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MINIREVIEW

A Leucine-Rich Repeat Protein Provides a SHOC2 the RAS Circuit: a Structure-Function Perspective

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ABSTRACT SHOC2 is a prototypical leucine-rich repeat protein that promotes downstream receptor tyrosine kinase (RTK)/RAS signaling and plays important roles in several cellular and developmental processes. Gain-of-function germ line mutations of SHOC2 drive the RASopathy Noonan-like syndrome, and SHOC2 mediates adaptive resistance to mitogen-activated protein kinase (MAPK) inhibitors. Similar to many scaffolding proteins, SHOC2 facilitates signal transduction by enabling proximal protein interactions and regulating the subcellular localization of its binding partners. Here, we review the structural features of SHOC2 that mediate its known functions, discuss these elements in the context of various binding partners and signaling pathways, and highlight areas of SHOC2 biology where a consensus view has not yet emerged.

KEYWORDS cancer, leucine-rich repeat, MAPK signaling, Noonan syndrome, PP1C, RAF, RAS, receptor tyrosine kinase, SHOC2

SHOC2 is a leucine-rich repeat protein that functions as a scaffold critical for RTK/RAS signaling. RAS proteins are a family of small GTPases that function as binary switches and are critical in development, multiple physiological processes, and diseases (1). The canonical RAS family members HRAS, KRAS, and NRAS have been shown to bind SHOC2 as well as a closely related RRAS subfamily member, MRAS, for muscle RAS homolog. RTKs are upstream regulators of RAS and drive RAS activation in response to growth factor binding (1, 2). Downstream effectors of RAS, including RAF and phosphatidylinositol 3-kinase (PI3K), bind GTP-loaded RAS and function as key mediators of RAS signaling. Upon activation, RAF kinases trigger a mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling cascade (1, 3). Subsequently, activated ERK paralogs translocate to the nucleus to phosphorylate targets, such as transcription factors that upregulate cell proliferation and survival (1, 3).

Although RAS effectors have been extensively studied, the functional roles of ancillary regulatory proteins such as SHOC2 are less characterized. Most SHOC2 studies underscore its role in promoting MAPK signaling, best exemplified by gain-of-function mutations of SHOC2 that are causal in Noonan-like syndrome, a RASopathy (4, 5). Although aspects of SHOC2 biology and its binding have been previously discussed (6, 7), here we outline the function of SHOC2 through its structural properties that mediate protein-protein interactions in further depth, provide an extensive discussion of the current conflicting reports regarding SHOC2 function, and highlight how SHOC2 is essential for the RTK/RAS signaling circuit. We also discuss recent work pertaining to the role of SHOC2 in RAS mutant cancers and propose potential therapeutic strategies to target SHOC2.

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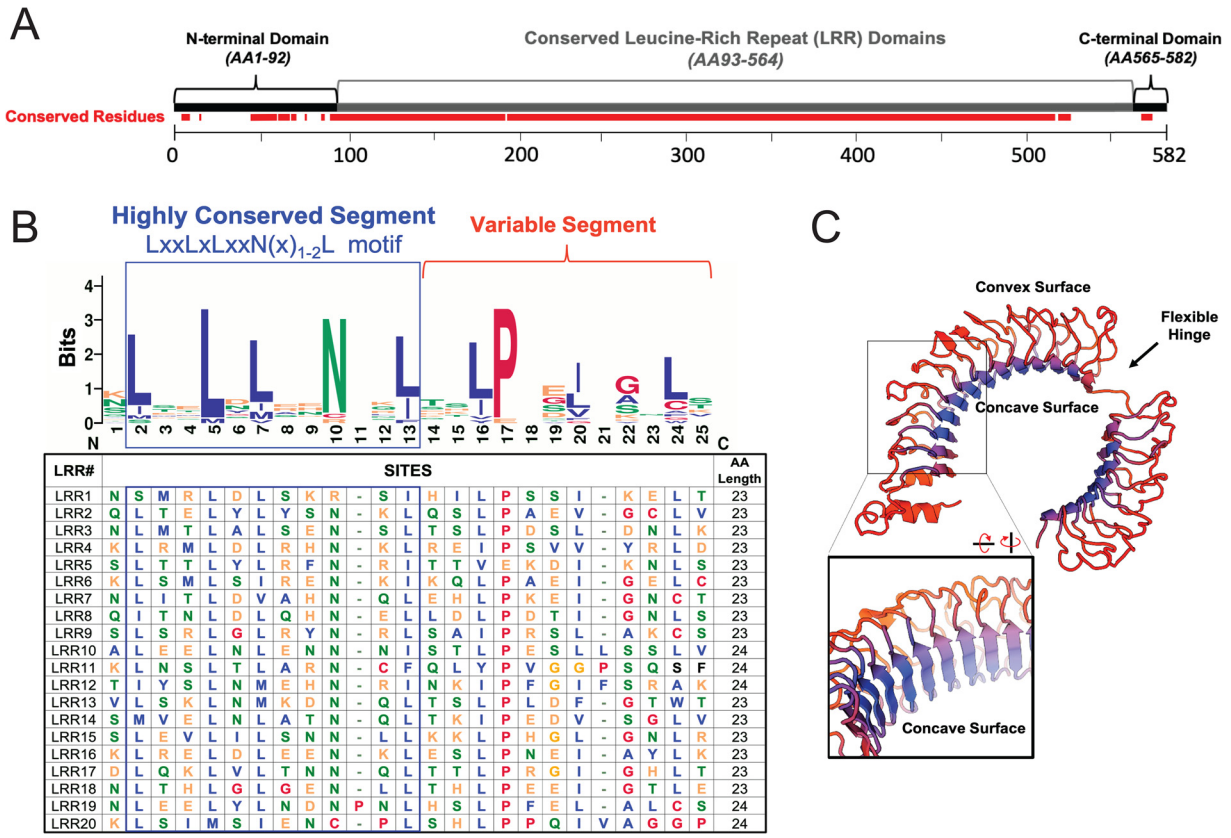

FIG 1 SHOC2 domains, sequence, and structural features. (A) Full-length SHOC2 (NCBI RefSeq NP_031399.2), depicting amino acid (AA) segments corresponding to domain regions and conserved leucine-rich repeats (LRRs). Conserved segments of SHOC2 from primary sequence analysis of several SHOC2 orthologs (COBALT) are annotated in red. (B) Primary sequence analysis of highly conserved SHOC2 LRRs (UniProtKB no. Q9UQ13-1) with consensus sequence logo (WebLogo v2.8.2) and sequence alignment (MAFFT v7.31). The canonical highly conserved leucine-rich repeat motif [LXXLXLXXN(X)$_2$L] is outlined in blue. (C) 3D modeling of SHOC2 (SWISS-MODEL; template 3b2d.1.A) with predicted flexible hinge indicated. The inset represents a rotated view, highlighting parallel beta sheets.

SHOC2 DISCOVERY

SHOC2 was first discovered through a genetic screen designed to identify genes that facilitate fibroblast growth factor (FGF) receptor (FGFR) signaling in *Caenorhabditis elegans* (8). Mutations of genes that enhance FGFR signaling drive a clear phenotype in *C. elegans*, whereby the organisms appear transparent compared to the wild-type control (8). Using this clear phenotype, DeVore and colleagues performed a genetic screen for genes whose deletion acted as a suppressor of clear (soc) phenotype (8). Among these genes that they identified were orthologs of the RTK adaptor protein GRB2 (soc-1) and SHOC2 (soc-2) (8, 9). Subsequent genotype-phenotype mapping and yeast two-hybrid experiments placed SHOC2 directly downstream of let-60 RAS and upstream of RAF (lin-45), and SHOC2 was found to directly interact with both RAS and RAF (10, 11). These initial studies noted a striking similarity between the primary structures of *C. elegans* ortholog soc-2/sur-8 and human SHOC2, with the highest sequence similarities found within repeated leucine-rich motifs (9, 10).

SHOC2: A CANONICAL LRR PROTEIN

Repetitive amino acid sequences, known as protein tandem repeats, give rise to unique, complex tertiary structures (12). Leucine-rich repeat (LRR) proteins are among the most well-studied examples of tandem repeat proteins. The major characteristic of LRR proteins is the repetitive sequences of leucine or other hydrophobic residues. SHOC2 is a quintessential LRR protein with an unstructured N-terminal domain followed by 20 predicted LRRs (UniProtKB) and a short C-terminal domain (Fig. 1A). Many

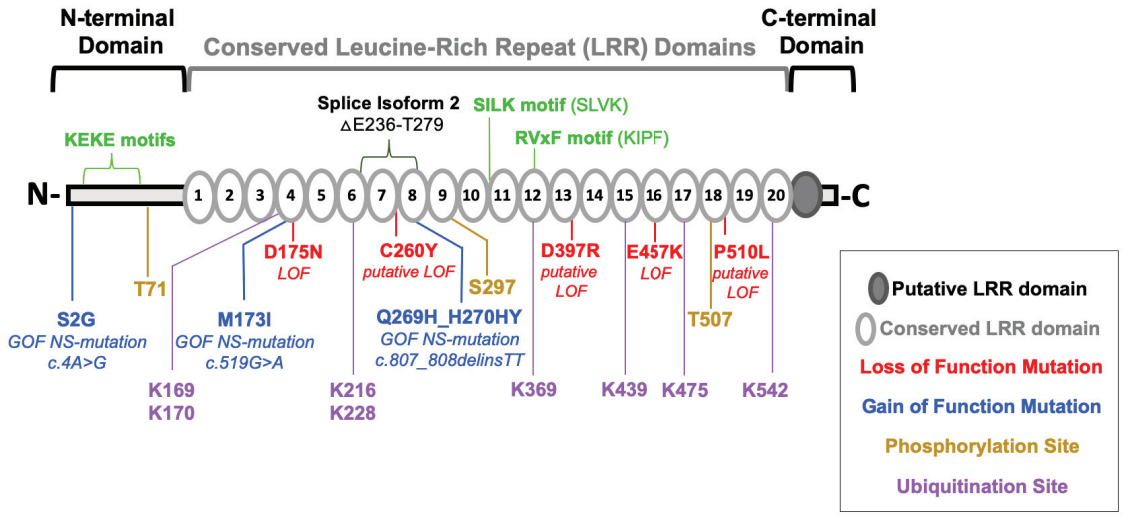

FIG 2 Critical SHOC2 sequence motifs and residues. Shown are a domain diagram of SHOC2 with the indicated domain regions, LRRs, and putative LRR and a domain diagram of full-length SHOC2 with motifs (green), deleted region of transcript variant 2 (536 aa) lacking exon 3 (NCBI RefSeq NR_136749.1), posttranslationally modified residues, and mutations reported to be functionally important in the literature, including gain-of-function (GOF) and loss-of-function (LOF) mutations. Putative mutations denote equivalent missense mutations of *SHOC2* identified in *C. elegans* ortholog soc-2/sur-8.

functionally important residues of SHOC2 map within its LRR domains; however, both the flanking N-terminal and C-terminal unstructured regions participate in maintaining key interactions as well (13). LRR domains of SHOC2 are 22 to 23 amino acids in length and start with a highly conserved segment (HCS) followed by a variable region with a reoccurring, medial proline residue (Fig. 1B). Each HCS consists of 11 to 12 amino acids with a sequence motif of LXXLXLXXN$(X)_{1-2}$L (Fig. 1B), where L can be a leucine or any other hydrophobic residue and N is an asparagine (14).

Leucine-rich HCSs are known to form short beta-strands that arrange in parallel to one another through interdomain hydrogen bonding (14). The arrangement of repetitive parallel beta-strands concatenates each LRR domain, resulting in a solenoid structure (14). Although the SHOC2 structure has yet to be solved, homology modeling methods predict that SHOC2 forms a horseshoe shape, with the reoccurring leucine-rich HCS making up the concave surface (15–17) (Fig. 1C). Some structure modeling algorithms predict a flexible hinge region between N- and C-terminal LRRs (Fig. 1C), lending to speculation that SHOC2 undergoes conformational changes depending on its interactions with various binding partners (13, 17, 18). However, not all structural models anticipate this cleft but instead predict a more rigid solenoid with a greater helical turn along the LRR domains (15, 19). Further ambiguity exists with SHOC2 structure regarding the precise number of LRR domains, with estimates ranging from 18 to 21 (9, 10, 13, 16, 17, 20), where the purported 21st LRR motif at the C terminus serves as a cap to stabilize the inner domains (20) (Fig. 1C), but this putative LRR-like domain lacks an HCS and does not align with the SHOC2 LRR consensus sequence. We note that many of these features are based on predictive computational models, and further work to solve SHOC2 structure in the presence and absence of these effectors remains an important area of investigation.

SHOC2 is expressed in many tissue types and is conserved across phylogenetic domains, ranging from *Cyanobacteria* and *Arabidopsis* to nonhuman primates and humans (15). SHOC2 orthologs exhibit a high degree of sequence homology within their LRR domains (Fig. 1A), which are known to serve as scaffolding surfaces that mediate protein interactions. Accordingly, numerous functionally important segments of SHOC2 have been found to reside within the LRRs. In the following sections, we will discuss the catalog of SHOC2 binding proteins and complexes that regulate

downstream RAS effector pathways. We will contextualize key SHOC2 gain-of-function (GOF)/loss-of-function (LOF) mutations and critical motifs that have been shown to mediate these interactions (Fig. 2).

THE MRAS/SHOC2/PP1 HOLOPHOSPHATASE

Initial *C. elegans* studies identified SHOC2 to be downstream of RAS but upstream of RAF, suggesting that SHOC2 facilitated proximal RAS-RAF interactions (10). Seminal work published by Rodriguez-Viciana and McCormick revealed that SHOC2 forms a holophosphatase with the catalytic subunit of phosphatase PP1 and MRAS, whereby SHOC2 serves as the linchpin for complex formation (21). Each of the three members plays indispensable roles in the proper function of the complex to enable RAS signaling.

Within the complex, PP1 serves as the enzymatic subunit for substrate serine/threonine dephosphorylation. PP1 is a class of serine/threonine phosphatases that has three isoforms (PP1α, PP1β, and PP1γ) (22). Similar to other phosphatases, PP1 requires regulatory subunits to control the specificity of target dephosphorylation, and several putative regulatory factors, including SHOC2, have been proposed (21, 22).

The best-known substrates of the MRAS/SHOC2/PP1 holoenzyme are the RAF kinases. MRAS/SHOC2/PP1 potentiates RAF activity through dephosphorylation of a critical regulatory site, S259, on RAF family members (S259 RAF1, S365 BRAF, and S214 ARAF) (16, 21) (Fig. 3A). During unstimulated conditions, inactive RAF kinases are found as monomers and are cytosolically sequestered by the binding of 14-3-3 to phosphorylated S259 and S621 on RAF (23, 24), which enforces a closed, inactive confirmation of RAF. Upon RAS activation, RAF associates with RAS-GTP at the plasma membrane, and S259 is dephosphorylated by the MRAS/SHOC2/PP1 complex (Fig. 3A), triggering an open RAF conformation (25). Active RAFs then dimerize, which is required for subsequent MAPK signaling (24). The regulation of RAF activity is intricate and has been extensively reviewed (24).

SHOC2 has two degenerate PP1 consensus binding motifs, SILK (SLVK) and RVxF (KIPF), found within medial LRRs (Fig. 2), and both sequence motifs cooperate in mediating PP1 binding (16). Although PP1α, PP1β, and PP1γ have been shown to bind SHOC2, only PP1α and PP1β dephosphorylate the inhibitory BRAF S365 phosphorylation site (16). Furthermore, PP1α/β-SHOC2 interactions are dependent on MRAS binding with SHOC2, but PP1γ is constitutively bound to SHOC2 irrespective of MRAS interaction (16), lending to the potential for differential SHOC2-PP1γ-specific substrates.

Although MRAS clearly interacts with SHOC2 (16, 21), several reports describe other RAS family interactions (10, 11, 26). MRAS, also known as R-RAS3, is a member of the RAS family and similarly functions as a binary switch dependent on its GTP/GDP bound state (1, 27). MRAS shares sequence similarity and an identical effector domain with the other more commonly studied RAS members, HRAS, NRAS, and KRAS, and also localizes to the plasma membrane (1, 25). MRAS-mediated localization of SHOC2 at the plasma membrane is critical for MAPK signaling, likely by increasing proximal interactions of PP1 to its substrates, ARAF, BRAF, and RAF1, that are bound to RAS (16, 28) (Fig. 3A).

RAS interacts with SHOC2 through its RAS effector domain upon GTP loading (11, 16, 21), and domain-mapping experiments identified the unstructured N-terminal region of SHOC2 to be particularly important in mediating MRAS interactions (10, 13, 15). Furthermore, *SHOC2* mutations of specific residues that disrupt or enhance MRAS binding have been discovered within the fourth, seventh, and eighth conserved LRRs (Fig. 2). These mutations include *SHOC2* GOF mutations discovered in Noonan-like syndrome, M173I and Q269_H270delinsHY, both leading to enhanced MRAS binding (18, 29).

C. elegans screens identified loss-of-function missense mutations of soc-2/sur8. Specifically, these mutants are equivalent to SHOC2 mutations D175N, C260Y, and

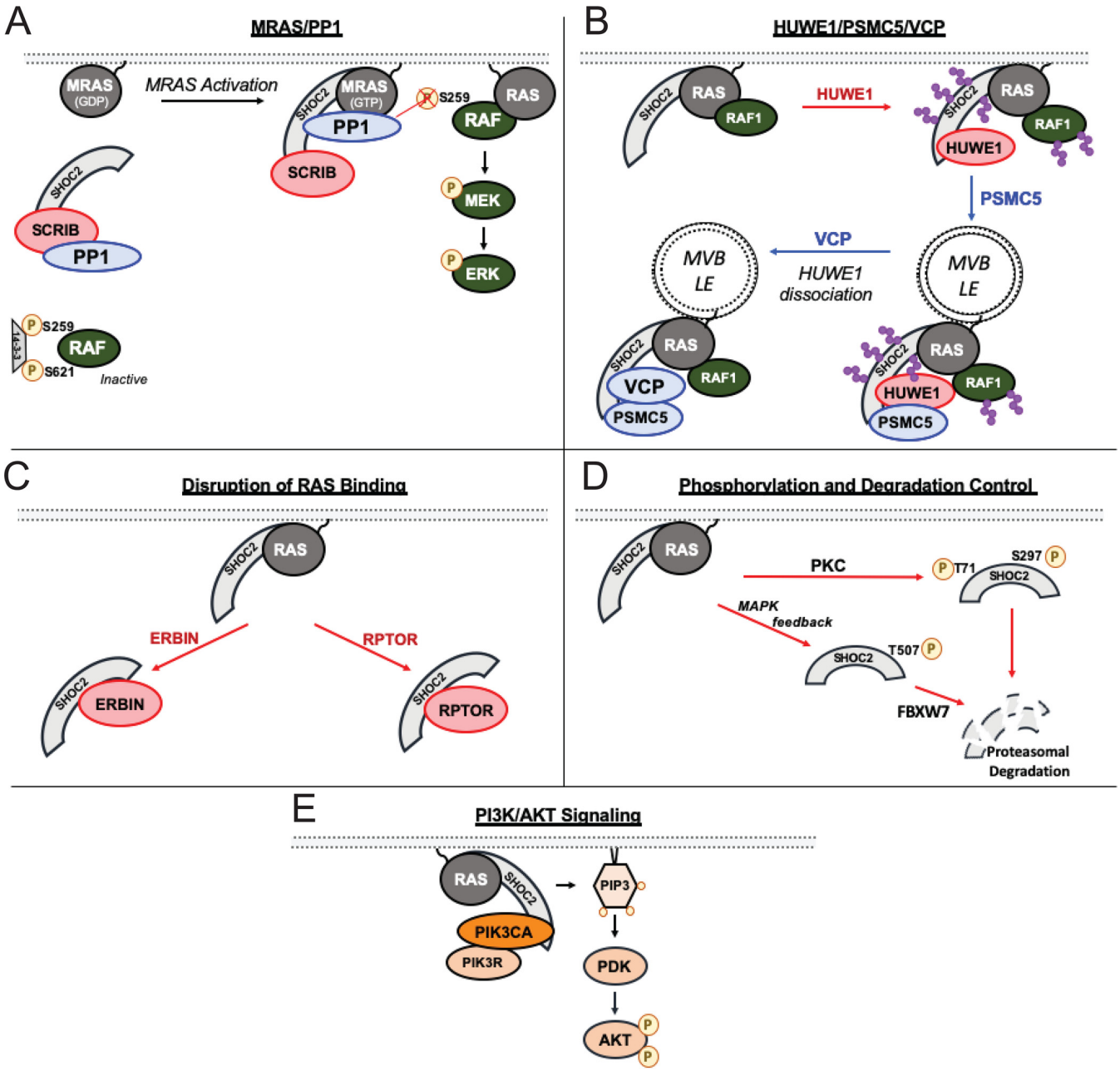

FIG 3 SHOC2-regulated RAS effector pathways and dynamic functional interactions. Shown is a schematic pathway overview of SHOC2's role in the MAPK/ERK pathway involving MRAS/PP1 holophosphatase complex (A), HUWE1/PSMC5/VCP (B), interactors that sequester SHOC2 from RAS (C), posttranslational modifications that result in SHOC2 degradation (D), and PIK3CA/p110α that mediates PI3K/AKT signaling (E). Direct SHOC2 interactors that result in diminished MAPK signaling are depicted in red, and those that enable MAPK signaling are in blue. Ubiquitin chains are represented in purple, and multivesicular bodies (MVB)/late endosome (LE) are indicated.

E457K (Fig. 2) (8–10). Indeed, D175N and E457K SHOC2 mutants have disrupted MRAS binding, and the E457K mutant fails to bind PP1 (21, 28). Intriguingly, forced localization of SHOC2 to the plasma membrane by N-terminal myristylation in combination with E457K mutation rescues MRAS-PP1 interactions (28).

Initial studies suggested that SHOC2 serves as a scaffold that accelerates RAS-RAF interactions to enhance MAPK signaling (10, 11). However, a series of immunoprecipitation studies demonstrated that RAF1 interactions with SHOC2 are indirect and mediated by binding of MRAS (21). The model that has been proposed articulates that SHOC2 facilitates MAPK signaling through its RAF holophosphatase activity, resulting in enhanced RAF dimerization and activation.

THE MRAS/RAF1/SHOC2/HUWE1 COMPLEX

In addition to direct regulation of RAF dimerization, SHOC2 also regulates MAPK signaling through an additional complex that elicits a negative-feedback regulation of RAF1 activity following epidermal growth factor (EGF) receptor (EGFR) activation (20, 30). HUWE1 is an E3 ubiquitin-protein ligase previously reported to ubiquitinate several critical proteins, such as MCL1 (31), TP53 (32), and MYCN (33). HUWE1 was initially identified as a SHOC2 interactor through a yeast 2-hybrid screen where SHOC2 served as the bait (30). Structurally, LRRs 12 to 14 of SHOC2 are required for its interaction with the HECT domain of HUWE1 (30). Epidermal growth factor (EGF) stimulation results in SHOC2-dependent, acute MAPK activation (20). Shortly following this activation, SHOC2 is ubiquitinated by HUWE1, internalized, and localized to late endosomes (15, 20, 34) (Fig. 3B). SHOC2 is modified with K11, K48, and K63 ubiquitin linkages, and HUWE1 induces polyubiquitination of SHOC2 at numerous lysine residues (Fig. 2), resulting in subsequent RAF1 ubiquitination (20, 30, 34). This ubiquitination event does not impact the protein stability/half-life of SHOC2 but is a necessary step for RAF1 turnover, and RAF1 levels increase upon HUWE1 knockdown, while BRAF and ARAF stability are unaffected (30). Subsequently, HUWE1/SHOC2-mediated reduction of RAF1 expression results in diminished MAPK signaling (30). A total of 8 lysine residues on SHOC2 have been identified to be ubiquitinated (K169, K170, K216, K228, K369, K439, K475, and K542) (30) (Fig. 2). A version of SHOC2 that contained arginine substitutions that cannot be ubiquitinated at 7 of these lysine residues (K169, K170, K216, K228, K369, K475, and K542) led to reduced RAF1 ubiquitination and sustained ERK activation (30).

Following HUWE1-mediated feedback inhibition, the complex undergoes rearrangement for subsequent reactivation of MAPK signaling. The resetting of the complex is regulated by PSMC5 and VCP, which regulate complex localization and reorganization, respectively. PSMC5 and VCP are both AAA+ ATPases, a superfamily of proteins that are characterized by Walker A/B motifs that hydrolyze ATP for energy to perform a diverse array of functions, including protein remodeling and denaturation (35, 36). PSMC5 is predominately known for its role as part of the 26S proteasomal complex (37) and modulating complex disassembly (38, 39). However, in the case of SHOC2, PSMC5 facilitates SHOC2 subcellular localization (34). Upon EGF stimulation, PSMC5 binds to the C-terminal LRRs of SHOC2 through its ATPase domain and facilitates MRAS/RAF1/SHOC2/HUWE1 complex localization to late endosomes (34). Subsequently, VCP recognizes and binds to polyubiquitinated SHOC2, resulting in HUWE1 dissociation from the complex (40). The displacement of HUWE1 at the late endosomes causes decreased SHOC2/RAF1 ubiquitination and resets the complex for further MAPK reactivation (34, 40) (Fig. 3B). The MRAS/RAF1/SHOC2/HUWE1 complex adds additional spaciotemporal complexity to SHOC2-mediated control over MAPK signaling.

ERBIN/SCRIB: LAP PROTEINS CONTROL SHOC2-MEDIATED MAPK SIGNALING

ERBIN and SCRIB are a part of the conserved family of LRR and Psd95-Dlg-Zo1 (LAP) domain-containing proteins and are known to localize at the plasma membrane to facilitate apicobasal polarity (41–43). LAP proteins, as their name implies, are structurally characterized by a series of N-terminal LRR domains and C-terminal PDZ domains (44).

ERBIN, also known as Erbb2 interacting protein, is known to regulate ERBB2 signaling by facilitating interactions with β2-adrenergic receptor (β2-AR) through its PDZ domains (45). ERBIN directly binds SHOC2 through its LRR domains (46), and this interaction has been shown to be functionally important in developmental processes including cardiac development and keratinocyte differentiation (47–49). ERBIN inhibits MAPK signaling by competing SHOC2 away from RAS (46, 49) (Fig. 3C). ERBIN has also been reported to interact with PP1, but this interaction was not further functionally characterized (16).

SCRIB similarly has been found to directly bind SHOC2 and inhibit MAPK signaling. However, unlike ERBIN, SCRIB does not disrupt SHOC2/RAS interactions. Instead, SCRIB

functions by sequestering PP1 from SHOC2 by binding to PP1 with higher affinity (16) (Fig. 3A). The interaction of SHOC2 with active MRAS enhances PP1 binding affinity, allowing for efficient holoenzyme formation by competing away PP1 from SCRIB (16). Furthermore, SCRIB LRR domains alone were not sufficient to enable its interaction with SHOC2. Rather, a segment of SCRIB between the N-terminal LRRs and C-terminal PDZ domains is necessary for SHOC2 binding (16). SCRIB binds to the C-terminal half of SHOC2, and LOF SHOC2 mutation E457K, found within conserved LRR16, results in hampered SCRIB binding (16, 28) (Fig. 2).

We note that both ERBIN and SCRIB are classic LAP proteins with similar structural features, yet they interact with SHOC2 through different domains. Further studies investigating the contact surfaces of LAP proteins that mediate SHOC2 interactions may reveal insights in LRR domain binding and define additional SHOC2 interactions.

NONCANONICAL ROLE OF RPTOR IN MAPK SIGNALING

SHOC2 has also been found to arbitrate cross talk between the MAPK pathway and mammalian target of rapamycin (mTOR) signaling (50). The mTOR kinase is a significant signaling hub downstream of various intracellular amino acid sensors and growth factor signaling and controls numerous signaling pathways designed to regulate nutrient homeostasis, metabolism, protein translation, and autophagy, among other cellular processes (51). Regulatory-associated protein of mTOR (RPTOR) is a well-known regulator of mTOR activity and functions by recruiting substrates to mTOR within the mTORC1 complex (52–54). RPTOR directly interacts with SHOC2 to inhibit SHOC2-RAS interactions and diminish MAPK signaling (50) (Fig. 3C). SHOC2/RPTOR interactions reciprocally lead to the dissociation of RPTOR from mTOR through competitive binding (50). The sequestration of RPTOR from the mTORC1 complex via SHOC2 binding decreases mTOR activity and increases autophagy (50), in line with the established role of mTOR as a negative regulator of autophagy (51). These findings highlight SHOC2 as a potential regulator of mTOR, which may serve as a mechanism that regulates both the MAPK and mTORC1 pathways.

SHOC2 PHOSPHORYLATION AND DEGRADATION CONTROL

SHOC2 protein degradation was first shown in neural progenitor cells, where differentiation resulted in a rapid decline of SHOC2 at the protein level (55). Recent studies have since identified key regulators of SHOC2 turnover and the phosphorylation of specific serine/threonine residues that trigger proteasomal degradation in the context of RTK signaling.

The first report of SHOC2 phosphorylation was discovered in the context of FGF/RTK signaling, whereby FGF2 stimulation stabilizes SHOC2 protein levels (56). Protein kinase C (PKC) family members PKC-α and PKC-δ phosphorylate SHOC2 at an N-terminal threonine residue (T71) and serine residue within LRR9 (S297), respectively (56) (Fig. 2 and 3D). Phosphorylation at either site is sufficient to cause SHOC2 polyubiquitination and proteasomal degradation, followed by diminished MAPK signaling (56). However, PKC protein levels are rapidly reduced upon FGF2 stimulation, resulting in SHOC2 stabilization (56).

In contrast, EGF stimulation was found to result in a MAPK negative feedback loop involving FBXW7-mediated SHOC2 degradation (50). FBXW7 is an F-box protein that is an integral member of the ubiquitin ligase Skp1–Cdc53/cullin–F-box protein (SCF/β-TrCP) complex and has been reported to function as a tumor suppressor (57). FBXW7 binds target substrates of SCF/β-TrCP complex to be marked for proteasomal degradation (58, 59). Upon EGF stimulation, SHOC2 is phosphorylated at a threonine residue in LRR18 (T507) in a MEK-dependent manner (50) (Fig. 2 and 3D). FBXW7 binds to the SHOC2 phosphorylation site through its F-box domain, leading to SHOC2 ubiquitination (50). In contrast to HUWE1 (30), FBXW7-dependent ubiquitination of SHOC2 results in proteasomal degradation (50). Although MEK inhibitors (MEKi) were found to be sufficient in reducing phosphorylation of T507, the kinase that directly phosphorylates SHOC2 was not identified (50). In

contrast, other studies failed to observe changes in SHOC2 protein levels in response to MEKi treatment (60, 61), lending to the possibility of context-specific regulation of SHOC2 expression.

SHOC2 IN PI3K-AKT AND MTOR SIGNALING

Phosphatidylinositol 3-kinase (PI3K) is another important RAS effector that has been shown to be indispensable in the tumor growth of RTK/RAS-driven cancers (62–64). In addition to RAF, studies have identified a novel role of SHOC2 in regulating the PI3K-AKT signaling pathway. PI3Ks are membrane-associated lipid kinases and are composed of regulatory subunits (p55 and p85) as well as catalytic subunit PIK3C/p110. As a RAS effector, PIK3C binds active RAS through its effector domain, which activates PI3K, resulting in the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) to produce PIP3 (65). PIP3 localizes AKT to the plasma membrane, where PDK1 phosphorylates and activates AKT (66). Subsequently, activated AKT phosphorylates numerous downstream substrates, resulting in proliferation, regulation of metabolism, and other cellular processes (65, 66). The presence of constitutively active HRAS or EGF treatment results in enhanced PIK3CA-SHOC2 interactions and activated AKT (67) (Fig. 3E). SHOC2 loss in colorectal cancer cells with mutant KRAS, BRAF, and PI3K also inhibited levels of active AKT (67). Although these findings pose a noncanonical role of SHOC2 beyond MAPK signaling, the mechanism of how PIK3CA-SHOC2 interaction enhances PI3K activity and whether these interactions are direct or indirect has not been fully explored. Furthermore, several independent studies have found SHOC2 to have a unique impact on MAPK signaling with no effect on the PI3K signaling axis (11, 21, 60, 61). Additional investigations are necessary to provide mechanistic insights on the determinants of differential SHOC2 regulation of the RAS effector pathways.

THE DYNAMICS OF SHOC2 SUBCELLULAR LOCALIZATION

Scaffold proteins serve as signaling hubs by coordinating binding partners and mediating proximal protein-protein interactions that would otherwise be stoichiometrically unfavorable (68). In addition, scaffold protein localization plays a vital role in signaling by facilitating spaciotemporal control of its interactors (68, 69). SHOC2 has been shown to be dynamically distributed across multiple intracellular compartments, including the plasma membrane, endosomes, multivesicular bodies, and the nucleus.

The importance of localization in SHOC2 biology is highlighted by a prominent GOF mutation discovered in Noonan-like syndrome. The *SHOC2* missense mutation (c.4A>G) results in a glycine substitution at the second residue (S2G) (Fig. 2) and confers an aberrant N-terminal myristylation site (4). Myristylated SHOC2 S2G constitutively localizes to the plasma membrane, causing increased MAPK signaling in response to EGF stimulation (4, 17). The plasma membrane is dynamic and heterogeneous, with regions of ordered lipid rafts rich in saturated fatty acids as well as disordered domains (70). Different RAS family members have been found to cluster in particular types of lipid domains, and this membrane organization has been shown to be important in regulating RAS signaling (70). Intriguingly, SHOC2 S2G mutants, under serum-starved conditions, localize to both cholesterol-rich and cholesterol-free lipid domains (17). However, upon EGF treatment, SHOC2 S2G redistributes to cholesterol-independent regions, revealing another potential level of localized regulation (17). Unlike myristylated mutant SHOC2, wild-type SHOC2 requires MRAS binding for plasma membrane localization (Fig. 3). SHOC2 LOF mutants unable to bind MRAS (D175N and E457K) are retained within the cytoplasm (28). Recent work found SHOC2 colocalizes with active MRAS at cell-cell junctions (28). Here, SHOC2 promotes concentrated MAPK activation, resulting in the phosphoregulation of p120-catenin and subsequent cell migration/scattering through junctional E-cadherin turnover (28).

The activation cycle of RTKs is dynamic, and activated receptors are known to be internalized to facilitate intracellular signaling and recycling (71). SHOC2 has also been found to localize to endosomes and multivesicular bodies. In the context of the MRAS/RAF1/SHOC2/HUWE1 complex, EGF treatment triggers SHOC2 internalization in a

clathrin-dependent manner (20). As discussed, this localization event was implicated in a stepwise resetting of the complex for subsequent MAPK reactivation (20). The C-terminal region of SHOC2 is necessary for SHOC2 localization at late endosomes and multivesicular bodies in response to EGF treatment (15, 34). Specifically, deletion of the putative LRR domain within the SHOC2 C-terminal region results in diffuse cytoplasmic distribution, presumably due to loss of PSMC5 interaction (15, 34).

Finally, SHOC2 has also been reported to be present within the nucleus (4, 15, 17, 20). Independent studies have found the N-terminal half of SHOC2 is required for both nuclear export and import (15, 17). The disordered N-terminal region of SHOC2 contains characteristic alternating lysine and glutamate residues (KEKE motifs) that are required for SHOC2 nuclear export and MAPK/ERK activity (13, 17). In addition, LRRs 2 to 5 are required for nuclear import, lending to speculation of a noncanonical nuclear localization sequence within this region (17). In initial studies, EGF stimulation of a panel of cell lines and primary patient fibroblasts was found to result in a robust translocation of SHOC2 into the nucleus (4). However, these findings conflict with reports involving the MRAS/RAF1/SHOC2/HUWE1 complex, whereby EGF treatment consistently results in SHOC2 detected in late endosomes and a clear absence of nuclear translocation (15, 20). Nuanced differences in EGF concentrations, treatment duration, and cell models are unlikely to explain this disparity, as the conflicting studies utilized similar EGF treatment conditions and reported disparate SHOC2 localization in the same COS-1 cell line (4, 15, 17, 20).

Nevertheless, these studies highlight the intricate role of SHOC2 within various compartments of the cell that affect RTK/MAPK signaling. Although the functions of SHOC2 within each compartment have been studied in isolation, we anticipate SHOC2 likely resides in multiple compartments, including at the plasma membrane, intracellular vesicles, and within the nucleus, undergoing constant dynamic trafficking.

SHOC2 IN RAS MUTANT CANCERS

Given the functional importance of SHOC2 in RAS signaling, it is not surprising that SHOC2 has been shown to play an instrumental role in transformation, metastasis, epithelial-to-mesenchymal transition (EMT), and MAPK pathway inhibitor resistance (16, 50, 56, 60, 61, 67, 72–74). RAS family proteins are known to be one of the most frequently mutated oncogenes, where ~30% of all cancers possess constitutively active mutations in *KRAS*, *HRAS*, or *NRAS* (75, 76). RAS hot spot mutations have been found to drive many tumor types, and RAS mutant cancers display a clear addiction to oncogenic RAS (75, 76). For these reasons, RAS proteins have been long-sought-after therapeutic targets but have proven challenging to directly target (75). The development of KRAS G12C covalent inhibitors, which covalently bind to the sulfur atom found within the mutated cysteine residue (77, 78), has shown responses in early clinical trials.

Several RAS pathway germ line mutations have been described in Noonan and Noonan-like syndromes. These mutations cause constitutive MAPK activation, and, accordingly, these patients have been found to be predisposed to certain cancers (79, 80). Although many of the Noonan/Noonan-like syndrome-associated genes have also been found to be somatically mutated in spontaneously arising cancers, somatic mutations in SHOC2 have not been described to date. Specifically, no SHOC2 mutations were found in a sizable cohort of leukemia biopsy specimens (81). In addition, SHOC2 is rarely found to be amplified or mutated in numerous cancer types, with mutation rates ranging from <1% in glioblastoma to 2.9% in uterine cancers (cBioPortal) (Fig. 4A), and there are no apparent hot-spot mutations of *SHOC2* (Fig. 4B). However, SHOC2 is ubiquitously and abundantly expressed in many cancer types (TCGA PanCan) and has been found to be significantly upregulated in metastatic lesions derived from colorectal cancer patients (56). Pancreatic cancers have been found to display increased expression of SHOC2 compared to normal adjacent tissue, and higher intratumoral SHOC2 protein levels are associated with a substantial reduction in overall

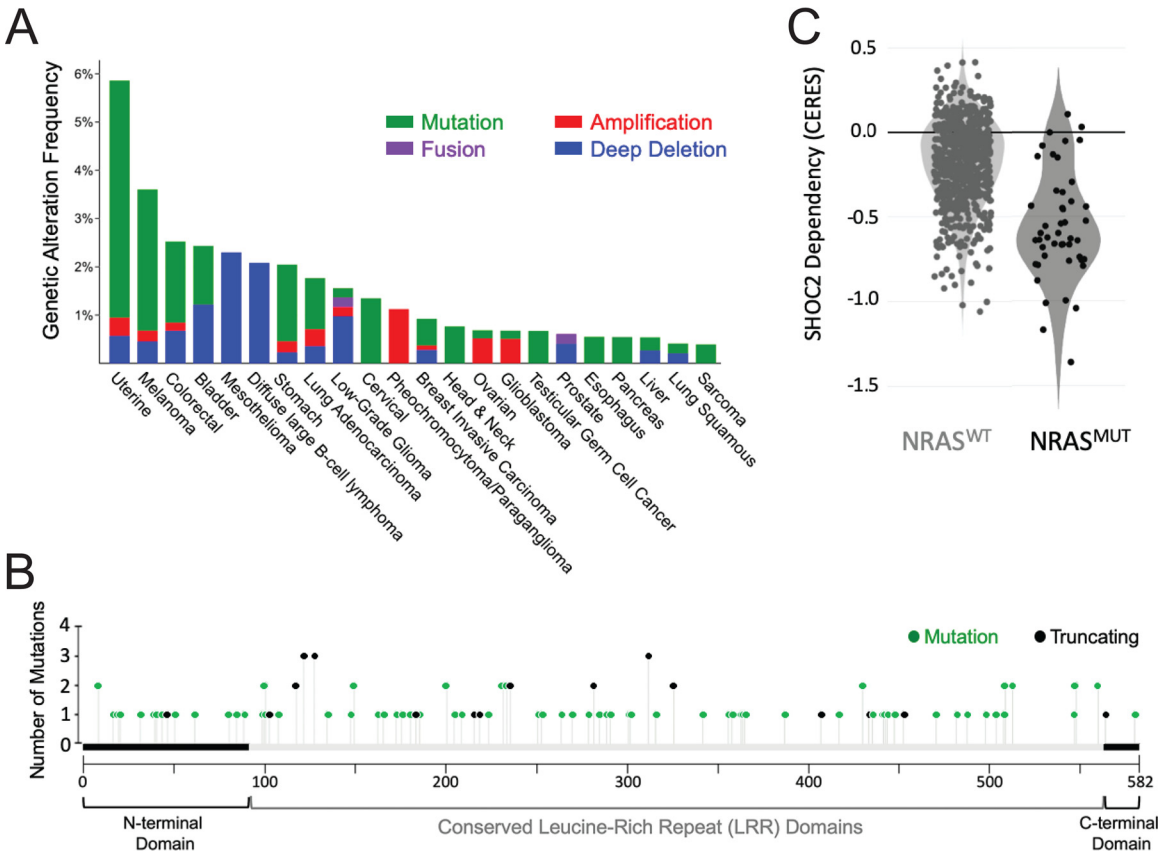

FIG 4 SHOC2 gene mutation, alterations, and dependency in cancer. (A) Percent occurrence of *SHOC2* alterations found in various human cancers (TCGA, cBioPortal). *SHOC2* mutation, amplification, and deletion frequencies are indicated in green, red, and blue, respectively. (B) Lollipop graph indicating *SHOC2* mutations found across cancers (TCGA, cBioPortal). The *x* axis represents the span of full-length SHOC2 (582 aa), and the *y* axis represents the number of samples harboring mutations, with missense mutations colored green and nonsense mutations colored black. (C) Violin plot of SHOC2 depletion score (CERES) in WT NRAS and mutant NRAS across cell lines in genome-scale CRISPR-Cas9 screens (DepMap; 20Q2).

survival (50). Finally, high expression of SHOC2 has also been clinically implicated in tyrosine kinase inhibitor resistance of EGFR mutant non-small-cell lung cancer (82).

Functionally, SHOC2 has been identified as a potent synthetic lethal interactor of mutant K/NRAS in acute myeloid leukemia (83) and as a dependency in NRAS mutant cells (84) (Fig. 4C). The loss of SHOC2 also results in reduced proliferation of KRAS mutant carcinoma cell lines grown in low attachment (60, 61). However, SHOC2 is found to be dispensable for the proliferation of the same carcinoma cell lines grown in two dimensions, suggesting that three-dimensional culture conditions affect the observed growth dependence on SHOC2 expression (60, 61).

In addition, recent studies have highlighted SHOC2 as a potent synthetic lethal interactor with MEKi. MEK1/2 are key downstream effectors of KRAS, and several studies demonstrated that MEK inhibition elicits a potent antitumorigenic effect (85–87). Furthermore, MEK has been shown to play a vital role in the initiation and maintenance of RAS mutant cancers (87). However, MEKi as a single therapeutic agent has been shown to be limited due to both acquired and intrinsic resistance mechanisms, leading to cancer resurgence (88–91).

In genome-scale CRISPR/Cas9 fitness screens performed in multiple KRAS mutant cancer cells, SHOC2 emerged as a highly penetrant and consistent synthetic lethal interactor of MEKi (60). BRAF mutant cancer cells upregulate RTK signaling in response to RAF inhibitor treatment as a means of reactivating the MAPK pathway, leading to adaptive resistance (92). These findings were extended to additional MAPK inhibitors

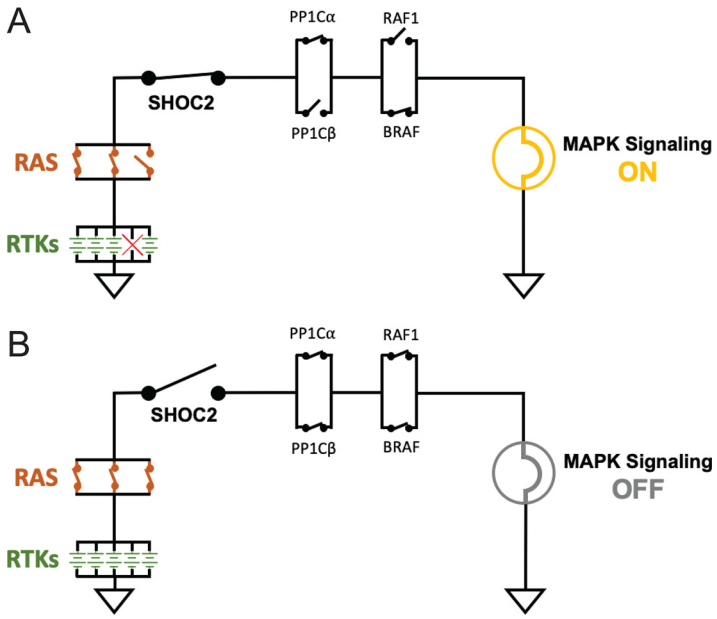

FIG 5 RTK/MAPK signaling circuitry. The receptor tyrosine kinase (RTK)/mitogen-activated protein kinase (MAPK) signaling pathway is represented as a circuit diagram, whereby RTKs are represented as parallel arranged power sources (green batteries) that transmit signal (e.g., electricity) downstream through functionally redundant paralogs, represented as parallel switches. (A) The loss of one paralog (single switch) still permits downstream signaling (MAPK signaling is represented as a lamp). (B) SHOC2 functions as a critical point of passage for RTK signal transduction to the MAPK pathway due to a lack of functionally redundant paralogs. The loss of SHOC2 alone is sufficient to abrogate the activation of the MAPK pathway.

in cancer cells exhibiting compensatory RTK-mediated adaptive resistance and have led to efforts to inhibit adapter proteins critical for RTK signaling, including GRB2 and SHP2 inhibitors (93–97). SHOC2 plays a critical role in RTK-mediated reactivation of the MAPK pathway in the context of KRAS mutant cancer cells treated with MEKi (60, 61), whereby the loss of SHOC2 leads to diminished MEKi-induced RAF dimerization, blunting MAPK reactivation (61). Functionally, RAF1 and BRAF were found to be important in the context of MEKi adaptive resistance, whereas ARAF was dispensable (61). Small-molecule inhibitor screens also identified MEK inhibitors to be synergistic with SHOC2 loss in KRAS mutant lung adenocarcinoma (61).

SHOC2 AS A POTENTIAL THERAPEUTIC TARGET

Deletion of SHOC2 induces a penetrant phenotype in forward genetic screens of *C. elegans* (8, 10) as well as multiple genome scale, single-gene CRISPR/Cas9 fitness screens in human cells (60, 74, 83, 84, 98), suggesting a uniquely essential role of SHOC2 in regulating the RTK/RAS signaling circuit (Fig. 5). Thus, SHOC2 may serve as a unique vulnerability within the RTK/RAS/MAPK pathway.

SHOC2 has a relatively simplistic solenoid structure with no conventionally tractable enzymatic domains. Since SHOC2 serves as a scaffold, small-molecule ligands that disrupt essential interactions may be sufficient to elicit a therapeutic effect. For instance, SHOC2-RAS interactions are required for SHOC2 function, and the design of a small-molecule ligand that competitively or allosterically inhibits SHOC2 binding to RAS should efficiently blunt downstream signaling events. Alternatively, enhancing the interaction of SHOC2 with inhibitory proteins such as ERBIN or SCRIB that off-compete RAS or sequester PP1, respectively, may prove effective as well. However, this approach may be limited by context-specific interactions due to variable expression levels of each binding partner. Another promising therapeutic strategy may involve ligand-mediated proteasomal degradation. Protein degraders are bifunctional small molecules that

eliminate the protein by recruiting E3 ligases to a target protein of interest, resulting in ubiquitin-linked proteasomal degradation (99, 100). However, not all proteins have been found to be amenable to this strategy (99). Utilizing a degrader model system, we demonstrated the feasibility of acute and efficient SHOC2 depletion by the degrader approach in KRAS mutant cancer cells, and SHOC2 degradation resulted in a profound MEKi sensitization (60).

FURTHER AREAS OF INTEREST IN SHOC2 BIOLOGY

Although it is clear that SHOC2 plays a key role in RTK/RAS signaling, several questions remain. First, the exclusivity of RAS family member interactions with SHOC2 remains unclear. Some studies have provided compelling evidence that SHOC2 exhibits preferential specificity to MRAS binding compared to the other RAS family members (15, 21, 28). As described above, there is both biochemical and genetic evidence for relevant interactions with MRAS, and clinical evidence of mutant *MRAS*, *RAF1*, and *PPP1CB* occurring in cases of Noonan-like syndrome provides strong support for this interaction (101–105). However, the exclusivity of SHOC2-MRAS interactions previously reported are in direct conflict with various independent groups demonstrating clear interactions between SHOC2 and KRAS (10, 11, 26). Furthermore, SHOC2 but not MRAS and PP1 scored in several genome-scale CRISPR/Cas9 fitness screens (60, 74, 83, 84, 98). The absence of PP1 in single-gene knockout screens may be explained by the pan-essential nature of PP1α and PP1β (16). One potential explanation could be that although SHOC2 binds MRAS to form a stable complex, transiently weak interactions with other RAS family proteins may be sufficient to localize SHOC2 to the membrane to properly regulate MAPK signaling. Detection of these transient interactions may require different assays than the previously reported immunoprecipitation studies. Indeed, prior work demonstrated clear SHOC2 interactions with H/K/NRAS proteins in yeast two-hybrid assays (10, 11), and bioluminescence resonance energy transfer assay displayed weak interactions between SHOC2 and mutant KRAS (28).

SHOC2 regulates MAPK signaling by recruiting partners that posttranslationally modify RAF kinases, and two distinct models of SHOC2-dependent regulation of RAF activity have been identified. In one model, SHOC2 complexes with PP1 to dephosphorylate the inhibitory "S259" site on RAF proteins at the cytoplasmic membrane, enabling its activation. In the second, SHOC2 binds HUWE1, which ubiquitinates and inhibits RAF1 signaling. How these two complexes interrelate and if they participate in the same subpools of SHOC2 to regulate MAPK signaling have not been well studied. One possibility is that the two complexes work in tandem: the SHOC2 holophosphatase enables initial RAF activation followed by negative feedback of MAPK signaling through SHOC2/HUWE1 ubiquitination of RAF1. However, further work investigating if SHOC2-mediated dephosphorylation and ubiquitination act in concert or in parallel to regulate the RAF proteins is needed.

Finally, SHOC2 is found to be expressed broadly with low tissue specificity (www.proteinatlas.org) (106), and binding partners that control complex disassembly or posttranslationally modify SHOC2 seem to impart context-specific regulation, raising the possibility that additional interactors mediate regulatory specificity over MAPK signaling. Given the expanding repertoire of SHOC2 interactions, systematically uncovering the binding partners of the SHOC2-regulome and elucidating their functions will be highly impactful in broadening our understanding of RTK/RAS signaling.

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