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MINIREVIEW

New Player in Endosomal Trafficking: Differential Roles of Smad Anchor for Receptor Activation (SARA) Protein

Victoria Rozés-Salvador,[a,b,c,d] Sebastian O. Siri,[a,b,c] Melina M. Musri,[a,b,c] Cecilia Conde[a,b,c]

[a]Universidad Nacional de Córdoba (UNC), Ciudad Universitaria, Córdoba, Argentina
[b]Instituto Mercedes y Martín Ferreyra (INIMEC-CONICET), Córdoba, Argentina
[c]Instituto Universitario Ciencias Biomédicas de Córdoba (IUCBC), Córdoba, Argentina
[d]Instituto A.P. de Ciencias Básicas y Aplicadas, Universidad Nacional de Villa María (UNVM), Ciudad Universitaria, Villa María, Argentina

ABSTRACT The development and maintenance of multicellular organisms require specialized coordination between external cellular signals and the proteins receiving stimuli and regulating responses. A critical role in the proper functioning of these processes is played by endosomal trafficking, which enables the transport of proteins to targeted sites as well as their return to the plasma membrane through its essential components, the endosomes. During this trafficking, signaling pathways controlling functions related to the endosomal system are activated both directly and indirectly. Although there are a considerable number of molecules participating in these processes, some are more known than others for their specific functions. Toward the end of the 1990s, Smad anchor for receptor activation (SARA) protein was described to be controlling and to facilitate the localization of Smads to transforming growth factor β (TGF-β) receptors during TGF-β signaling activation, and, strikingly, SARA was also identified to be one of the proteins that bind to early endosomes (EEs) participating in membrane trafficking in several cell models. The purpose of this review is to analyze the state of the art of the contribution of SARA in different cell types and cellular contexts, focusing on the biological role of SARA in two main processes, trafficking and cellular signaling, both of which are necessary for intercellular coordination, communication, and development.

KEYWORDS SARA, endosomal trafficking, signaling, development, TGF-β

SARA PROTEIN: AN OVERVIEW OF ITS BIOCHEMICAL FEATURES AND CELLULAR FUNCTIONS

Smad anchor for receptor activation (SARA) is a protein of 180 kDa ubiquitously expressed during both the developmental and the adult stages of several organisms, including *Drosophila melanogaster*, mouse, rat, and humans (1–3). In humans, SARA has been detected in the heart, brain, placenta, lung, liver, muscle, kidney, and pancreas and thus appears to be widely expressed (1, 4). In mouse, three predicted SARA proteins have been described and are named $SARA_1$ (the full-length version, encoded by 17 exons and comprising 1,397 amino acids), $SARA_2$ (lacking the Smad binding domain [ΔSBD], encoded by 16 exons, and comprising 1,338 amino acids), and $SARA_3$ (lacking the *Fab1*, *YOTB*, *Vac1*, and *EEA1* protein [FYVE] domain [ΔFYVE], encoded by 16 exons, and comprising 706 amino acids). Mouse $SARA_1$ and $SARA_2$ transcripts are splicing variants homologous to the human SARA isoforms. In contrast, $SARA_3$ has not been detected either in humans or in mice (4) (Fig. 1). An association of their temporal and spatial expression with likely differential roles of these two SARA isoforms ($SARA_1$ and $SARA_2$) has not yet been analyzed. The commercial antibodies used to study SARA recognize only the N-terminal domain, which is shared by $SARA_1$ and $SARA_2$. Also, only Tang et al. (5) specifically use the $SARA_2$ isoform (expressed ectopically) as a negative

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Address correspondence to Victoria Rozés-Salvador, vrozes@immf.uncor.edu, or Cecilia Conde, cconde@immf.uncor.edu.

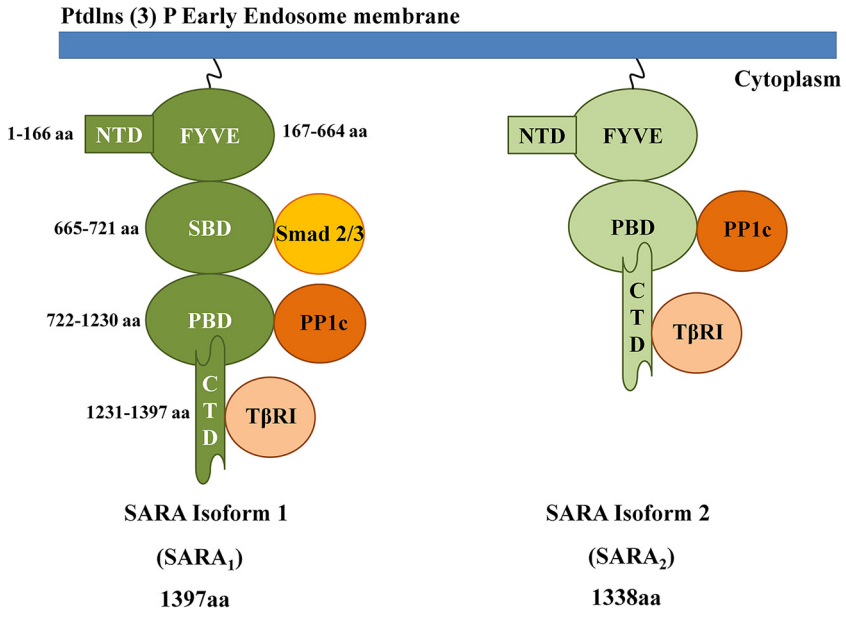

FIG 1 Representative image of the SARA isoforms ($SARA_1$ and $SARA_2$) present in mouse with their domains and sites of interaction with different proteins, such as Smad2/3, PP1c, and TβRI. aa, amino acid.

control of the interaction with transforming growth factor β (TGF-β) signaling. Given this, the association is merely hypothetical, and further studies are needed to answer this question.

Structurally, SARA belongs to the large family of proteins containing the *Fab1*, *YOTB*, *Vac1*, and *EEA1* protein (FYVE) domain (1), which confers the ability to interact with phosphatidylinositol 3-phosphate (PI3P), a resident phospholipid of membranes highly enriched in endosomes and directly involved in the recruitment of proteins, membrane dynamics, and trafficking regulation (6, 7). The fact that SARA contains a FYVE domain and the fact that this protein localizes on early endosomes (EEs) suggest that it plays a role in trafficking. SARA was described to be a novel member involved in the endosomal trafficking pathway in neurons and other cellular models (8, 9).

In addition to trafficking, SARA is also able to mediate cell signaling. For this, SARA contains structural motifs for biochemical interactions, including a Smad binding domain (SBD), to interact with the transcription factors Smad2 and Smad3 (Smad2/3), and a C-terminal region, required for interaction with the TGF-β type I transmembrane receptor (TβRI) (1, 10). Both domains are indispensable for promoting the interaction of cytosolic Smad2/3 with the TβRI on the EE membrane, turning on the signaling through Smad2/3 phosphorylation, and further nuclear translocation (1). However, SARA may also inactivate TGF-β signaling by recruitment of the catalytic subunit of protein phosphatase 1 (PP1c) (11) (Fig. 1). The regulatory context for this antagonistic effect of SARA on TGF-β signaling is far from being understood. However, we discuss this phenomenon below in the section "Going out of endosomal trafficking: differential roles of SARA for cell signaling." Although proteins interacting with SARA and the protein belonging to the TGF-β pathway have been widely described, SARA may also interact with several other molecular partners, briefly summarized in Table 1.

CONTRIBUTION OF SARA TO EE-MEDIATED TRAFFICKING

Trafficking mediates a broad range of physiological processes, such as differentiation, proliferation, development, and apoptosis, among others. It is carried out via two basic routes, depending on whether cargo is moved out of the cell (exocytosis) or into it (endocytosis).

TABLE 1 Endosomal proteins directly or indirectly associated with SARA that participate in certain cellular functions

Protein	Associated function(s) of SARA (reference[s])	SARA domain	Cell types	Localization
Erbin (ErbB2/Her2-interacting protein)	Associates with Smad2/3 and is a negative modulator of TGF-β signaling, blocking oligomerization of Smad2/3 with Smad4 (88, 89). Competes with ERBIN for binding to Smad2/3; overexpression of SARA reverses the inhibitory effect of ERBIN on Smad2/3-dependent transcription (90).	Amino acids 730–926 (ERBIN-binding domain)	HEK 293, NIH 3T3, a keratinocyte cell line from adult human skin (HaCaT)	Early endosomes
RNF11 (RING finger protein 11)	Competes with Smad7 binding to Smurf2, thereby disrupting the Smurf2-Smad7 complex and restoring TGFβ signaling (91). The RNF11-SARA complex is associated with endosomal sorting complexes required for transport (ESCRT-0) core proteins, participating both structurally and functionally in the ESCRT-dependent lysosomal degradation of receptors (TβRs and EGFR) (92, 93). Perturbation of RNF11 and SARA levels decreases EGFR degradation, thereby generating conditions that may favor their mitogenic signaling (EGFRs that are constitutively active in certain cancers) (94, 95).	Amino acids 667–907	HEK 293, human umbilical vein endothelial (HUVE)	Early, late, and recycling endosomes
cPML (cytoplasmic promyelocytic leukemia protein)	An essential function in the modulation of TGF-β signaling in the cytosolic fraction that is profoundly impaired in the acute promyelocytic leukemia (APL) blasts (96, 97). Modulates TGF-β signaling, facilitating the localization of the TβRI-TβRII-SARA-Smad complex in the early endosome (97). Physically interacts with Smad2/3 and SARA, acts as a bridging factor between them, and is necessary for the formation of a stable and functional SARA-Smad2/3 complex (97).	No specific direct interaction	HEK 293T, COS-1, mouse embryonic fibroblasts (MEFs)	Discrete cytosolic punctate regions
Hgs (hepatocyte growth factor-regulated tyrosine kinase substrate)	FYVE domain protein involved in Smad activation through cooperation with SARA (98). Hgs and SARA, both of which bind to Smad2, synergistically cooperate in activin signaling (98). Both SARA and Hgs are reported to attenuate TGFβ signaling (99).	No specific direct interaction	HepG2, HEK 293T, human $CD4^+$ T, a human keratinocyte cell line (HaCaT)	Early endosomes
Endofin	May fulfill scaffolding functions together with SARA to promote R-Smad-Smad4 complex formation (100).	No specific direct interaction	HepG2, Hep3B (human hepatoma-derived containing hepatitis B virus surface antigen)	Early endosomes

Ligand-receptor complexes located at the plasma membrane are endocytosed by different mechanisms to transduce extracellular stimuli into the cell (12). The best-known mechanism for cargo internalization is clathrin-mediated endocytosis (CME), a process dependent on the adaptor protein clathrin and several associated proteins to load cargo in clathrin-coated vesicles (13, 14). Canonical examples of this transport include internalization of the transferrin receptor (TfR), the epidermal growth factor receptor (EGFR), the β2-adrenergic receptor (β2AR), and TGF-β receptors (TβRs) (15–19). However, clathrin-independent routes have also been described. The most representative example of this is caveola/raft-dependent endocytosis (20–22), an internalization mechanism mediated by a lipoprotein system of lipid rafts coated with caveolins and associated cavins (which are adaptor proteins). Several receptors located at the plasma membrane are internalized by this mechanism, including G protein-coupled receptors (GPCRs), receptor-tyrosine kinases (RTKs), TβRs, Wnt, and Notch (18, 23–25).

Independently of the internalization route, endocytosed molecules are sorted into endosomes containing different types of Rabs, a family of small GTPases belonging to the superfamily of Ras proteins, directly involved in trafficking (26). In their active state (bound to GTP), Rabs recognize molecular targets that are recruited to endosomes (26, 27). Some of these Rabs are selectively expressed in specific endosome populations, defining different pools of these. This property makes Rabs good molecular markers for

defining the identity of endosomes (28). However, there are reports of multiple combinations of Rabs on the same endosomal membrane, such as the simultaneous presence of Rab4, Rab5, and Rab11 (29–31). Mosaics of Rabs give endosomes biochemical and functional diversity (30), which may enable them to sort cargo for two main purposes: first, to regulate intracellular signaling and, second, to define whether cargo is rapidly recycled back to the plasma membrane or to final degradation through late endosomes and lysosomes (32, 33).

As we previously mentioned, the finding of the FYVE domain in SARA was the first indication of its binding to the EE membrane (1, 7, 8). Specifically, SARA has been found on Rab5 EEs. This pool of EEs receives cargo before it is destined either to rapid recycling or to lysosome-dependent degradation, an early step in cargo internalization (8, 9, 34, 35). However, several reports have shown the presence of SARA on EEs lacking either Rab5, Rab11, or EEA1 (9, 18, 34, 36). The physiological importance of these EE pools, as well as their spatiotemporal regulation, remains unclear and requires further research (37).

In addition to its location in the EE membrane, SARA has been proposed to be a key protein in EE dynamics and morphology. The gain of function of SARA after ectopic expression induced EE enlargement (38) and reduced the recycling of the transferrin receptor (TfR) to the cell surface, decreasing transferrin uptake in HEK 293T and MDCK cells, a phenotype previously observed after Rab5 overexpression (8, 38). This phenomenon was reversed after expression of Rab5-GDP, suggesting a functional link between SARA and Rab5 on EEs (8). SARA thus appears to play a crucial role in both EE dynamics and morphology.

CONTRIBUTION OF SARA-MEDIATED ENDOCYTOSIS TO NEURONAL DEVELOPMENT

Neurons present two different domains required for neurotransmission, the axon and the somatodendritic compartment, which makes neurons one of the most polarized cell types found in animal biology. The proper development and maintenance of these compartments are crucial for the physiology of the nervous system. To achieve this morphology, neurons experience several transformations throughout their lives, in a process known as "the establishment of neuronal polarity" (39). Briefly, neuronal development *in vitro* begins with spheres surrounded by an actin-rich structure (lamellipodium). Subsequently, neurons develop several minor neurites, conserving cellular symmetry. Then, one of these neurites grows faster than the others, breaking the symmetry of the cell (polarization) and developing the axon of the neuron (40–42). Neuronal polarization *in vitro* is comparable to the same phenomenon *in vivo* (43, 44).

For all these reasons and unlike in unpolarized cells, trafficking in neurons has specific adaptations (45, 46). Neural endosomes are also polarized. For example, an EEA1-positive EE population segregates to the somatodendritic domain of hippocampal neurons, while Rab5-positive endosome populations are located in both domains, the somatodendritic domain and the axonal compartment (47, 48).

Considering the participation of SARA in the endosomal system, several studies have explored the role of SARA in neuronal trafficking models both *in vitro* and *in vivo*. SARA is expressed in cultured hippocampal neurons and is uniformly distributed throughout the cell body, minor neurites, axon, and axonal growth cone. Functionally, SARA regulates neuronal development (9, 49). The loss of function of SARA in cultured neurons transfected with a short hairpin RNA targeting SARA (shSARA) or isolated from the SARA knockout (SARA-KO) mouse led to the formation of long supernumerary axons, a characteristic phenotype observed after the loss of polarization mechanisms (9).

In neurons, SARA has been found on endosomes carrying EEA1, Rab5, Rab4, and Rab11 (9), in agreement with reports suggesting at least 3 different populations of endosomes (either containing SARA or not): (i) endosomes carrying either EEA1 or Rab11, (ii) endosomes containing SARA, in addition to either EEA1 or RAb11, and (iii) endosomes carrying only SARA (8, 9). The suppression of SARA changed the distribu-

tion of recycling endosomes (REs) and altered the delivery of somatodendritic components, such as TfR (50) and the axonal protein L1, a cell adhesion molecule strongly implicated in cell migration, adhesion, neurite growth, myelination, and neuronal differentiation (51, 52). SARA also regulates the trafficking of rhodopsin-carrying vesicles in photoreceptors of the rat eye, targeting rhodopsin to nascent discs at the base of the outer segment (OS), a compartment specialized in light absorption (2). Together, this evidence supports the notion that SARA regulates and promotes axonal and dendritic development by delivering specific molecules mediated by its role in endosomal trafficking.

The genetic suppression of SARA *in vivo* after *in utero* electroporation of shSARA in embryonic mouse brains suggests that the loss of SARA function impairs neuronal migration during neocortex development (49). Postnatal analysis of *in utero*-modified brains (up to postnatal day 15 [P15]) revealed that cortical neuronal migration is still incomplete, suggesting a strong delay in neuronal development after embryonic knockdown of SARA. This suppression leads to an enhancement of L1 at the cortical surface, increasing contacts with neighboring neurites and changing the adhesion properties of migrating neurons, which may be explained by the abnormal delivery of L1 to the growing axons after SARA suppression. In addition, morphological changes were also detected after silencing SARA expression *in vivo*, affecting both neuronal orientation and the transition from multipolar to bipolar morphologies during cortical development through an L1-mediated mechanism (49).

Research on *D. melanogaster* and zebrafish revealed a protein that is homologous to SARA (named Sara) and, therefore, that has developmental functions homologous to the developmental functions of SARA in rodents, and it has been described that Sara participates during the division of neural precursors of the spinal cord in zebrafish (53). Asymmetric division of cells and neural precursors is crucial during early stages of embryonic development. In zebrafish, a daughter cell may differentiate into a neuron (called an "n cell") or divide again before differentiation into two neurons (called a "p cell"). In this context, differential levels of Sara endosomes in daughter cells determine the fate of the neural precursors, suggesting that Sara is essential for cell fate commitment in this type of lineage (53). Developmental studies using wing discs of *D. melanogaster* revealed that Sara accumulates in EEs, colocalizing with Rab5 (36). In addition, the phosphorylation of SARA is required during sensory organ precursor (SOP) division, controlling the dynamics of Sara-containing endosomes from the spindle during asymmetric division (54, 55) Finally, it has been described that the molecular motor Kinesp98A (a homolog of the mammalian KIF16B) is located in Sara-containing endosomes, bringing them to the central axis of the cell during division, which suggests that SARA participates in SOP development in *D. melanogaster* (56).

In summary, SARA mediates the trafficking of the neural and nonneural cargo required for development. Clearly, SARA is part of a highly dynamic network of endosomes that sort and deliver components for both neuronal and nonneuronal physiology. It seems that different combinations mixing SARA and Rab isotypes define the usefulness of endosomes, impacting their performance at both the endosomal trafficking and cell function levels. Further analysis is required to identify the context and the requirements for the assembly of these combinations.

GOING OUT OF ENDOSOMAL TRAFFICKING: DIFFERENTIAL ROLES OF SARA FOR CELL SIGNALING

Although SARA has been linked to the trafficking of TβR-associated components, several articles have shown that it also participates in nontrafficking signaling pathways. In this section, we discuss emerging concepts in intracellular signaling mediated by SARA obtained from different experimental models, focusing on the TGF-β, Notch/Delta, and epidermal growth factor (EGF) pathways.

Evidence of cooperation between SARA and TGF-β signaling. TGF-β is involved in numerous cellular processes, such as growth inhibition, proliferation, cell migration,

invasion, neuronal polarity, the epithelial-to-mesenchymal transition (EMT), remodeling of the extracellular matrix (ECM), and immune response suppression (57, 58). The binding of soluble TGF-β to its receptor in the membrane leads to the formation of receptor heterocomplexes, in which TβRII phosphorylates TβRI (59). TβRI then acquires a conformation that facilitates its activation and assembly with Smad2/3 at their COOH-terminal domains (60). Subsequently, Smad2/3 rapidly dissociates from the receptor to form a trimeric complex with the common mediator Smad4 (through phosphorylation of threonine 276) (61). This complex translocates to the nucleus, acting in cooperation with several transcription factors, coactivators, and corepressors to regulate the expression of target genes, such as those for fibronectin I, Rho-guanine nucleotide exchange factor 7 (GEF7), fibroblast growth factor 1, and glucocorticoid receptor DNA binding factor 1, among others (62–64).

The activation of TGF-β signaling can be switched off by the phosphatase PP1c, whose function is to dephosphorylate TβRI by a negative feedback mechanism that regulates TGF-β signaling. The formation of a complex between growth arrest and DNA damage-inducible protein (GADD34) and Smad7, with the consequent recruitment of PP1c to the site where TβRI is located, allows this regulation (65). In addition, TβRI can also be dephosphorylated by the protein phosphatase 2A (PP2A) (66).

The participation of SARA in TGF-β signaling is controversial. On the one hand, there is evidence supporting the hypothesis that SARA is necessary for TGF-β signaling, based on the following empirical data. First, SARA presents a Smad binding domain (SBD), by which it interacts with Smad2/3, a member of TGF-β signaling (10, 67). Second, the C-terminal domain of SARA allows an association with TβRI. It should be noted that SARA may interact with TβRI regardless of the binding of Smad2, as has been reported using Mv1Lu and COS-1 cells (1). Third, SARA also participates in the regulation of the subcellular distribution of Smad2/3. SARA helps to expose Smad2/3 to the activated TβRI complex for subsequent phosphorylation (7). Finally, SARA recruits PP1c through its phosphate binding domain (PBD). Experiments in *D. melanogaster* showed that overexpression of a mutant form of SARA (SARA-F678A, with a mutation within the PBD domain that prevents interaction with PP1c) results in the inhibition of PP1c recruitment to the TGF-β complex and, consequently, TβRI hyperphosphorylation. Similar results were obtained in COS-7 cells (11) (Fig. 2).

On the other hand, it has been suggested that SARA is dispensable for TGF-β signaling, based on the following findings. First, a SARA binding-deficient Smad2 mutant was phosphorylated by the tyrosine kinase receptors, suggesting that Smad2 phosphorylation and further activation may be mediated by both SARA-dependent and -independent mechanisms (68). Second, using B-cell lymphomas, no correlation was found between SARA expression and the TGF-β-induced phosphorylation levels of Smads. Similarly, the loss of function of SARA did not affect TGF-β-induced Smad activation, Smad nuclear translocation, or the expression of TGF-β–target gene expression in HeLa cells (69). Third, endogenous regulation of SARA is produced by phosphatidylinositol 3-kinase (PI3K) activity independently of TGF-β, indicating that the mechanism of PI3K inhibition-mediated SARA downregulation differs from that induced by TGF-β in human kidney cells (HCKs) (70). Fourth, the internalization of TβRs into EEs is not affected in mouse embryonic fibroblasts (MEFs) derived from SARA mutant mice lacking the FYVE domain (4). Fifth, the depletion of PI3K isoform PI3K-C2β or Vps34 caused a partial reduction of SARA binding to EEs without affecting Smad2/3 phosphorylation, suggesting that a reduction in the association between SARA and EEs does not compromise Smad2/3 phosphorylation in mouse and human vascular endothelial cells (71, 72).

Participation of SARA in Notch/Delta signaling. Notch/Delta signaling is an evolutionarily conserved pathway in multicellular organisms that regulates cell fate determination during development and maintains adult tissue homeostasis (73).

Using the *D. melanogaster* model, several studies about Sara participation in endosome-mediated signaling showed that Sara is associated with phosphatidylinosi-

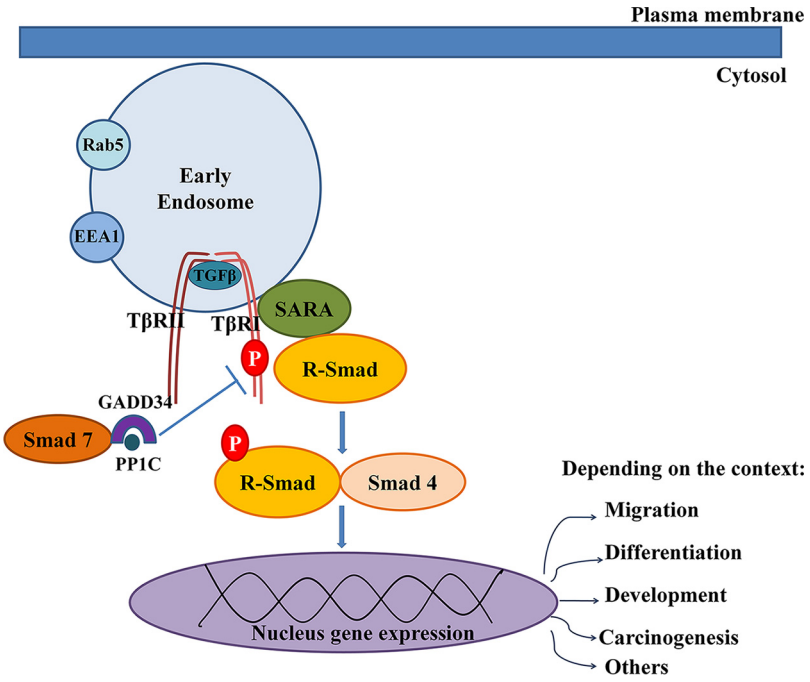

FIG 2 Representative image of the participation of SARA in the TGF-β signaling pathway. Once the receptor complex has been endocytosed with the TGF-β ligand, SARA binds to TβRI and recruits Smad2/3, and this is phosphorylated. Then, Smad2/3 binds to Smad4, and together they translocate to the nucleus and modulate the expression of target genes. On the other hand, the complex formed by Smad7-GADD34-PP1c acts as a negative regulator of the route, dephosphorylating TβRI.

tol 3-phosphate (PI3P) in multivesicular endosomes, which are directed to the central axis of the cell during mitosis and involved in the symmetric partition of decapentaplegic (Dpp) signaling molecules (TGF-β-type morphogens) between daughter cells during fly wing development (3, 36).

With the same model, during asymmetric division of the sensory organ precursor (SOP), Sara endosomes also segregate asymmetrically during cell division and contain both the Notch receptor and the Delta ligand, segregating these molecules to daughter precursors of the external sensory organ cell pIIa (the precursor of the sensory organ external cells), where Notch signaling is activated (53). Also, changes in Sara expression cause poor targeting of Notch signaling, disturbing its location in daughter cells, suggesting that Sara endosomes mediate asymmetric Notch/Delta signaling during the asymmetric division of SOP (3, 55). These results suggest that SARA is required not only for endosome segregation but also for the proper distribution of the signaling molecules necessary for development.

Participation of SARA in the EGF signaling pathway. The EGF signaling pathway is one of the most important pathways that regulate growth, survival, proliferation, and differentiation in mammalian cells. However, deregulation of the EGF/EGFR axis has been linked to different forms of pathogenesis, diseases, and cancer development (74). Recently, a link between SARA and the ubiquitin ligase RNF11, which is involved in the degradation of EGFR, has been reported in the A431 and HeLa cell lines. This study suggests that depletion of RNF11 increased the recycling of EGF to the plasma membrane and enhanced the extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation induced by EGF stimulation. Accordingly, the loss of function of SARA traps the EGF-EGFR complex in EEs, delaying its degradation and favoring ERK1/2 signaling. These results have expanded knowledge about the roles of SARA, offering new insights into EGF-EGFR trafficking and its intracellular signaling (75).

SARA thus participates in highly conserved signaling pathways, which regulate critical processes affecting cellular signaling during developmental and adult stages.

An interesting but unexplored issue is whether SARA might be involved in Smad-dependent pathways, such as signaling of bone morphogenetic protein (BMP), a critical morphogen for neuroectodermal specification, or even in Notch/Delta signaling in superior organisms as well as *D. melanogaster*. Moreover, considering that several of the phenomena described here also occur in humans, we wonder whether SARA may also contribute to asymmetric cell division in superior mammals, like that which occurs during hematopoietic stem cell division in humans, among other examples. These signaling pathways are also involved in the development of pathologies such as cancer (76–78); however, little is known about any implication that SARA may have in such diseases or whether it could be a therapeutic target.

EMERGING EVIDENCE OF SARA PARTICIPATION IN SEVERAL DISEASES

There is evidence that SARA is involved either directly or indirectly in pathological processes. During the epithelial-mesenchymal transition (EMT), a biological process by which cells lose their relatively differentiated epithelial characteristics and show increased migratory or synthetic properties (79), changes in SARA expression have critical consequences in the maintenance of the epithelial phenotype, altering Smad2 and Smad ubiquitination regulatory factor 2 (Smurf2) expression levels (5, 80, 81). SARA also regulates high-glucose-induced EMT and extracellular matrix (ECM) excretion, by modulating the activation of Smad2/3 in renal tubular epithelial cells, positioning SARA as a potential novel target in pre-EMT stages for the improvement of renal fibrosis in chronic kidney diseases (5). Stimulation of HEK cells with TGF-β decreases both SARA and Smad2 expression, producing changes in the cell phenotype and enhancing the expression of EMT markers, such as smooth muscle α-actin (80). A similar effect was described in renal tubular epithelial cells after induction of the EMT phenotype (5).

Interestingly, different disease models have revealed changes in SARA expression levels. As liver fibrosis develops, SARA expression tends to decrease (82), in contrast to observations obtained from epithelial cells derived from asthmatic human patients (83), in synovial fluid fibroblasts of rheumatoid arthritis patients (84), and in subcutaneous white adipose tissue from type 2 diabetes mellitus-induced obese rats (85), where SARA expression is upregulated. Moreover, using vascular smooth muscle cells of human patients with Marfan syndrome, an association between Rab5 and SARA in EEs facilitating TGF-β trafficking and signaling in these patients has been shown (86). In addition, evidence from SARA knockout mice suggests that SARA plays a causal role in skin carcinogenesis, promoting the dedifferentiation of cells to a malignant cell phenotype of skin cancer, suggesting a protective role for SARA in this disease (4). Finally, both SARA and phospho-Smad3 (p-Smad3) expression are increased in the hippocampus of rats after inducing status epilepticus (SE) by pilocarpine treatment (87). The suppression of SARA by lentiviral transduction delayed the start of SE through a mechanism dependent on TGF-β/p-Smad3, suggesting that the SARA/Smad3 pathway contributes to SE development (87).

Although several reports associate SARA with pathological processes, the studies correlate SARA expression levels only with pathological phenotypes. Moreover, the modification in SARA levels seems to be an indirect readout instead of a direct cause of these pathologies. Further studies are required to analyze the direct participation of SARA in these or other pathologies.

FINAL COMMENTS

In this review, we have summarized evidence on the role of SARA in both endosomal trafficking and cellular signaling processes (Table 2), with particular emphasis on the participation of SARA during neuronal development in several models. We have also mentioned the link between SARA and pathological processes. While it is true that both cellular and physiological scenarios may condition the interactions and functions of proteins, it is interesting that proteins like SARA, associated with endosomes, can

TABLE 2 Summary of the events in which SARA links to trafficking or signaling processes

Pathway	Evidence of function related to SARA	Cell type or organism	Reference(s)
Trafficking	Localized on early endosomes	HeLa, HEK, fly sensory organ precursors, rat hippocampal neurons	3, 8, 9, 34, 49
	SARA delays the recycling of Tf and TfR on the cell surface, while SARA suppression increases the levels of TfR	HEK, rat hippocampal neurons	8, 9
	The suppression of SARA affects the recycling endosome distribution, changing the endosome distribution after SARA suppression	Rat hippocampal neurons	9
	Interacts with rhodopsin and syntaxin 3 on axonemal vesicles, playing a role as a novel vesicle-tethering molecule	Vertebrate rod photoreceptor cells	2
	Sara endosomes traffic Delta/Notch during asymmetric cell division	Fly SOP in *D. melanogaster*	3
	Regulates the surface distribution of L1, affecting cell adhesion and migration	Cortical neurons of mouse brain	49
	Participates structurally and functionally in the ESCRT-dependent lysosomal degradation of EGFRs	Human umbilical vein endothelial (HUVE)	93
Signaling	Participates in TGF-β signaling, interacting with Smad2/3 and PP1c	COS-1, Mv1Lu, HepG2, COS-7	1, 11
	Sara endosomes mediate Notch/Delta signaling	Fly SOP in *D. melanogaster*	3
	The absence of SARA delays the degradation of EGF-EGFR, resulting in an increase in ERK1/2 signaling	Primary endothelial and HeLa cells	75
	Negatively regulates Dpp signaling	Wings in *D. melanogaster*	11
	Binds to Smad2 and synergistically cooperates with Hgs in activin signaling	Human $CD4^+$ T, a human keratinocyte cell line (HaCaT)	98

participate in several signaling pathways, regulating processes from development to adult homeostasis.

Although an active and critical role of SARA has been demonstrated during neuronal development, we should highlight that nothing is known about the expression, location, and function of SARA in the peripheral nervous system, which could have significance not only in growth but also in regeneration processes. Further studies are required to address this point.

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