

LZTR1: A promising adaptor of the CUL3 family (Review)

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Received October 22, 2020; Accepted May 7, 2021

DOI: 10.3892/ol.2021.12825

Abstract. The study of the disorders of ubiquitin-mediated proteasomal degradation may unravel the molecular basis of human diseases, such as cancer (prostate cancer, lung cancer and liver cancer, etc.) and nervous system disease (Parkinson's disease, Alzheimer's disease and Huntington's disease, etc.) and help in the design of new therapeutic methods. Leucine zipper-like transcription regulator 1 (LZTR1) is an important substrate recognition subunit of cullin-RING E3 ligase that plays an important role in the regulation of cellular functions. Mutations in LZTR1 and dysregulation of associated downstream signaling pathways contribute to the pathogenesis of Noonan syndrome (NS), glioblastoma and chronic myeloid leukemia. Understanding the molecular mechanism of the normal function of LZTR1 is thus critical for its eventual therapeutic targeting. In the present review, the structure and function of LZTR1 are described. Moreover, recent advances in the current knowledge of the functions of LZTR1 in NS, glioblastoma (GBM), chronic myeloid leukemia (CML) and schwannomatosis and the influence of LZTR1 mutations are also discussed, providing insight into how LZTR1 may be targeted for therapeutic purposes.

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Key words: Noonan syndrome, glioblastoma, LZTR1, CUL3, ubiquitin ligase, ubiquitination, RAS/MAPK signaling pathway

1. Introduction

Ubiquitylation is a key post-translational modification that plays a significant role in the stability of the intracellular environment, cell proliferation and differentiation, as well as various other cellular functions (1). An imbalance in ubiquitination-mediated protein degradation can represent the molecular basis of certain human diseases, such as cancers (prostate cancer, lung cancer and liver cancer) and nervous system diseases (Parkinson's disease, Alzheimer's disease and Huntington's disease) (1). Ubiquitin is activated in an ATP-dependent reaction catalyzed by the ubiquitin-activating enzyme E1 (2,3). Subsequently, under the action of the ubiquitin-conjugating enzyme E2, activated ubiquitin is transferred to a specific substrate along with E3 ubiquitin ligase (1). Substrate recognition for ubiquitin ligation is determined by E3 ubiquitin ligase (2,3), thus E3 ubiquitin ligase is specific compared with E1 and E2 enzymes and constitutes an important topic in medical research. One of the best known E3 ligase family is cullin (CUL)-RING E3 ubiquitin ligase, which consists of a molecular scaffold connecting a substrate-specific adaptor protein to a catalytic component comprising a RING finger domain and an E2 ubiquitin-conjugating enzyme (4-8). The human genome contains eight CUL family members, namely, CUL1, -2, -3, -4A, -4B, -5, -7 and -9, all of which have an evolutionarily conserved CUL homology domain at the C-terminus, which promotes the interaction of CUL with RING box protein (RBX)1 or RBX2 (9-18). E3 adaptors of CUL3, such as speckle-type protein (SPOP) (19-29), Kelch repeat and BTB domain-containing protein 8 (KBTBD8) (30-32) and Kelch-like ECH-associated protein 1 (KEAP1) (33-35), are composed of a similar structure called broad-complex, tramtrack and bric-a-brac (BTB) domain (15-18), which combines the substrate receptor and adaptor functions into the CUL3-RBX1 E3 ubiquitin ligase complex (36,37).

Recently, increased attention has been paid to leucine zipper-like transcription regulator 1 (LZTR1), due to its far-reaching implications in physiological and pathological conditions of cells and human diseases, such as Noonan syndrome (NS), glioblastoma (GBM), chronic myeloid leukemia (CML) and schwannomatosis (38,39). LZTR1, also a member of the BTB-Kelch superfamily proteins, is the substrate-specific adaptor for CUL3 ubiquitin ligase complex (38-40). Given its weak homology to known members of the basic leucine zipper-like family, LZTR1 was initially identified as a transcriptional regulator (40). Proteins of the

BTB-Kelch superfamily often interact with actin filaments and play important roles in fundamental cell functions, such as transcriptional regulation and protein ubiquitination (41-45). However, unlike other BTB-Kelch proteins, LZTR1 shows no interaction with actin but is localized on the Golgi complex (40), suggesting a unique function and status. Mutations in the *LZTR1* gene occur in $\leq 8\%$ of patients with NS (46,47), 4.4% of those with GBM (39,48) and 24.4% of those with schwannomatosis (Table I) (49-51). The occurrence of these diseases is related to abnormal function of RAS proteins, demonstrating a close relationship between LZTR1 and proteins of the RAS superfamily (38,52-54). Notably, almost all members of the RAS superfamily, including KRAS, NRAS, RAS-like without CAAX1 (RIT1) and RAF1, are substrates of LZTR1 (38,55,56). LZTR1 regulates the RAS/MAPK signaling pathway by inducing the polyubiquitination and degradation of RAS-superfamily proteins (KRAS, MRAS, NRAS and RAF1) (Fig. 1A) (57), whereas disease-associated LZTR1 mutations lose this capability, leading to excessive activation of RAS/MAPK signaling (Fig. 1B). The effect of LZTR1 on RAS/MAPK signaling is specific as demonstrated by the failure of the other two prominent CUL3 adaptors (KEAP1 and SPOP) in the process of RAS ubiquitination (38).

2. Structure and mutations of the LZTR1 protein

The Kelch domains of the BTB-Kelch superfamily proteins are located at the C-terminus of the BTB domains, and the conserved BACK domains are present in almost all members (17). Different from the general BTB-Kelch-superfamily proteins, the primary structure of LZTR1 includes six Kelch motifs at the N-terminus and two C-terminal BTB-BACK domains (40). The Kelch domains selectively recruit substrates, whereas the BACK domains are considered to mediate dimerization and the binding to CUL3 (58). Furthermore, the second BTB domain (from N to C) mediates the interaction between the Golgi complex and LZTR1, suggesting that LZTR1 may be a novel Golgi matrix-associated protein (Fig. 1C) (40).

The majority of NS- and GBM-related LZTR1 mutations are clustered in the Kelch domains (Table I), and these mutations are defective in LZTR1-mediated substrate binding, thus preventing the formation of substrate-LZTR1-CUL3 complexes and their efficient ubiquitination and degradation, which results in excessive activation of RAS/MAPK signaling in NS or GBM (39,55,58). Furthermore, except for the Leu812Pro mutation, mutations in the LZTR1 BACK domains exhibit reduced interaction with CUL3 and although LZTR1-L812P retains the ability to bind to CUL3, it fails to dimerize LZTR1 (38). The endogenous and exogenous expression of wild-type LZTR1 displays punctate endomembrane immunostaining, but diffuse and uniform cytoplasmic staining when LZTR1 mutations occur in the BACK domains, including in the Leu812Pro mutant (58). Interestingly, unlike NS- and GBM-associated LZTR1 mutations, which are concentrated in the Kelch domains, schwannomatosis-associated LZTR1 mutations are more evenly distributed across all domains, and these mutations may result in failure to bind with CUL3 (38,39,46-48). In summary, disease-related LZTR1 mutations are defective in mediating substrate ubiquitination by disrupting the formation of substrate-LZTR1-CUL3 complexes (Fig. 1D; Table I).

3. LZTR1 in Noonan syndrome

RASopathies are defined as a class of inherited diseases with germline mutations in genes encoding components of the RAS/MAPK signaling pathway (58). RAS-superfamily proteins are a class of evolutionarily conserved proteins with high affinity for GDP and GTP (52,59,60). RAS proteins, similar to molecular switches, are activated when interacting with GTP and serve as positive regulators in the physiological activities of cells, such as proliferation and cell division (59,61-64). RASopathy-associated RAS mutations located in the secondary locus (mutations at these loci only slightly affect the physiological function of RAS superfamily proteins) weakly affect GTPase activity without causing embryonic-lethal death (53,54,65-69). NS, one of the most common and typical RASopathies, is caused by germline gain-of-function pathogenic RAS variants affecting the RAS/MAPK signaling pathway (38).

The pathogenesis of NS depends on abnormal protein degradation of RAS-superfamily proteins, including KRAS, NRAS, RIT1 and RAF1 (38,55,56,58). LZTR1 has also been added to the list of genes causing NS, and mutations in LZTR1 are present in $\leq 8\%$ of patients with NS (46,70). NS patients harboring LZTR1 mutations present typical NS facial features, a webbed neck, cardiovascular defects and coagulation dysfunction (71).

Previous studies have suggested that almost all NS-related mutations in LZTR1 are dominant mutations that occur mainly in the Kelch domains (38,58,70). These mutations neither alter CUL3 binding nor affect subcellular localization and stability of LZTR1 (72), although they may impair its ability to specifically recognize substrates, such as KRAS, NRAS and HRAS, ultimately contributing to the excessive activation of RAS/MAPK signaling (58,72). However, a recent study reported biallelic loss-of-function LZTR1 mutations in three patients NS (Table I), suggesting recessive inheritance of some NS cases (73). Thus, considering the different effects of LZTR1 mutations, determining the association between LZTR1 and the autosomal-dominant and autosomal-recessive forms of NS may prove useful.

RAF1 is the downstream substrate of most classical RAS proteins, mediating the activation of the RAS/MAPK signaling pathway. Protein phosphatase 1 (PP1), which interacts with the leucine-rich repeat protein SHOC2 to form a complex encoded by an NS-causing gene, dephosphorylates RAF1 to activate ERK (74,75). A recent study on a case report identified eight LZTR1 variants, including a *de novo* variant, in seven probands who were susceptible to NS and one known *de novo* PP1 catalytic subunit β (PPP1CB) variant in a patient with NS, indicating that LZTR1 and PPP1CB may be closely related to the pathogenesis of NS (56). Moreover, co-immunoprecipitation demonstrated that endogenous LZTR1 interacted with the RAF1-PPP1CB complex and that RAF1 phosphorylation levels (Ser259), but not ubiquitination, suggesting that LZTR1 may be involved in additional pathways that mediate the phosphorylation of RAF1 to regulate its activity (Fig. 1E and F) (56).

RIT1 also belongs to the RAS superfamily and is highly similar to other members (K-, H-, R- and NRAS), regulating the physiological activity of cells (76-79).

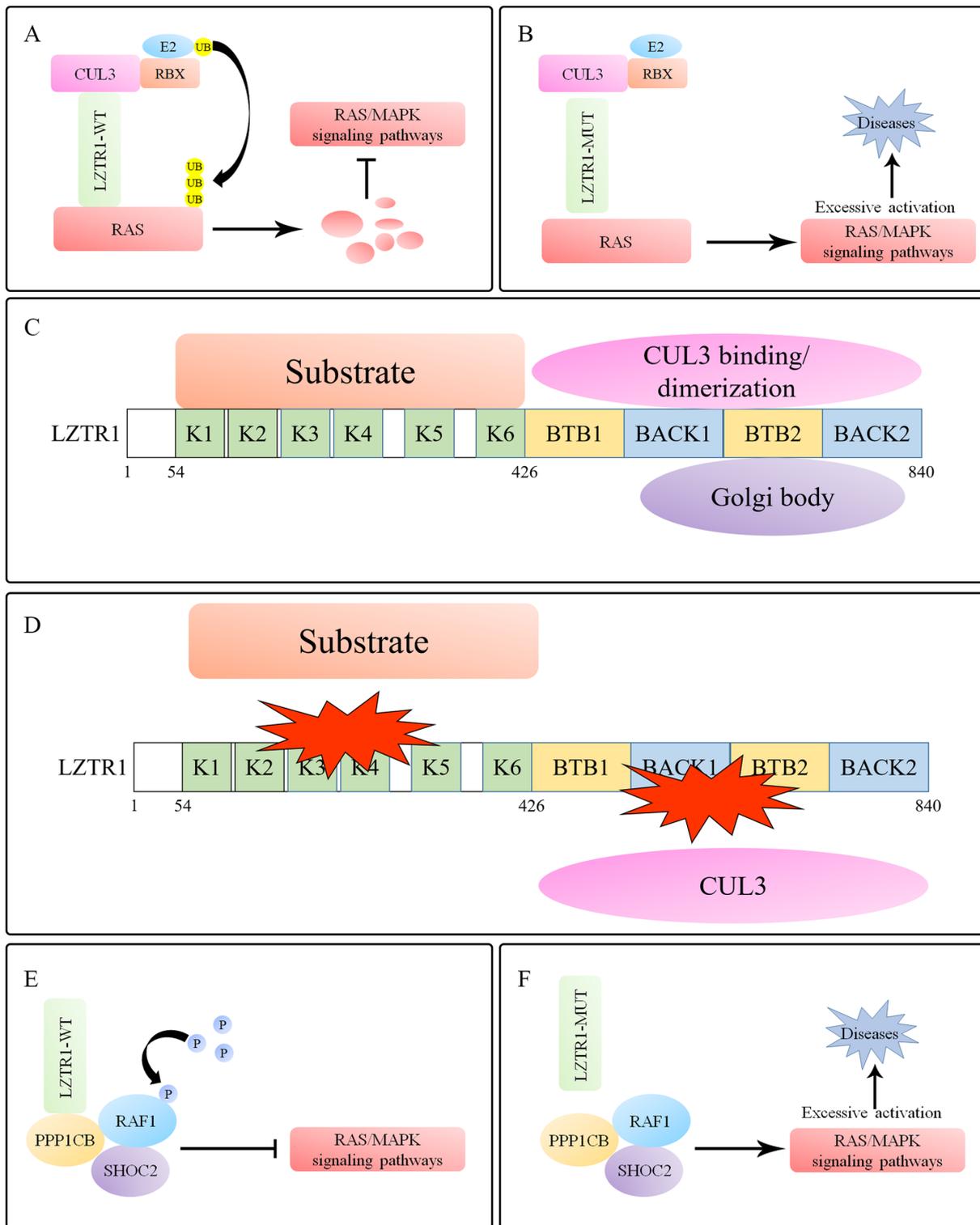


Figure 1. LZTR1 regulates RAS/MAPK signaling. (A) LZTR1 induces polyubiquitination and degradation of RAS proteins to inhibit the RAS/MAPK signaling pathway. (B) Mutated LZTR1 loses the ability to regulate RAS superfamily proteins, leading to excessive activation of the RAS/MAPK signaling pathway. (C) A total of six N-terminal Kelch motifs and two BACK domains are located at the C-terminus within LZTR1. The Kelch domains selectively recruit substrates, whereas the BACK domains are predicted to mediate dimerization and CUL3 binding. (D) Mutations in Kelch domains decrease binding to substrates. The mutations located in the BTB/BACK domains of LZTR1 prevent the binding of LZTR1 to CUL3. All of these mutations prevent the formation of the substrate-LZTR1-CUL3 complex. (E) LZTR1 mediates RAF phosphorylation by binding to the RAF1/PPP1CB/SHOC2 complex to inhibit the RAS/MAPK signaling pathway. (F) Mutations in LZTR1 lead to loss of RAF1 regulation, leading to excessive activation of the RAS/MAPK signaling pathway, eventually resulting in Noonan syndrome. LZTR1, leucine zipper-like transcription regulator 1; BTB, broad-complex, tramtrack, and bric-a-brac; BACK, BTB and C-terminal Kelch; CUL, cullin; PPP1CB, protein phosphatase 1 catalytic subunit β ; RBX, RING box protein; WT wild-type; Mut, mutant; UB, ubiquitin; P, pyrophosphate.

Germline mutations in RIT1 account for 5-9% of mutations in patients with NS (54,80). The NS-associated

mutation Met90Ile in RIT1 (Fig. 2A), which is located in an atypical site around the switch II region, contributes

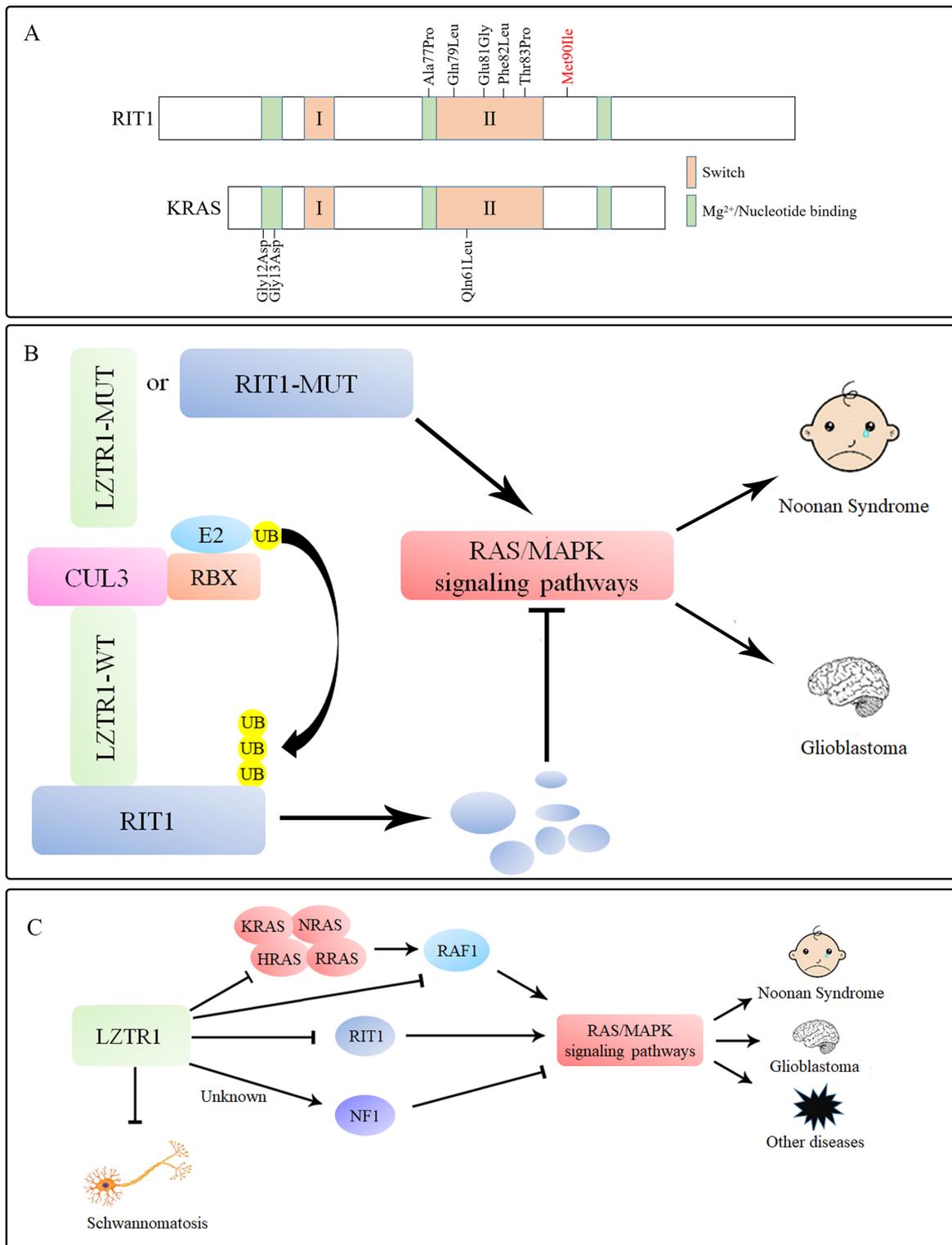


Figure 2. LZTR1 inhibits the RAS/MAPK signaling pathway by regulating RIT1 in NS and GBM. (A) NS-related RIT1 mutations do not occur in codons analogous to the classic Gly12, Gly13, and Gln61 alleles compared with other RAS-superfamily proteins, although they are clustered around the switch II region. (B) LZTR1 induces polyubiquitination and degradation of RIT1 to inhibit the RAS/MAPK signaling pathway. By contrast, mutations in LZTR1 or RIT1 prevent the formation of the substrate-LZTR1-CUL3 complex, resulting in insufficient degradation of RIT1, abnormal activation of RAS/MAPK signaling, leading to the occurrence of NS or GBM. (C) LZTR1 regulates RAS/MAPK signaling, and mutations in LZTR1 or RAS-superfamily proteins lead to excessive activation of the RAS/MAPK signaling pathways, eventually resulting in NS and GBM. LZTR1 inhibits the occurrence of schwannomatosis, although the specific mechanism remains unclear. The relationship between NF1, which serves as a negative regulator of the RAS signaling pathway, and LZTR1 remains to be studied. LZTR1, leucine zipper-like transcription regulator 1; RIT1, RAS-like without CAAX1; CUL3, cullin 3; NS, Noonan syndrome; GBM, glioblastoma; NF1, neurofibromin 1; RBX, RING box protein; WT, wild-type; Mut, mutant; UB, ubiquitin; P, pyrophosphate.

to the symptoms of typical NS, suggesting a strong association between RIT1 and NS (55). LZTR1 acts a negative

regulator in the control of RIT1 activity by inducing RIT1 ubiquitination via K48 (Lys48)-linked ubiquitination and

Table I. Leucine zipper-like transcription regulator 1 mutations.

Disease	Mutation frequency in disease (%)	Mutations in Kelch domains	Mutations in BTB-BACK domains
Noonan syndrome	8.0	Arg97Leu, His121Asp, Tyr136Cys, Tyr136His, Asn145Ile, Arg170Trp, Ile205Thr, Arg210*, Glu217Ala, Ser244Cys, Ser247Asn, Gly248Arg, Arg284Cys, His287Tyr	Trp437*, Ala461Asp, Ile462Thr, Trp469*, Glu563Gln, Val579Met, Arg688Gly(Cys), Arg688Cys, Asp531Asn, Arg697Glu, Tyr749Cys, Arg755Gln, Ile821Thr
Glioblastoma	4.4	Trp105Arg, Asp139Ala, Asn143Thr, Gly195Ser, Arg198Gly, Gly248Arg, Arg284Ser, Thr288Ile, G404Glu	Arg810Trp
Schwannomatosis	24.4	His71Arg, Pro115Leu, Ser122Leu, Arg170Gln, Leu187Leu, Met202Arg, Arg284Cys, Gly285Arg, Met400Arg, Gly404Arg	Val456Gly, Arg466Gln(Trp), Pro520Leu, Met665Lys, Arg688His(Cys), Leu812Pro, Ser813Leu

BACK, broad-complex, tramtrack, and bric-a-brac and C-terminal Kelch.

degradation (Fig. 2B). The exogenous overexpression of LZTR1 in 293T cells leads to a reduction in RIT1 protein levels, which is reversed by the proteasomal inhibitor bortezomib and by the CUL3 inhibitor MLN4924, but not by the lysosomal inhibitor bafilomycin (81,82).

Notably, almost all NS-associated RIT1 mutants are defective in their interaction with endogenous LZTR1 compared with the wild-type RIT1, thus preventing the proteolysis and ubiquitination of RIT1 mutants (39,54,55,77). Similarly, a group of NS-associated LZTR1 mutations are associated with impaired RIT1 degradation, thus revealing the close relationship between LZTR1 and RIT1 (49,55). Under physiological conditions, RIT1 is ubiquitinated by the LZTR-CUL3 complex, then degraded by the ubiquitin-proteasome system (UPS). Moreover, under pathological conditions, RIT1 escapes from UPS-mediated degradation, thus leading to significant enhancement of MAPK signaling in NS (Fig. 2B). This suggests that LZTR1 serves a negative role in MAPK signaling, whereas mutations in LZTR1 or members of the RAS superfamily affect MAPK signaling and contribute to NS.

4. LZTR1 in glioblastoma (GBM)

RAS is considered the most closely related oncoprotein to human cancer (such as pancreatic, lung and colon cancer, as well as hematological malignancies) and is commonly activated in tumor cells (47,59,64,83). Given their vital effects on GTPase activity, cancer-related RAS mutations are often lethal in embryos, avoiding the transmission of germline (60,84-86).

Brain cancer is one of the most common tumors in adults; among the different types of brain cancer, GBM is the most common (48%) and deadliest (median overall survival of 12-14 months) given its high tumor heterogeneity and poor survival (87-93). Numerous genetic mutations,

including those in the *LZTR1* gene, have been identified in GBM using whole-exome sequencing. LZTR1 mutations in GBM include 4.4% non-synonymous mutations and 22.4% focal deletions in the coding sequence (94). RIT1 is regarded as an important pathogenic factor of GBM, which participates in the activation of the MAPK/ERK signaling pathway and plays crucial roles in various physiological processes, including cell survival, proliferation and differentiation (95-99). Moreover, LZTR1 can interact with endogenous RIT1, which is considered a tumor promoter in GBM (39,94). Frattini *et al* (94) demonstrated that 9 out of 10 LZTR1 mutations occur in the Kelch domains and greatly impair the RIT1-LZTR1 interaction. Thus, LZTR1 suppresses the MAPK/ERK signaling pathway by degrading RIT1 to inhibit proliferation and migration of GBM cells; however, GBM-associated LZTR1 mutations impair this function (Fig. 2B) (39,77,94). Moreover, LZTR1 decreases tumor size by inducing glioma spheres, enhancing glioma cell adhesion and reducing cell cycle progression-related proteins (such as cyclin A, PLK1 and p107), whereas mutations or deletions in LZTR1 impair glioma sphere formation and promote the self-renewal ability of GBM cells (39,77). These findings suggest that GBM-associated LZTR1 mutations in human GBM drive self-renewal and the growth of glioma spheres (39,55,77). Moreover, LZTR1 simultaneously found in GBM with mutations and deletions confirms the two-hit hypothesis of cancer (the two alleles of tumor suppressor genes are required to be mutated into 'loss of function' in one cell (100). When a cell in a person is heterozygous for a germline mutation ('one hit'), it is required to undergo a second, somatic event ('second hit') that inactivates the other allele to initiate cancers (100), further indicating that LZTR1 acts as a tumor suppressor in GBM (89-91,94). Given the significance of LZTR1-RIT1 signaling in GBM, LZTR1 and RIT1 may represent promising therapeutic targets for GBM.

5. LZTR1 in chronic myeloid leukemia (CML)

The activation of key cellular pathways, including the RAS/MAPK signaling pathway, affects the survival and proliferation of BCR-ABL+ CML cells (101-103). The occurrence of tyrosine kinase inhibitor (TKI) resistance is also associated with dysregulation of RAS/MAPK signaling (38). In recent studies, the inactivation of endogenous or exogenous LZTR1 increased the phosphorylation of MAPK kinase 1 and -2, as well as ERK1 and -2 in CML cells (indicating enhanced RAS/MAPK signaling activation) and promoted the resistance of these cells to TKIs (104,105). This phenotype may depend on the failed formation of the CUL3 ligase complex, as similar phenotypic changes were also observed when CUL3 was genetically silenced (38). Thus, there is limited knowledge of the link between LZTR1 inactivation and TKI resistance, and further study of this molecular mechanism may help alleviate TKI resistance in CML.

6. LZTR1 in schwannomatosis

Schwannomatosis is a hereditary disease characterized by schwannomas in the spinal and peripheral nerves and predisposition to benign tumors throughout the nervous system (106-108). Local or diffuse chronic pain is the most common symptom reported by patients with schwannomatosis (49). Germline mutations in the *LZTR1* gene occur in 41 out of 168 sporadic patients with schwannomatosis (24.4%), highlighting the complex heterogeneity of schwannomatosis (106,108). Interestingly, schwannomatosis-associated LZTR1 mutations are uniformly located in almost every domain (Table I). Thus, in contrast with the mutations in NS and GBM, there is no positional preference for LZTR1 mutations in schwannomatosis (50). Notably, the occurrence of schwannomatosis-associated LZTR1 mutations at the same genetic locus as NS-associated ones and the coexistence of NS and schwannomatosis in some patients have also been reported in previous studies (108,109). According to the two-hit hypothesis, biallelic loss of a tumor suppressor gene can lead to tumorigenesis; hence, according to the two-hit hypothesis, the NS patients carrying LZTR1 germline mutations are more likely to have schwannomatosis (50,109,110). However, the pathogenesis of this disease and the signaling pathways involving LZTR1 have rarely been rarely studied.

SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily B member 1 (*SMARCB1*) was identified as the first predisposing gene for schwannomatosis (111). Both *SMARCB1* and *LZTR1* are located at the 22q centromere, and a previous study identified germline variants of *LZTR1* in 24 patients with *SMARCB1*-negative schwannomatosis from 22 unrelated families (112). Notably, recent studies reported the occurrence of GBM in patients with schwannomatosis and germline *LZTR1* mutations or *SMARCB1* mutations (48,113,114). Given the lack of evidence of protein interaction between *SMARCB1* and *LZTR1* or their participation in specific signaling pathways, further research on *LZTR1* and *SMARCB1* may clarify the molecular basis of schwannomatosis and GBM.

7. Conclusions and prospects

LZTR1 acts as a negative factor that suppresses RAS function and MAPK signaling; mutations in this protein may dysregulate RAS ubiquitination and lead to impaired protein degradation of RAS superfamily proteins, leading to NS, GBM, schwannomatosis and CML cell resistance to TKIs (Fig. 2C) (38,39,58). Notably, NS- and GBM-related LZTR1 mutations are concentrated in the Kelch domains, whereas schwannomatosis-associated LZTR1 mutations are uniformly located in almost every domain (38,50,58,106,107,113-115). The distribution of these mutations is associated with the severity of these diseases. Indeed, LZTR1 mutations located in Kelch domains directly affect the substrate binding of the LZTR1 (40,58), which has the greatest effect on the degradation level of the substrate, thus leading to more serious diseases, such as NS and GBM. However, schwannomatosis-associated LZTR1 mutations occur in sites that only slightly affect the function of LZTR1 and to some extent, substrate-LZTR1-CUL3 complexes still work. This hypothesis may be tested by studying LZTR1 mutations in patients with concurrent GBM and schwannomatosis.

For several genetic diseases, such as NS, early detection may be more important than treatment. *LZTR1* has also been added to the list of genes causing NS and is present in $\leq 8\%$ of NS patients (46,70). Thus, prenatal screening for LZTR1 mutations and prophylactic use of RAS inhibitors may be a possible way to avoid the occurrence of NS after birth. Moreover, for patients with LZTR1 mutations in GBM and other cancer types, RAS pathway inhibitors may be a good choice for treatment. Thus, comprehensive understanding of other mechanisms of RAS activation may benefit patients carrying LZTR1 mutations and offer new therapeutic strategies (38,58).

LZTR1 is also closely related to the central nervous system. LZTR1 interacts with CUL3 and neurofibromin 1 (NF1) to regulate night-time sleep by increasing GABA receptor signaling and has been associated with RAS-related neurological diseases created by Nf1 deficiency (116). NF1 is a negative growth factor of the RAS/MAPK signaling pathway, which, together with LZTR1, may inhibit RAS/MAPK signaling pathways (117). However, the molecular mechanism of the interaction between LZTR1 and NF1 remains unknown. The functions of LZTR1 in the nervous system are not restricted to those already described and may be closely related to neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease (117). Notably, the frequency of LZTR1 mutations in schwannomatosis are as high as 25% (49), yet the specific molecular mechanism of the disease has not been explored. Characterizing LZTR1-associated functional changes in schwannomatosis will provide additional insights into the disease and design of new therapeutic strategies, thus benefitting patients carrying LZTR1 mutations.

LZTR1 has previously been considered as a ubiquitin-ligase enzyme, rather than phosphokinase; however, it also regulates the phosphorylation but not the ubiquitination of RAF1 (56). LZTR1 could mediate the phosphorylation of RAF1 by inducing ubiquitination of PPPICB in complexes, and LZTR1 itself may also phosphorylate substrates. However, such

Table II. Leucine zipper-like transcription regulator 1 substrates.

Substrate name	Substrate function
KRAS	Cell division, angiogenesis
NRAS	Cell division, angiogenesis
HRAS	Cell division, angiogenesis
RRAS	Vascular homeostasis and regeneration, angiogenesis, cell adhesion
RIT1	Neuronal development and regeneration
RAF1/PPP1CB/SHOC2	Cell cycle, cell division, glycometabolism, angiogenesis
NF1	Cell division

RIT1, RAS-like without CAAX1; PPP1CB, protein phosphatase 1 catalytic subunit β ; NF1, neurofibromin 1.

phenomenon has not been confirmed. Phosphorylation and ubiquitination are common post-translational protein modifications that play crucial roles in the functions of substrates. Thus, the molecular basis of determining whether LZTR1 is capable of phosphorylating substrates directly is worth exploring in the future.

Compared with the current knowledge of SPOP, another E3 adaptor of CUL3 (2,15,19-29), our understanding of LZTR1 substrates is still limited. The interaction between proteins depends on their specific structure, and a class of proteins that interacts with the same protein often share similar domains, such as SPOP-binding consensus motifs (Φ - π -S-S/T-S/T, where Φ : nonpolar residues; π : polar residue; S: Ser and T:Thr) (22). Searching for regions on the substrates that bind to LZTR1 from the existing known substrates (RAS superfamily proteins) will accelerate LZTR1 research, together with structural studies to identify interacting domains in LZTR1-mediated substrates (38,39,55-58,116). Currently, the known substrates of LZTR1 are restricted to the RAS superfamily (Table II). Therefore, additional potential substrates for LZTR1 should be further studied.

Additionally, studying alternative mechanisms of RAS regulation will also assist with the development of new treatments for RAS-driven diseases. Notably, although KRAS is the most frequently mutated member of the RAS family and is closely related to human malignant tumors, it has long been considered to be untargetable (23,53,118-125). LZTR1 is the most specific and potent regulator of KRAS (38). Thus, further research on LZTR1 may help in the successful targeting of KRAS in the future.

Notably, LZTR1 is a novel Golgi matrix-associated protein (40), and it may be closely related to the function of Golgi body. The Golgi body is responsible for the processing, sorting and transportation of proteins and plays an important role in tumorigenesis, progression and invasion (126). The functional changes in the Golgi body caused by LZTR1 variants should also be further studied.

In conclusion, LZTR1 might become an important focus of biomedical research. Further studies are required for improved understanding of the biological function of LZTR1 and its role in the occurrence of diseases, as well as the development of disease treatments, such as rational design of LZTR1 promoters for patients harboring loss-of-function LZTR1 mutations.

Acknowledgements

We would like to thank Dr Yuqi Wang (West Lake University, China) for the kind help and good advice provided.

Funding

This work was supported by The Natural Science Foundation of Zhejiang Province (grant no. LY20C070001), The National Natural Science Foundation of China (grant no. 31801165), The Natural Science Foundation of Ningbo (grant no. 2018A610213), The National Undergraduate Training Program for Innovation and Entrepreneurship (grant no. 202011646030), The Program of Xinmiao (Potential) Talents in Zhejiang Province (grant nos. 2019R405061, 2019R405011 and 2020R405039), The Student Research and Innovation Program of Ningbo University (grant nos. 2020SRIP1923, 2020SRIP1919, 2020SRIP1902 and 2020SRIP1901) and The K.C. Wong Magna Fund in Ningbo University.

Availability of data and materials

Not applicable.

Authors' contributions

XJ conceived the present study. HZ and XC drafted the manuscript. QL, JW and YZ made substantial contributions to the interpretation, drafting the study and revising it critically for important intellectual content. XJ, HZ and XC were major contributors in the manuscript. Data authentication is not applicable. 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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