use of an alternative mechanism to target RASA3 to the membrane in blood vessel ECs, or, by analogy to NF1, by the lack of a requirement for modular binding domains to enable RASA3 to inactivate Ras in this specific cell type.

RASA4 and RASAL1

RASA4-deficient mice are viable but show decreased clearance of lung bacterial infections attributable to impaired antibody Fc receptor (FcR)–mediated phagocytosis in macrophages (67). RASA4 is required for FcR-induced activation of the Rho-related small GTPases, Cdc42 and Rac1, which are necessary for phagocytic cup formation. How RASA4 participates in Cdc42 and Rac1 activation is not understood. RASA4 does not act as a GEF for Cdc42 and Rac1, although it does interact physically with both molecules through its GAP domain (67). RASA4 also suppresses Ras activation during FcR signal transduction (67), albeit the significance of this inhibition with respect to impaired phagocytosis in RASA4-deficient mice is unclear.

In an RNA interference–based screen of human mammary epithelial cells, RASA4 was identified as one of several targets which promoted cell transformation when depleted, which implicates RASA4 as a tumor suppressor (112). However, increased susceptibility of RASA4-deficient mice to tumor formation has not been reported. A similar genetic screen performed in fibroblasts identified the paired-like homeodomain transcription factor 1 (PITX1) as a tumor suppressor; loss of PITX1 promotes cell transformation in a Ras-dependent manner (113). RASAL1 was identified as a direct transcriptional target of PITX1, and knockdown of RASAL1 also resulted in Ras-dependent cell transformation. RASAL1 abundance is frequently decreased in various tumor cell types, which indicates that RASAL1 may function as a tumor suppressor. In cancer cell lines, *RASAL1* was silenced through promoter CpG methylation rather than through PITX1 deficiency (114–116). RASAL1 deficiency caused by hypermethylation of *RASAL1* in fibroblasts may be the mechanism underlying uncontrolled fibroblast activation in kidney fibrosis in mice and humans, particularly because knockdown of RASAL1 in normal fibroblasts in vitro results in a fibrotic phenotype, as evidenced by increased proliferation and expression of fibrotic markers (117).

Physiological Roles of the SynGAP Family

SynGAP

Genetically SynGAP-deficient mice die within the first week of postnatal life (118–120). Conditional SynGAP-deficient mice, in which brain SynGAP abundance is reduced to 20% of that observed in wild-type mice, also results in death; however, when brain SynGAP is reduced only to 40% of wild-type abundance, mice survive (121). SynGAP abundance correlates inversely with the extent of neuronal apoptosis, which explains the mortality observed in severely SynGAP-deficient mice (121). Heterozygous SynGAP-deficient mice, although viable, exhibit abnormalities in cognition and behavior, functions that are controlled by the hippocampus (122–124). These mice show an increase in the number of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)–type glutamate receptor complexes in synapses and defects in hippocampal long-term potentiation that could provide a basis for the cognitive and behavioral phenotype (118, 119). Homozygous SynGAP-deficient neurons in vitro show increased Ras activation and decreased activation of the p38 MAPK in response to synaptic *N*-methyl-D-aspartate (NMDA)–type glutamate receptor activation, both of which may lie upstream of altered AMPA-type receptor clustering and

response (125, 126). SynGAP-deficient neurons also show increased activation of Rac, which causes increased neuronal spine formation in vitro (120, 127). Notably, this spine formation phenotype can be corrected by expression of wild-type SynGAP but not by SynGAP mutants lacking GAP activity or the PSD-95-binding region (120), which indicates that functional SynGAP and its interaction with PSD-95 affect the regulation of neuronal morphology. As a corollary to these findings in mice, inherited de novo truncation mutations in the *SYNGAP* gene cause some cases of nonsyndromic mental retardation in humans (128).

DAB2ip

Studies performed with blood vessel ECs in vitro indicate an important function for DAB2ip in TNFR1 signaling (55, 59, 129, 130). In unstimulated blood vessel ECs, DAB2ip associates with TNFR1 in a closed conformation formed by binding between the N-terminal C2 domain and a C-terminal proline-rich region in DAB2ip. In response to TNF binding to TNFR1, DAB2ip is released from TNFR1 and forms an open conformation subsequent to phosphorylation by the receptor-interacting protein 1 (RIP1) kinase, which binds the DAB2ip GAP domain. The C2 domain of DAB2ip then binds ASK1, which promotes ASK1 dephosphorylation and activation by the protein phosphatase PP2A, which also associates with the DAB2ip GAP domain. Simultaneously, the open conformation of DAB2ip associates with TNFR-associated factor 2 (TRAF2), which results in inhibition of TNF-induced activation of the transcription factor nuclear factor κ B (NF- κ B) signaling pathway. Through activation of ASK1 and inhibition of NF- κ B, DAB2ip is thought to promote TNF-induced apoptosis in blood vessel ECs. Similarly, DAB2ip promotes endoplasmic reticulum stress-induced activation of ASK1 and apoptosis in both blood vessel ECs and fibroblasts (131).

Abundance of DAB2ip is commonly decreased in primary cells and cell lines derived from prostate, breast, lung, and gastrointestinal tumors (54, 58, 132–135). In each of these cell types, promoter methylation is one mechanism of gene suppression (132–135). Both histone methylation, mediated by the Polycomb repressive complex 2,3 protein Ezh2 (enhancer of Zeste homolog 2), and histone deacetylation have major roles in prostate cancer cells (132, 136, 137). Reexpression of DAB2ip in these cells restricts their growth in vitro, which indicates that loss of DAB2ip promotes transformation (54, 58). This is also consistent with the findings of two genome-wide association studies in humans, which show that a *DAB2ip* single-nucleotide polymorphism (SNP) is significantly associated with genetic susceptibility to aggressive prostate cancer (138).

Mechanistically, loss of DAB2ip appears to promote both transformation and tumor metastasis (137). Short hairpin RNA-mediated knockdown of DAB2ip in human prostate epithelial cells results in metastatic prostate cancer, when injected into mice. Reexpression of a catalytically inactive form of DAB2ip containing a GAP domain point mutation does not suppress tumor growth in vivo but does inhibit metastasis. Conversely, reexpression of a catalytically active form of DAB2ip containing a point mutation in its C-terminal region, which abrogates its ability to inhibit NF- κ B, inhibits tumor growth but not metastasis. Together, these findings indicate that DAB2ip inhibits tumor growth by suppressing Ras and

inhibits metastasis by suppressing NF- κ B. In addition, the ability of DAB2ip to suppress PI3K and the PKB pathway and to promote ASK activation in prostate epithelial cells likely contributes to its function as a tumor suppressor (139). Increased activation of NF- κ B in DAB2ip-deficient prostate epithelial cells results in epithelial-to-mesenchymal transition (EMT), marked by increased mesenchymal marker abundance on prostate epithelial cells, which is associated with metastasis (137, 140), and that suggests that DAB2ip inhibits EMT and metastasis.

Although blood vessel development is normal in DAB2ip-deficient mice, inflammation-induced angiogenesis is increased and is attributed to augmented signaling through VEGFR2 in blood vessel ECs (60). However, as in the TNFR signaling pathway, the role of DAB2ip in VEGFR2 signaling appears unrelated to its ability to directly inhibit Ras activity. Instead, DAB2ip inhibits VEGFR2-mediated activation of PI3K and phospholipase C through binding to the receptor complex. Loss of DAB2ip in aortic grafts results in graft arteriosclerosis accompanied by intima expansion (141). In the absence of DAB2ip, vascular smooth muscle cells show increased migration and proliferation in response to the inflammatory cytokine, interferon gamma (IFN- γ), which is critical to neointima formation. DAB2ip normally inhibits IFN- γ signaling through direct binding to and inhibition of receptor-associated Janus-activated tyrosine kinase 2 (JAK2) (141). DAB2ip is also implicated as a regulator of the blood vascular system in humans. A genome-wide association study shows an association between a DAB2ip SNP and, separately, early onset myocardial infarction, peripheral arterial disease, pulmonary embolism, and abdominal aortic aneurysm (142).

RASAL2

Knockdown of RASAL2 in astrocytoma cells increases the activation of Rho and induces a mesenchymal-to-amoeboid transition associated with a change in tumor cell migratory properties (61). The change in Rho activation could indicate a function for RASAL2 as a RhoGAP in this cell type. Alternatively, through modulation of the GEF activity of ECT2, RASAL2 could affect Rho activation indirectly.

The Nonredundant Nature of RasGAPs in Tissue-Specific Homeostasis

Of the 10 members of the RasGAP family, loss of function of all but 2 is sufficient to confer phenotype at the cellular or organism level. For RASA2 and RASAL3, knockdown and knockout studies have yet to be reported. This apparent lack of functional redundancy between RasGAPs can be explained by the fact that different cell surface receptors appear to target one or a limited number of RasGAPs in order to terminate Ras signaling. At the molecular level, it seems likely that selectivity is dependent on receptor-generated signals that recruit specific RasGAPs to the membrane or receptor complex. Why only a limited number of RasGAPs are used by an individual receptor is unknown. It is possible that different receptors may activate distinct Ras family isoforms at different plasma membrane microdomains and endomembrane sites (143), and these isoforms, in turn, may be inhibited by distinct RasGAPs. For example, among the Ras isoforms, RASA1 shows increased activity toward R-Ras compared with H-Ras, and NF1 shows strongest activity toward K-

Ras (144–146). In addition, a select few RasGAPs also have RapGAP activity. Thus, there may be a requirement for specialized RasGAPs with particular enzymatic specificities to selectively inhibit the activation of individual Ras family members or Rap at distinct sites within the cell. Furthermore, several RasGAPs also perform nonredundant Ras-independent functions in cellular signal transduction, which provides a further basis for RasGAP-specific loss-of-function phenotypes.

A remaining question concerns the basis for tissue-specific phenotypes in mice and humans when a RasGAP is knocked out in all tissues. In part, this may result from differing consequences of increased Ras activation in different cell types. For example, Ras overexpression in ECs does not affect blood vessel development but does lead to lymphatic vessel hyperplasia (70). Furthermore, the oncogenic potential of Ras varies among different cell lineages (9). Alternatively, there is the possibility of genuine functional redundancy between RasGAPs in certain cell types and tissues. Evidence of this redundancy exists in blood vessel development, where it is observed that nonconditional double *RASA1*- and *NF1*-deficient mice show more severe blood vessel abnormalities and die at an earlier time point in embryogenesis than mice that lack either single RasGAP (68). This suggests that *RASA1* and *NF1* have partially overlapping and redundant functions in the signaling pathway that regulates blood vessel development.

Conclusion

Continuing analyses of the physiological function of RasGAPs are revealing essential roles for individual family members as regulators of vascular, hematopoietic, immune, and neuronal systems. Different RTKs appear to use distinct RasGAP family members to inactivate Ras, which makes RasGAPs important tumor suppressors and critical regulators of tissue homeostasis in humans. In addition, several RasGAPs have distinct Ras-independent functions in cell signal transduction. Together, these functions exemplify the varied, yet nonredundant, roles of RasGAPs in mammalian physiology.

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Fig. 1. Ras is bound to GDP in its inactive state. RasGEFs dissociate GDP to enable Ras to bind GTP. GTP-bound Ras signals through downstream kinase cascades that promote cell proliferation and differentiation. RasGAPs inactivate Ras by catalyzing GTP hydrolysis, returning Ras to its inactive GDP-bound state.

Fig. 2.

(A) Domain organization of the RasGAP family members. **(B)** Domain-directed membrane localization of RasGAPs. Shown are examples of the different mechanisms by which RasGAPs can be targeted to membranes and Ras-GTP. RASA1 SH2 domains can bind phosphotyrosine residues located in intracellular domains of activated RTK. The RASA3 PH domain binds PIP₂, which is present constitutively in membranes. The RASA2 and RASA3 PH domains can also recognize PIP₃ generated in membranes in activated cells as a result of phosphorylation of PIP₂ by PI3K. Both C2 domains of RASA4 and RASAL1 are able to recognize membrane phospholipids. NF1 can be targeted to membranes through an undefined interaction with the SPRED1 protein that binds to membrane lipids through its SPR domain.

Table 1

Phenotypes of RasGAP mutant mice. Mutations are all knockouts except RASA3 G125V, which is a spontaneous mutant. Abbreviations: BV, blood vessel; LV, lymphatic vessel; E, embryonic day; P, postnatal day.

RasGAP	Mutation	Phenotype	Receptors	References
RASA1	Nonconditional	Abnormal BV development E10.5 lethal	Unknown	(68)
	EC-specific	Abnormal BV development E10.5 lethal	Unknown	(69)
	T cell-specific	Abnormal T cell development and survival	TCR, IL-7R	(72, 73)
	Broad inducible	LV hyperplasia and chylothorax	VEGFR3	(69)
		Increased BV angiogenesis	FGFR	(71)
	LV-specific inducible	LV hyperplasia and chylothorax	VEGFR3	(69)
NF1	Nonconditional	Cardiovascular and neuronal abnormalities E13.5 lethal	Unknown	(87, 88)
	EC-specific	Cardiovascular abnormalities E13.5 lethal	Unknown	(89)
	Schwann cell-specific	Plexiform neurofibromas	Unknown	(92, 93)
	Broad inducible	Myeloproliferative disorder	GM-CSF, IL-3, SCF	(95–97, 99)
		Benign cutaneous lymphomas	Unknown	(94)
RASA3	Nonconditional-GAP-deficient	BV leakage E13.5 lethal	Unknown	(108)
	G125V	Severe combined anemia and thrombocytopenia (Scat) P30 lethal	Unknown	(109)
RASA4	Nonconditional	Impaired macrophage phagocytosis	FcR	(67)
SynGAP	Nonconditional	Neuronal apoptosis P1-7 lethal	NMDA-type glutamate receptor	(118–120)
	Brain-specific	Neuronal apoptosis P1–7 lethal	NMDA-type glutamate receptor	(121)
DAB2ip	Nonconditional	Increased inflammatory BV angiogenesis	VEGFR2	(60)
		Increased aortic graft arteriosclerosis	IFN- γ R	(141)

Table 2

RasGAPs and human disease.

RasGAP	Alteration	Disease	Comments	References
RASA1	Germline-inactivating mutation	CM-AVM	Autosomal dominant	(74, 75)
	MicroRNA-mediated loss of RASA1 RNA	Pathological angiogenesis	Involved in angiogenic switch	(71)
NF1	Germline-inactivating mutation	Neurofibromatosis	Autosomal dominant Evidence of somatic second hit mutation	(77–80, 147)
RASA4	Reduced protein abundance	Breast cancer	Knockdown in human mammary epithelial cells promotes transformation	(112)
RASAL1	Reduced expression or protein abundance	Multiple cancers	Knockdown in human fibroblasts promotes transformation Reduced RASAL1 observed in multiple cancer types	(113–116)
		Kidney fibrosis	Knockdown in human fibroblasts promotes fibrosis Reduced RASAL1 observed in fibroblasts from fibrotic kidneys	(117)
SynGAP	Germline-inactivating mutation	Nonsyndromic mental retardation	de novo truncation mutations	(128)
DAB2ip	Reduced expression	Prostate, lung, breast, and gastrointestinal tumors	Functions as negative regulator of tumor growth and metastasis Reduced DAB2ip observed in primary cancers	(54, 58, 132–135, 137)
	SNP (rs1571801)	Aggressive prostate cancer	Genome-wide association studies	(138)
	SNP (rs7025486)	Abdominal aortic aneurysm Early onset myocardial infarction Peripheral arterial disease Pulmonary embolism	Genome-wide association study	(142)