

Shank-associated RH domain interactor signaling in tumorigenesis (Review)

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Abstract. Shank-associated RH domain interactor (SHARPIN) is a component of the linear ubiquitin chain activation complex, which is essential for p53 signaling and inflammation. Previous studies have demonstrated that SHARPIN functions in tumor cell survival, growth, invasion and tumorigenesis. These functions include the regulation of p53 proteins via poly-ubiquitination, interaction with a type II protein arginine methyltransferase 5 in melanoma cells, modulating ras-associated protein-1 through p38 and c-Jun N-terminal kinases/c-Jun signaling, and mediating phosphoinositide 3-kinase/AKT signaling via phosphatase and tensin homologue deleted on chromosome 10. Hence, SHARPIN not only participates in the inflammatory response but also serves a critical role in tumor cells. The present review summarizes the biological functions of the absence or presence of SHARPIN with regard to activating the canonical NF- κ B signaling pathway and the effects on p53 and other signaling pathways for the modulation of tumorigenesis. Therefore, this review provides insight into the underlying role and mechanisms of SHARPIN in tumorigenesis, as well as its potential application in cancer therapy.

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1. Introduction

Cancer is a major global public health burden, with ~21.6 million new cancer cases predicted for 2030 (1). In general, tumorigenesis is the process that promotes the transformation of normal cells into invasive cells, overcoming the constraints that usually limit proliferation and survival. These alterations can give rise to a number of potentially deleterious circumstances or vulnerabilities that can be lethal to patients if left unchecked (2). Despite extensive research investigating tumorigenesis, the precise underlying molecular mechanisms remain unclear.

Shank-associated RH domain interactor (SHARPIN) is an ~40-kDa multifunctional adaptor protein that is amplified and overexpressed in a number of human cancer types. Studies have shown that the SHARPIN promotes cancer cell proliferation, tumor formation and metastasis (3-6). SHARPIN was first identified in C57BL/KaLawRij mice, functioning as a shank binding protein at the postsynaptic density of excitatory synapses in the central nervous system (7). In addition, SHARPIN has important physiological functions in several organisms (7) and is ubiquitously expressed in various types of cells and tissues (8). *SHARPIN* is an autosomal gene that is conserved among a number of mammalian species, including humans, chimpanzees, dogs, rats and mice (8). SHARPIN is located on cell membranes and in the nuclei, and primarily functions in immune and inflammatory responses (8). A previous study has reported that mutations in this gene contribute to chronic proliferative dermatitis, which is accompanied by immune system malfunction and multi-organ inflammation (7). Moreover, SHARPIN deficiency results in

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Abbreviations: SHARPIN, shank-associated RH domain interactor; LUBAC, linear ubiquitin chain assembly complex; MDM2, mouse double minute 2 homolog; PRMT5, protein arginine methyltransferase 5; PI3K, phosphoinositide 3-kinases; PTEN, phosphatase and tensin homologue deleted on chromosome ten; IKK, inhibition of κ B kinase; NEMO, NF- κ B essential modulator; HOIL-1L, heme-oxidized IRP2 ligase 1L; HOIP, HOIL-1 interacting protein; PAX3, paired box gene 3; MITF, microphthalmia-associated transcription factor; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PI3K, phosphoinositide 3-kinase; Rap1, ras-associated protein-1

Key words: SHARPIN, tumorigenesis, LUBAC, p53, PRMT5, PTEN

an autoinflammatory phenotype in an inflammatory mouse model (7). There is evidence demonstrating that SHARPIN is a component of the linear ubiquitin chain assembly complex (LUBAC), which participates in a range of complex biological functions (9,10) (Fig. 1). Moreover, the LUBAC is also involved in various molecular and cellular processes, such as embryogenesis (11) and apoptosis (12). SHARPIN has been well characterized as a crucial regulator of canonical NF- κ B signaling during the inflammatory response (10,13) and T-cell differentiation (14,15). A recent study reported that SHARPIN serves an important role in promoting breast cancer progression (16). A previous study also showed that SHARPIN regulates p53 protein levels in tumor cell lines through the mouse double minute 2 homolog (MDM2)-dependent pathway (17). Additionally, it has been reported that SHARPIN interaction with protein arginine methyltransferase 5 (PRMT5) mediates tumor cell growth (3). Notably, Zhou *et al* (18) demonstrated that SHARPIN upregulates ras-associated protein-1 (Rap1), which promotes melanoma development through p38 and c-Jun N-terminal kinases (JNK)/c-Jun signaling pathways. A previous study also revealed that SHARPIN mediates the phosphatase and tensin homologue deleted on chromosome 10 (PTEN) signaling pathway, which promotes tumorigenesis by phosphoinositide 3-kinase (PI3K)/AKT signaling (19). Therefore, research investigating SHARPIN has improved our understanding of its functions in humans; however, a comprehensive summary of mechanisms by which SHARPIN regulates tumorigenesis has not been available until now. Hence, the following sections summarize the current data on the possible functions of the aforementioned proteins and SHARPIN in tumorigenesis.

2. SHARPIN is a key member of the LUBAC family of proteins

Numerous studies have demonstrated that LUBAC consists of three structurally related proteins: Heme-oxidized IRP2 ligase 1L (HOIL-1L), HOIL-1 interacting protein (HOIP) and SHARPIN, with molecular weights of 120, 58 and 40 kD, respectively (20). LUBAC is involved in post-translational modifications that regulate a multitude of cellular processes, including cell death, development, carcinogenesis and autoimmune diseases (21,22). The HOIL-1L subunit is an accessory molecule that is involved in the stabilization of LUBAC. For example, cells lacking HOIL-1L have significantly decreased linear ubiquitylation (23), and the primary function of SHARPIN is to maintain the linear ubiquitylation activity in LUBAC. HOIP is a catalytic subunit that is associated with regulatory proteins, such as HOIL-1L and SHARPIN (24). A recent study demonstrated that the aforementioned subunits interact with each other in the trimeric core of LUBAC, contributing to the overall stabilization of the complex (25).

A recent study demonstrated that deficiency in SHARPIN or HOIP in mice causes severe inflammation in adulthood or embryonic lethality, respectively. By contrast, HOIL-1 deficiency contributes no overt phenotype (11). Previously, the LUBAC was shown to serve a pivotal role in complete activation of the canonical NF- κ B signaling pathway, whereas the absence of one subunit resulted in attenuated activation of this pathway, particularly when SHARPIN was absent (26).

These data suggest that HOIP, HOIL-1L and SHARPIN are all necessary for efficient activation of the NF- κ B signaling pathway. Notably, Rodgers *et al* (27) suggested that linear ubiquitination is required for activation of the NACHT, LRR and PYD domains-containing protein 3 inflammasome, and this finding further expands the role of LUBAC as an innate immune regulator.

3. SHARPIN regulates the canonical NF- κ B signaling pathway

In brief, the canonical NF- κ B signaling pathway can be triggered by different stimulators, such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 and pathogen-associated molecular patterns (28,29). When stimulators combine with the TNF receptor (TNFR), TNFR type 1-associated death domain protein, FAS-associated death domain protein and TNFR-associated factor 2 proteins and protein kinases are recruited in the cytoplasm as a result of phosphorylation and activation of the inhibition of the κ B kinase (IKK) complex (30). The classical IKK complex consists of two catalytic subunits and a regulatory subunit, IKK α , IKK β and the NF- κ B essential modulator (NEMO), respectively. Furthermore, the activated IKK complex facilitates the phosphorylation of I κ B, which releases NF- κ B dimers that freely translocate into the nucleus and bind with DNA, promoting the transcription of relevant target genes (30,31). Recent studies have demonstrated that the LUBAC mediates linear ubiquitylation, which is involved in the canonical NF- κ B signaling pathway (Fig. 2A).

Numerous studies have suggested that NEMO possesses a specific ubiquitin-binding region that interacts with the LUBAC (23,32-34). The NF- κ B activated state is influenced by the process of NEMO conjugating with a linear poly-ubiquitin chain (35). Furthermore, NEMO deficiency leads to decreased interaction with LUBAC, preventing SHARPIN-mediated linear ubiquitination and NF- κ B activation (14,36). Consistent with this, SHARPIN deficiency leads to inhibition of LUBAC-mediated linear poly-ubiquitination of endogenous NEMO and attenuates the activation of the NF- κ B signaling pathway (37). Therefore, p65/p50 cannot translocate into the nucleus to induce target gene expression due to decreased phosphorylation and degradation of NF- κ B inhibitor α (I κ B α) (28) (Fig. 2B). These findings suggest that the NEMO-SHARPIN interaction is essential to mediate canonical NF- κ B signaling. Hence, these results suggest that SHARPIN is important in regulating canonical NF- κ B signaling and that this disruption may impact downstream physiological functions.

4. SHARPIN is a mediator of p53

It is well known that p53 is a vital tumor suppressor and its presence was initially reported in response to various types of stress >40 years ago (38). The p53 protein acts as a transcription factor in cells and is functionally inactivated in the majority of cancer types (39). Several studies have demonstrated that activated p53 is associated with numerous downstream responses and is involved in important physiological and pathological processes, including cell cycle arrest, DNA repair, apoptosis, metabolism, invasion, metastasis and tumorigenesis (40-42).

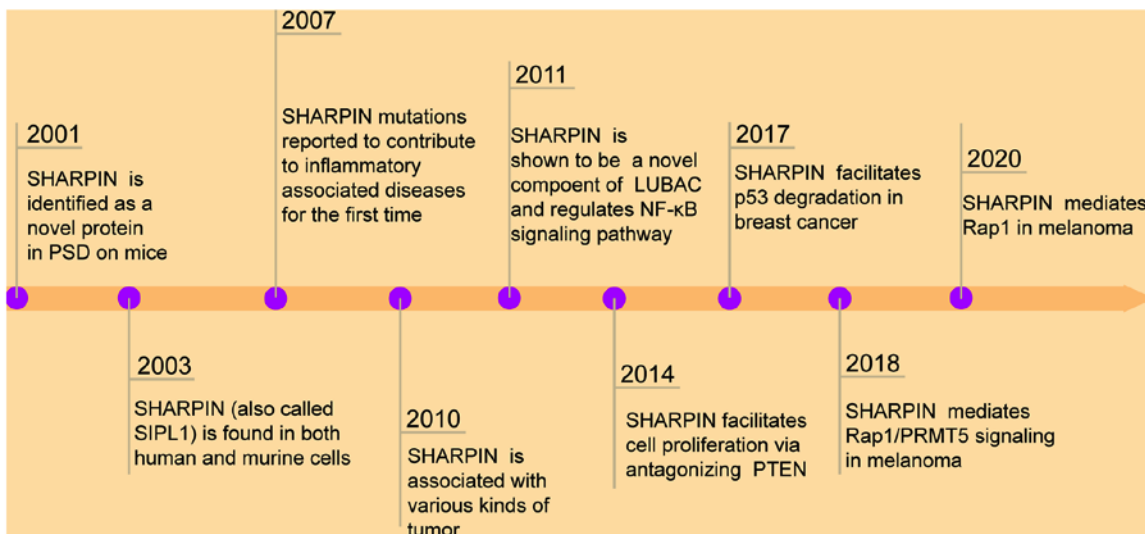


Figure 1. Historical process of resolving SHARPIN function. SHARPIN was originally identified in the PSD of mice, functioning in the nervous system. SHARPIN is reported to participate in inflammatory-associated diseases and tumors. Researchers gradually realized that SHARPIN is a novel component of the LUBAC and mediates the NF- κ B signaling pathway. Subsequently, SHARPIN-mediated regulation of p53, PRMT5, Rap1 and PTEN signaling in tumors has been reported. SHARPIN, shank-associated RH domain interactor; PSD, postsynaptic density; LUBAC, linear ubiquitin chain assembly complex; PRMT5, protein arginine methyltransferase 5; PTEN, phosphatase and tensin homolog, Rap1, ras-associated protein-1.

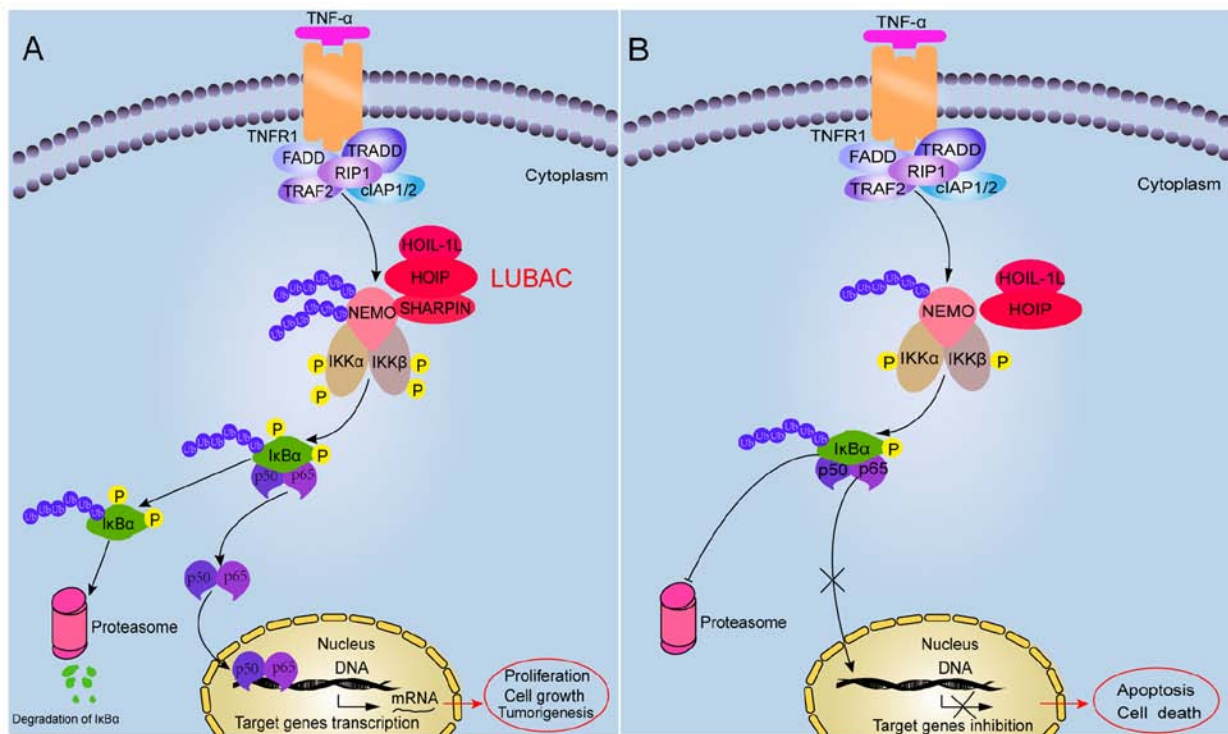


Figure 2. Difference between SHARPIN presence and absence in regulating the NF- κ B signaling pathway. (A) Presence of SHARPIN promotes canonical NF- κ B signaling activation. Once stimulators bind with the receptors, associated molecules are recruited to activate the IKK complex. The complete LUBAC is involved in this process that serves a role in ubiquitination and fully activates the IKK complex. Subsequently, the phosphorylation and ubiquitination of I κ B α contributes to the degradation of I κ B α , which generates dimers. The dimers translocate into the nucleus and mediate target gene transcription. (B) Absence of SHARPIN attenuates canonical NF- κ B signaling activation. When SHARPIN is absent, the LUBAC attenuates the phosphorylation and ubiquitination of I κ B α , resulting in reduced I κ B α degradation and inhibiting dimer translocation into the nucleus. Therefore, transcription of the target genes is inhibited. SHARPIN, shank-associated RH domain interactor; IKK, inhibition of κ B kinase; LUBAC, linear ubiquitin chain assembly complex; TNF, tumor necrosis factor; TNFR1, TNF receptor 1; TRADD, TNFR type 1-associated DEATH domain protein; TRAF2, TNFR-associated factor 2; RIP1, receptor-interacting serine/threonine protein kinase 1; cIAP1/2, cellular inhibitor of apoptosis protein-1/2; FADD, FAS-associated death domain protein; HOIL-1L, heme-oxidized IRP2 ligase 1L; HOIP, HOIL-1 interacting protein; NEMO, NF- κ B essential modulator; p, phosphorylated; Ub, ubiquitinated.

p53 is maintained at very low cellular levels in normal cells due to binding with E3 ubiquitin ligase mouse double minute

2 homolog (MDM2), which contributes to p53 ubiquitination and rapid degradation by the proteasome (43). SHARPIN may

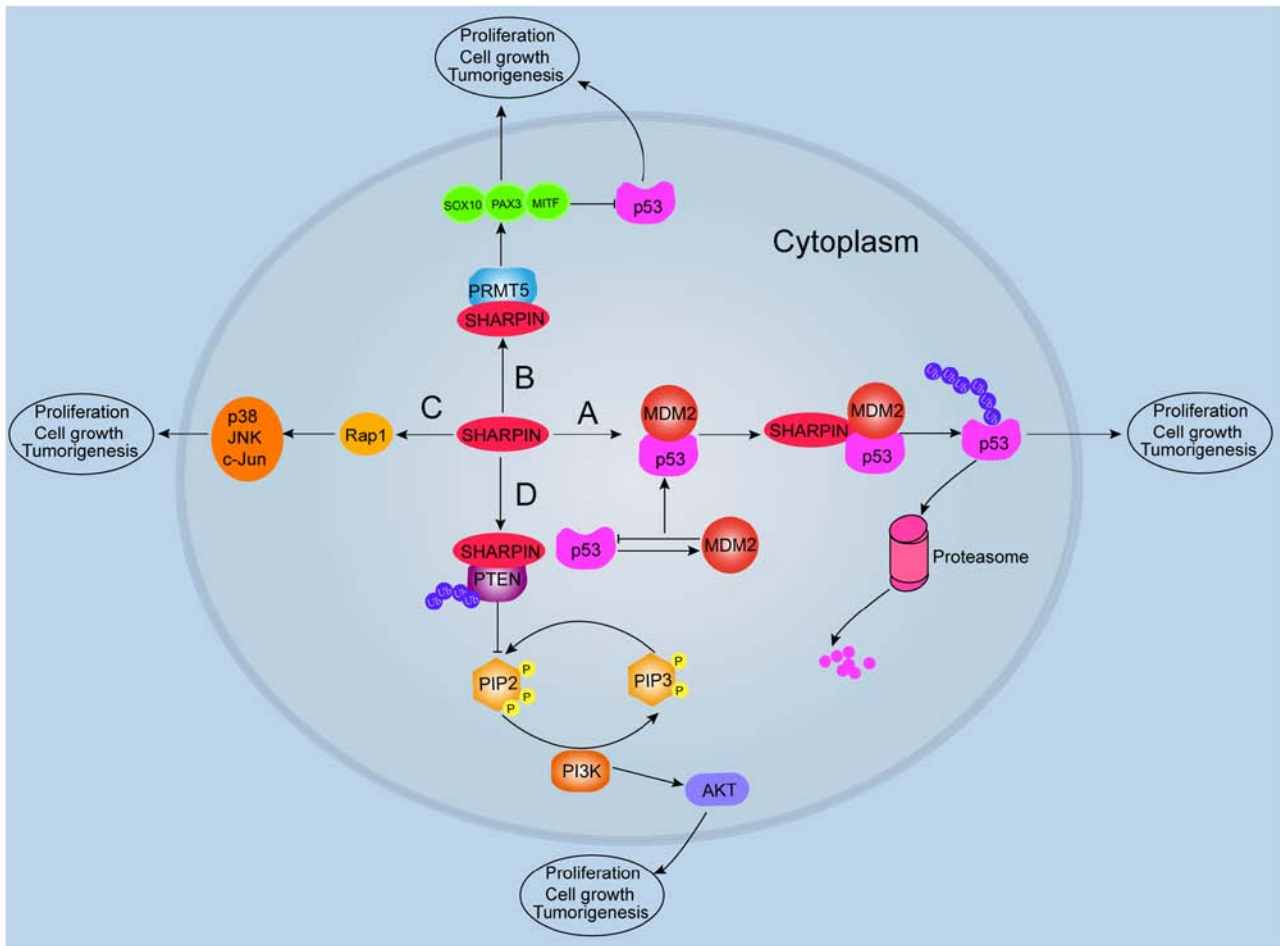


Figure 3. SHARPIN regulates multiple signaling pathways in tumorigenesis. A: SHARPIN mediates p53 ubiquitination and rapid degradation by the proteasome, which promotes tumorigenesis. B: SHARPIN interacts with PRMT5 and activates SOX10, PAX3 and MITF, which promotes tumorigenesis. PRMT5 also regulates PAX3 and MITF and inhibits p53 in tumorigenesis. C: SHARPIN upregulates Rap, which promotes melanoma development through p38 and JNK/c-Jun signaling. D: SHARPIN can bind with PTEN, which mediates tumorigenesis through the PI3K/AKT signaling pathway. SOX10, SRY-box transcription factor 10; MITF, melanocyte inducing transcription factor; PAX3, paired box 3; PRMT5, protein arginine methyltransferase 5; SHARPIN, shank-associated RH domain interactor; Rap1, ras-associated protein 1; JNK, c-Jun N-terminal kinases; MDM2, mouse double minute 2 homolog; PTEN, phosphatase and tensin homologue deleted on chromosome 10; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PIP2, phosphatidylinositol (4,5)-bisphosphate; PI3K, phosphoinositide 3-kinases; p, phosphorylated; Ub, ubiquitinated.

be involved in promoting p53 ubiquitination and maintaining cellular levels of p53 (44). Mechanistically, MDM2 recognizes the N-terminal trans-activation domain of p53 and mediates p53 transcription as an inhibitor of p53 transcriptional activation (45). Nevertheless, in response to cellular stress signals, p53 is rapidly activated by phosphorylation, which releases MDM2 and promotes p53 stabilization (46). MDM2 regulates p53 through direct binding, mono-ubiquitylation, polyubiquitylation (44) or negative feedback regulation (47), which maintains appropriate cellular levels of p53.

A recent study demonstrated that SHARPIN may be upstream of p53 signaling in breast cancer cells, as depleted SHARPIN resulted in decreased cell proliferation and increased expression of p53 (17). Furthermore, the study reported that SHARPIN modulates p53 protein levels through poly-ubiquitination and degradation in a MDM2-dependent manner, which determines the fate of p53 in tumor cells (Fig. 3). Therefore, p53 protein levels in cells are indirectly regulated by SHARPIN. Nevertheless, whether other accessory molecules or signaling pathways are involved in

SHARPIN-mediated regulation of the p53/MDM2 complex needs further clarification.

SHARPIN interacts with PRMT5. PRMT5 is a member of the PRMT family of proteins and can catalyze symmetric methylation of histone and non-histone proteins. PRMT5 catalyzes the transfer of a methyl group from S-adenosylmethionine to the guanidino nitrogen atoms of arginine (48). PRMT5 is commonly activated in cancer, which is mediated in part by the PRMT5 co-factor methylome protein 50 (also known as p44) (49). Researchers have demonstrated that PRMT5 serves an essential role in lung cancer, leukemia (49), tumorigenesis (50), cell survival and human embryonic stem cell proliferation (51). Stopa *et al* (52) provides an overall literature review of PRMT5 overexpression, which appears to be an important factor in the tumorigenicity of a large number of cancer types, such as gastric (53) and breast cancer (54). In addition, PRMT5 mediates the methylation of Arg1175 of epidermal growth factor receptor, which controls extracellular signal-regulated kinase activation (55). Similarly, E2F-1

may be directly methylated by PRMT5, which influences the stability of E2F-1. Depleting PRMT5 using small interfering RNA resulted in decreased arginine methylation of E2F-1 but increased E2F-1 levels in one study (56).

Recent reports have shown that the interaction of SHARPIN with PRMT5 contributes to regulating the transcription of cancer-associated genes (3,56). For example, Fu *et al* (57) reported that SHARPIN activates PRMT5 to specifically target histone H3R2 for mono-methylation, which is responsible for the subsequent activation of cancer-associated genes and mediates metastasis in invasive lung cancer cells. Tamiya *et al* (3) also demonstrated that PRMT5 activity is increased after SHARPIN binding. This study observed that SHARPIN activates PRMT5 by regulating SRY-box transcription factor 10, paired box gene 3 (PAX3) and microphthalmia-associated (MITF) transcription factors in melanoma development (Fig. 3). These results demonstrate that both SHARPIN and PRMT5 are involved in tumorigenesis.

PRMT5 regulates p53 signaling. Scoumanne *et al* (42) reported that PRMT5-knockdown prevented p53 stabilization and reduced p53 expression in response to DNA damage and cell cycle arrest. Subsequently, expression of both the *MDM2* and *p21* target genes was inhibited. The study also demonstrated that PRMT5 is required for p53 protein synthesis and suggested that PRMT5-mediated regulation of p53 serves a critical role in colon carcinoma cell survival. Furthermore, another study revealed that PRMT5 influences the methylation of p53 in response to DNA damage (58). Similarly, PRMT5 functions in a negative regulatory mechanism that underlies p53-dependent apoptosis in *Caenorhabditis elegans* (59). PRMT5 regulates Mdm4 (p53 regulator) expression, which influences p53-induced cell cycle arrest (60,61). PRMT5 is also important for regulating the p53-dependent mechanism of apoptosis (13). Furthermore, PRMT5 modulates p53 function in cell proliferation (42) and tumorigenesis (62), and p53 signaling has important functions in tumorigenesis (63,64). Overall, these results demonstrate that PRMT5 is directly involved in regulating p53 function in cell apoptosis, the cell cycle, survival and proliferation.

SHARPIN regulates PRMT5 activity, which induces the transcriptional activity of PAX3 and MITF in melanoma growth; in turn, PAX3 and MITF can inhibit p53 activation (3). Consistent with these findings, a recent study demonstrated that PAX3 specifically binds to the promoter of p53 leading to repressed p53 expression in glioblastoma (65). In addition, MITF binds p53 to regulate cyclin-dependent kinase inhibitor 1A in melanoma cells (66). Overall, these data suggest that PRMT5 is capable of regulating p53 indirectly, and SHARPIN mediates p53 through PRMT5-dependent signaling with PAX3 and MITF.

5. SHARPIN interacts with Rap1 in tumorigenesis

A study by Lilja *et al* (67) demonstrated that SHARPIN is indispensable for regulating integrin inactivation by Shanks interaction with Rap1 in cells. This finding suggests that SHARPIN and Rap1 functions may be closely associated. Furthermore, a recent study also supported this viewpoint, demonstrating that SHARPIN promotes melanoma progression

through Rap1 via the p38 and JNK/Jun signaling pathways (18) (Fig. 3). However, the specific mechanism by which SHARPIN mediates Rap1 remains unclear, and whether this interaction is direct or indirect needs to be further explored.

6. SHARPIN functions in the PTEN signaling pathway

Studies have shown that PTEN is a pivotal tumor suppressor gene, and that it is frequently deleted in late-stage human cancer types and has important functions in crosstalk with the PI3K/AKT signaling pathway, mediating several fundamental cellular processes under different circumstances via multiple downstream targets (68-70). For example, a study has suggested that PI3K/AKT signaling is attenuated in the brains of patients with Alzheimer's disease (71). Moreover, PI3K/AKT/PTEN is mediated by intracellular ROS production (72). The main function of PTEN is to catalyze the conversion of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to phosphatidylinositol (4,5)-bisphosphate (73). PTEN is an antagonist of PI3K and, when dephosphorylated, serves as a negative regulator of PI3K/AKT signaling in normal cells, while loss or inactivation of PTEN contributes to hyperactivation of PI3K/AKT in primary T-cell leukemia (74) and adult B-cell acute lymphoblastic leukemia (75). Once activated, the PI3K/AKT pathway promotes cell survival by blocking the function of pro-apoptotic proteins and promoting protein synthesis and cell proliferation. Consequently, these results indicate that inactivation of PTEN is a critical step during tumorigenesis.

There has been much interest in the transcriptional and post-translational modulation of PTEN expression, protein stability and activity mediated by miRNA (76), phosphorylation (77), acetylation (78) and ubiquitination (79). A recent study reports that PTEN knockdown could significantly promote mouse neuronal cell proliferation and differentiation *in vitro* (80). Moreover, a previous study demonstrated that SHARPIN is a negative regulator of PTEN in human tumor cell lines and human primary cervical cancer cells both *in vitro* and *in vivo* (81). Furthermore, it has been reported that SHARPIN interacts with PTEN through its ubiquitin-like domain (81). It is well known that the major function of PTEN is inhibition of the PI3K signaling pathway, while loss of PTEN activates the PI3K/AKT pathway (82). Additionally, attenuation of PTEN function activates PI3K/AKT signaling and elicits tumorigenesis (82). Hence, reducing PTEN-induced PIP3 phosphatase activity and enhancing the activity of the PI3K/AKT signaling pathway promotes tumorigenesis (83). In accordance with this, De Melo *et al* (84) suggested that SHARPIN facilitated PTEN poly-ubiquitination via lysine63 and formation of the SHARPIN/PTEN complex, which did not lead to PTEN degradation. These biochemical processes alter the affinity of the SHARPIN/PTEN complex and reduce the phosphatase activity of PTEN (Fig. 3). The study proposed that SHARPIN promotes poly-ubiquitination of PTEN in a manner that does not alter PTEN stability, and that SHARPIN/PTEN binding is enhanced by poly-ubiquitination of PTEN.

7. Conclusion

Overall, there has been a marked increase in the understanding of the function of SHARPIN in recent years. The present review

summarizes our understanding of how SHARPIN mediates canonical NF- κ B signaling and crosstalk with various mechanisms, regulating tumorigenesis. Furthermore, the function of SHARPIN in the NF- κ B, p53, PRMT5, Rap1 and PTEN signaling pathways was explored. The aforementioned results shed light on possible functions of these proteins in tumor cells, and may provide novel targets for cancer treatment in the future. However, the detailed molecular function of associated co-activators or co-repressors contributing to these pathways needs further investigation.

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Authors' contributions

CZ, DX, KZ and JY jointly conceived and designed the review, researched the literature and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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