Review Article

The Role of SliTrk5 in Central Nervous System

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Received 30 January 2022; Revised 6 June 2022; Accepted 23 June 2022; Published 14 July 2022

Academic Editor: Immacolata Castellano

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SLIT and NTRK-like protein-5 (SliTrk5) is one of the six members of SliTrk protein family, which is widely expressed in the central nervous system (CNS), regulating and participating in many essential steps of central nervous system development, including axon and dendritic growth, neuron differentiation, and synaptogenesis. SliTrk5, as a neuron transmembrane protein, contains two important conservative domains consisting of leucine repeats (LRRs) located at the amino terminal in the extracellular region and tyrosine residues (Tyr) located at the carboxyl terminal in the intracellular domains. These special structures make SliTrk5 play an important role in the pathological process of the CNS. A large number of studies have shown that SliTrk5 may be involved in the pathogenesis of CNS diseases, such as obsessive-compulsive-disorder (OCD), attention deficit/hyperactivity disorder (ADHD), glioma, autism spectrum disorders (ASDs), and Parkinson’s disease (PD). Targeting SliTrk5 is expected to become a new target for the treatment of CNS diseases, promoting the functional recovery of CNS. The purpose of this article is to review the current research progression of the role of SliTrk5 in CNS and its potential mechanisms in CNS diseases.

1. Introduction

Increasing evidence has identified that SliTrk5 can modulate many important stages of central nervous system development, including axon and dendritic growth, neuron differentiation, and synaptogenesis, which may affect the pathological mechanism of many central nervous system diseases [1]. The expression of SliTrk5 is highly restricted to neural tissue of CNS, compared with various systems throughout the body. It has two conserved domains: leucine repeats (LRRs) located at the amino terminal in the extracellular region and tyrosine residues located at the carboxyl terminal in the intracellular domains. The LRR domain is highly similar to the SLIT family proteins that control axonal guidance and branching. Additionally, the tyrosine residues in intracellular carboxyl terminus of SliTrk5 influencing nervous system development and function have a high degree of homology with that of the neurotrophin receptor, tropomyosin-related kinase (Trk) [1, 2]. Scholars identified the SliTrk family proteins originally in a screen for genes that were differentially expressed in the neural tube defects mice [2]. Correspondingly, it has also been verified that six members of the SliTrk family located in three different loci: chromosome 3 (SliTrk3), chromosome 13 (SliTrk1, SliTrk5, and SliTrk6), and the X chromosome (SliTrk2 and SliTrk4) [3], all of which are generally expressed in the developing central nervous system at times and locations that are important to neuronal morphogenesis and synaptogenesis [4, 5], as well as in brain tumors, embryonic stem cells, subsets of endothelial cells, hematopoietic stem cells, and in leukemia and lymphoma cells [6], but vary within the family. For example, SliTrk5 is significantly expressed in the CA1 region of hippocampal [7] and also in occipital and frontal lobes of brain, spinal cord, medulla, and in early hematopoietic progenitors [8, 9], whereas SliTrk1 is expressed in the mature neurons,
SliTrk2 is highly expressed in the ventricular layer, and SliTrk6 shows compartmentalized expression in diencephalon. In the in vitro experiment, overexpression of SliTrk1 was reported to induce unipolar neurites in cultured neuronal cells but SliTrk5 and other members inhibited neurite outgrowth [10]. Therefore, the members of SliTrk family are not only different in the expression, but also have different functions in the central nervous system. SliTrk5 and its family members regulated the process of synaptogenesis and neurite outgrowth by precisely participating in the formation of synaptic adhesion molecule complexes with the LAR-type receptor phosphotyrosine-phosphatases (LAR-RPTPs) [11–15]. Currently, many investigations have indicated that SliTrk5 may be involved in the pathomechanism of many CNS diseases, including obsessive-compulsive-disorder (OCD), attention deficit/hyperactivity disorder (ADHD), glioma, autism spectrum disorders (ASDs), and Parkinson’s disease (PD) [16]. It has also been announced that regulating the expression of SliTrk5 can promote the functional recovery of the nervous system. All these data suggested that SliTrk5 may be involved in the pathogenesis of above diseases and may be an underlying target for the treatment of CNS diseases. Therefore, it is necessary to understand the role of SliTrk5 in the development of the central nervous system and in the pathogenesis of central nervous system diseases.

2. Overview of SliTrk5

Exon-mapping using cDNA sequences has revealed the SliTrk5 gene is located in chromosome 13, and there are 8-50 introns in the upstream of initiation methionine, and the protein-coding region of SliTrk5 is located in a single exon. The molecular weight of SliTrk5 predicted by the deduced amino acid sequences is 108.3 kd [7]. SliTrk5 has a characteristic domain architecture consisting of an intracellular carboxy-terminal domain and two consecutive extracellular LRR modules, LRR1 and LRR2. These structures are connected by a single transmembrane domain [6]. The carboxy-terminal regions where conserved tyrosine residues presented are completely conserved between humans and mouse. The homologies of SliTrk5 between mouse and human are 95–97%. Therefore, it can be speculated that the organization and function of the SliTrk5 are similar between human and mouse. All of these results imply that the carboxy-terminal regions and the LRR domain play an important role in the function of SliTrk5 [7]. And it is of great significance to study the molecular structure and function of SliTrk5.

2.1. Interaction with Synaptic Adhesion Molecules, RPTP. It has been confirmed that synaptogenesis is an elementary process that establishes definite connections between neurons and supports the organization of neural circuits. In the development of synaptogenesis, the membrane-anchored proteins play a crucial role in initial axon–dendrite target recognition, differentiation of presynaptic and postsynaptic specializations, which called synaptic adhesion molecules (SAMs) [17]. The unique extracellular LRR domain structure of SliTrk5 can combine with the SAMs to control synapse formation, thus regulating the connections between neurons. The LRR domain of SliTrk5 is a typical LRR structure, which contains six LRR repeating motif sandwiched between N-terminal and C-terminal cysteine-rich capping domains, LRRNT and LRRCT, with a defining sequence LxxLxLxxN/C (x being any amino acid) [10, 18, 19]. This domain is thought to be homologous to the well-known family of RPTP containing proteins, SLIT family proteins [20], which play a crucial role in axonal guidance and repulsion, tangential neuronal migration, cytoskeletal dynamics, and modifying cell adhesion properties [1, 21]. In addition to SliTrk5, many other leucine-rich neuronal transmembrane proteins have been selected as key synaptic organizers, including LRR transmembrane protein (LRRTM), synaptic adhesion-like molecule (SALM), neurotrophin receptor tyrosine kinase C (TRKC), netrin-G ligand 3 (NGL-3), and fibronectin LRR transmembrane (FLRT) [22–26].

The LRR domain contained in SliTrk5 plays a pivotal role in the regulation of various neuronal functions, such as neurite outgrowth, synapse formation, and dendritic morphogenesis. To execute such functions, SliTrk5 majorly employed the following two basic mechanisms. On the one hand, LRR domain works on the trans cell-cell adhesion molecule PTPRD modulating axon–dendrite adhesion [27, 28], which belongs to type Ia receptor protein tyrosine phosphatase (RPTP) family. The RPTP protein family, including PTPRD, PTPRS, and PTPRF, has been shown to be presynaptic binding partners, interacting with the LRR domain of SliTrks on the postsynaptic membrane to control the formation of inhibitory synapse [29]. In addition, the latest research point of view on structure of synaptic adhesion partner has considered that the binding affinity between RPTP with SliTrks is further modulated by alternative splicing variants (MeA and MeB) in the immunoglobulin-like (Ig-like) domains of RPTP [30, 31]. The crystal structure of the complex formed by the binding of RPTP family proteins with SliTrks reveals the structural basis of its binding mode and the importance of MeB splicing insertion in type Ia RPTP on its binding selectivity and function. Three Ig-like domains of PTPRD bind the LRR1 domain of SliTrks with a 1 : 1 stoichiometry in a MeB splicing insert–dependent manner [11, 32]. Key interaction residues on the type Ia RPTP/SliTrk complex have been shown to be highly conserved in all SliTrk members and type Ia RPTP members, indicating that the binding pattern between PTPRD and SliTrk5 is also similar to the structure described above [12] (Figure 1). Another hand, LRR domain can regulate the function of neuron cell-surface receptor [27, 28]. Song et al. have shown the SliTrk5 acted as a coreceptor of TRKB, regulating brain-derived neurotrophic factor- (BDNF-) dependent biological responses by directly modulating the circulation of TRKB receptor via recruitment of Rab11-FIP3 (an effector protein). They claimed, under normal circumstances, the LRR domain of SliTrk5 interacts primarily with PTPD, whereas it shifts to cis-interactions with TRKB upon the stimulation of BDNF. At this point, PTPD and TRKB compete to combine the LRR domain of SliTrk5 [33]. Therefore, SliTrk5 acts as a common receptor of TRKB, regulating its BDNF-dependent transport and signal.
transduction in CNS, which plays numerous important roles in synaptic development and plasticity [34]. The LRR domain is the most important structural basis for the function of SliTrk5 in central nervous system.

2.2. Function of Tyr Residues in Intracellular Region. Compared with the extracellular LRR domain, the intracellular region of SliTrk5 has a more complex architecture. There are many conserved tyrosine residues in the intracellular region of SliTrk5, which are similar to TRK both in structures and functions, including neurite outgrowth and dendritic elaboration, synapse formation, and neuronal survival [6, 35]. One of the similarities between SliTrk5 and TRK is the presence of NPxY motif (the defining sequence is ASN-Pro-X-TYR, where X is any amino acid) near their intracellular juxtamembrane region [36]. Once phosphorylated, the NPxY motif of TRK could serve as a binding site for adaptor proteins, such as Shc, which initiates Ras and phosphoinositide 3 kinase downstream signal, regulating signal transduction of CNS [37, 38]. In addition, phosphorylation of tyrosine residues in this particular motif has been reported as a signal of receptor endocytosis [39], suggesting that SliTrk5 may recruit Shc or other scaffold proteins to initiate intracellular signal and may be related to endocytosis. Besides, a conserved Tyr residue in a position near the C-terminus that is homologous to that of Y791 structure of TRKA is contained in SliTrk5. The recruitment and activation of the γ isoform of phospholipase C (PLC) could be caused by the phosphorylation of Y791 in TRKA, which lead to the decrease of Ca²⁺ of internal stores and protein kinase C (PKC) activation, finally affecting the transmission of this signal pathway [40–42]. When studying the role of SliTrk5

![Diagram of functional structure of SliTrks and cell adhesion molecule IIa receptor protein tyrosine phosphatases (RPTPs) and their interaction in synapse formation.](image-url)
in vitro HeLa cells, there was another intracellular tyrosine residue (Y833) of SliTrk5 was phosphorylated, which may affect the axon growth [43]. Therefore, we speculate that these conserved tyrosine residues in SliTrk5 may couple to PLC-g signal cascades or other downstream signal proteins, regulating the signal transmission of the CNS.

3. Role of SliTrk5 in the Development of CNS

3.1. Regulating Neurite Outgrowth and Dendritic Morphology. The function of SliTrk5 in regulating the process of neuronal outgrowth has been established from the beginning. The LRR domain of SliTrk5 is semblable to that of the SLIT family proteins, which are famous for modulating axon guidance and branching [1]. Neurons that overexpressed SliTrk5 have induced more inhibitory input, which potentially decreases neuronal activity and inhibits dendritic growth [44]. In addition, preliminary studies conducted by overexpressing SliTrk5 in PC12 cells showed that compared with the control group, overexpression of SliTrk5 reduced both the number and length of neurites [6]. The Golgi tracing analyses has found in the striatum of adult SliTrk5 deficient mice; there was a significant decrease in dendritic complexity of medium spiny neurons [16]. In a word, these results suggest that the SliTrk5 is the neuronal components controlling the neurite outgrowth and dendritic morphology.

3.2. Promoting Synaptogenesis. There was a study showing that LRR-rich proteins may induce synaptic formation, and the purpose of which was to perform an expression screen for synaptogenesis proteins [45]. SliTrk5 is, as a matter of course, one of those proteins because of its typical LRR domain structure. By immunocytochemical studies in vitro, scholars discovered where the SliTrk5 localized in cultured neurons was on synaptic sites [16]. Moreover, SliTrk5 can not only induce presynaptic neuronal differentiation in cellular coculture system but also promote the formation of inhibitory synapse [44, 46]. Via its extracellular LRR domain, SliTrk5 could bind presynaptic membrane partners, such as PTPRD, and recruit intracellular postsynaptic proteins, participating in the modulation of synapse formation, which has been confirmed [47]. In the process of synapse formation, SliTrk5 was associated with the synaptic adhesion molecules (SAMs), which plays an important role in initial axon–dendrite target recognition, differentiation of presynaptic and postsynaptic specializations. Therefore, SliTrk5 contributes a lot to the development of synaptogenesis, especially the inhibitory synapses.

3.3. Affecting the Neuron Survival and Signal Transmission. SliTrk5 has been implicated in promoting neuronal survival. If the SliTrk5 was knocked out, the total brain volume of the mice would be reduced, which was especially obvious in the striatum [16]. Furthermore, researchers have observed the overactivation of the orbitofrontal-subcortical circuits by functional imaging in SliTrk5 knockout mice who exhibited OCD-like behavior, which was posited to be the result of an imbalance of signal transmission in the basal ganglia pathways [48, 49]. In addition, a recent study has demonstrated the ability of SliTrk5 affecting signal transmission in dopaminergic circuits by stimulating the formation of inhibitory synapses in midbrain dopaminergic neurons [44]. In brief, SliTrk5 plays an important role in the survival and signal transmission of neuron.

3.4. Participating in Tumorigenesis in Brain. In different types of human brain tumors, the expression of SliTrk5 was most widely in different human brain tumors, compared with other members of the SliTrks family [7]. Especially in the gliomas, the expression of SliTrk5 was upregulated and associated with the pathological grading [50]. In addition to playing an important role in the development of brain tumors, SliTrk5 is also involved in the process of tumorigenesis in other organs, such as colorectal cancer, thyroid tumors, gastric cancer, nasopharyngeal carcinoma, and lung squamous cell carcinoma [51–55]. Thus, besides its role in normal development, SliTrk5 might also be implicated in malignancy.

3.5. Involving in Neural Tube Defects. The original cDNA of SliTrk5 and other members of SliTrks were found in a study conducted to identify differentially expressed genes in mice with neural tube defects [6]. Until now, SliTrk5 is considered to be involved in the mechanism of neural tube defects but the details of their association are still being studied and may be reported elsewhere in the future.

Besides above functions in the CNS, SliTrk5 has also reported to affect the process of angiogenesis and bone formation. A study analysing markers of pork-granulosa cells cultured in vitro found that the expression of SliTrk5 was downregulated over time and proved that SliTrk5 is involved in angiogenesis and vascular development of the ovary, which can be screened as a potential marker [56]. Additionally, SliTrk5 is selectively expressed in osteoblasts and as a negative regulator of hedgehog signal which is necessary for bone formation in osteoblasts. In vitro culture, deletion of SliTrk5 leads to an increase in hedgehog signal, but its overexpression in osteoblasts inhibits downstream targets of hedgehog signal, thus modulating osteoblast differentiation [57]. In a word, SliTrk5 is an emerging protein that is not well understood by people. It plays an important role in diseases of many systems throughout the body, especially in the central nervous system.

4. Potential Mechanisms of SliTrk5 in CNS Diseases

SliTrk5 has been identified as one of the factors modulating basic function of the physiological and pathological mechanism of CNS, such as neurite outgrowth, dendritic elaboration, synaptogenesis, and neuronal signal transmission, which was associated to the development of neuropsychiatric disorders, including obsessive-compulsive spectrum disorders (OCDs), attention deficit/hyperactivity disorder (ADHD), autism spectrum disorders (ASDs), and Parkinson’s disease (PD) [35].
4.1. SliTrk5 in Obsessive-Compulsive Spectrum Disorder (OCD). Obsessive-compulsive spectrum disorder (OCD), characterized by repeated unwanted thoughts and/or repetitive behavior, is one of the most common mental disorders [58]. Researchers have studied the rare nonsynonymous mutations of the protein-coding sequence of the human SliTrk5 gene in 377 OCD subjects. The results demonstrated that rare functional mutations in SliTrk5 contribute to the impairing of synaptic activity and genetic risk for OCD in human [59, 60]. To study the relationship between SliTrk5 and Tourette’s syndrome (TS), which commonly occurs in conjunction with obsessive-compulsive disorder, investigating its role in a family-based sample of 377 affected children, but they did not find any evidence of a link between TS and SliTrk5 [61]. The SliTrk5 deficiency mice whose coding region of the SliTrk5 was replaced by the b-galactosidase gene (lacZ) reporter gene initially showed an increase in anxiety-like behavior (assessed by the elevated-plus-maze and the open-field tests) and followed by repetitive and excessive self-grooming behavior, which eventually lead to severe facial skin damage and hair loss [16]. Excessive grooming that led to the formation of facial lesions, increasing anxiety-like behavior, defects in corticostriatal transmission, and altered expression of gluta-mate receptors in the striatum, and all of these behavior and pathway manifestation found in the SliTrk5 deficiency mice are similar to another genetic mouse model of OCD which has been reported lacking the synapse-associated protein 90-postsynaptic density-95-associated protein 3 (Sapap3) [62]. Sapap3 is an intracellular scaffolding molecule localized to the postsynaptic position of excitatory synapses and is crucial for maintenance of synaptic structure [63, 64]. In addition, another mouse model of OCD with repetitive grooming behavior was Hoxb8 deficiency mice, which was almost identical to that observed in SliTrk5 deficiency mice [65–67]. However, the treatment of fluoxetine, as a selective serotonin reuptake inhibitor (SSRI), which is mainly used for the treatment of depression and obsessive-compulsive disorder, can effectively alleviate reduce the excessive grooming behavior of SliTrk5 deficiency mice [68]. Because the SliTrk5 intracellular domain resembles TRK neurtrophin receptors, it is plausible that they may have similar ligands. It would be interesting because neurtrophin can be modulated by existing drugs such as FLX [69]. Meanwhile, FLX can alleviate the obsessive-compulsive like behavior in SliTrk5-deficient mice. All of above evidences suggested that the expression or the activity of SliTrk5 may be regulated by FLX, but it is still obscure. Future studies that investigate the disease relevance of the human gene are essential for SliTrk5 to emerge as an important therapeutic target and/or biomarker for OCD [70].

The possible pathological mechanisms of SliTrk5 in OCD include the following:

(1) Affecting the anatomy of striatum

The effect of SliTrk5 on the anatomy of striatum mainly contains two aspects. Firstly, the volume of the striatum was reduced in both young and aged mice whose SliTrk5 gene has been knocked out. The striatum volume relative to the whole brain estimated by Cavaliere measuring showed a significant decrease in striatum volume compared with wild-type mice, while the ratio of other brain structures to total brain volume did not change, suggesting that SliTrk5 deficiency significantly affected the anatomical structure of the striatum [16]. These results found in SliTrk5 deficiency mice, in line with many previous studies, reported that the volume of the striatum is decreased, as well as in some but not all individuals with OCD [71–73]. Secondly, it has been revealed that there was a significant decrease in dendritic complexity at 50 μm and greater distance from the soma in the individual medium spiny neurons of the striatum in SliTrk5 deficiency mice, either using the Sholl analysis to observe the dendritic complexity or using fractal dimension analysis to quantify how completely a neuron fills its dendritic field, all of which has reflected a decrease in synaptic connectivity. However, there was no difference in striatal cell soma area between SliTrk5 deficiency mice and their wild-type litter born larva [16]. In conclusion, it is possible the lessened striatal volume in the SliTrk5 deficiency mice might be accounted for by altered neuronal morphology (Table 1).

(2) Increasing the orbitofrontal cortex activity

To investigate the role of SliTrk5 in the central nervous system, scholars evaluated the difference of baseline activity in specific brain regions between wild-type and SliTrk5 knockout mice by assessing expression of FosB, which is a transcription factor used to assess neuronal activity routinely. As a result, they found that FosB was upregulated exclusively in the orbitofrontal cortex of SliTrk5 knockout mice, which did not show in other brain regions [16, 74]. This finding also has been consistently verified in functional imaging studies that discovered there is an increasing activity in orbitofrontal cortex in individuals with OCD (Table 1) [49, 75].

(3) Modulating region-specific glutamatergic neurotransmission

Shmelkov et al. have discovered the protein amounts of glutamate receptor subunits NAMDA2A, NAMDA2B, Glutamate Receptor-1, and Glutamate Receptor-2 involved in excitatory neurotransmission were decreased by 20–60% in SliTrk5 deficiency mice, greatly modulating the glutamatergic neurotransmission of specific region [16]. The sequence variation of SliTrk5 was also considered may be associated with the structural neuroimaging phenotype of obsessive-compulsive disorder [76]. Probably because of these defects, the corticostriatal neurotransmission of SliTrk5 deficiency mice was impaired, agreeing with the observation of altered corticostriatal transmission in OCD patients [77, 78]. Current studies have highlighted the important role of excitatory synapses in the striatum and cortical striatum nerve conduction in the pathogenesis of OCD-like behavior (Table 1) [79]. Consequently, SliTrk5 may be involved in the pathogenesis of OCD by regulating the glutamatergic neurotransmission, thus affecting the corticostriatal neurotransmission.
### 4.2. SliTrk5 in Brain Tumor

Recent years, SliTrk5 was reported to participate in the development of brain tumors. A previous study has validated the expression of SliTrk5 is upregulated in gliomas and correlated with pathological grading [7, 50]. SliTrk5 can promote BDNF-dependent signal by acting as a TRKB coreceptor in striatum neurons [33]. Brain-derived neurotrophic factor (BDNF) is widely distributed in the central nervous system, affecting the malignant degree of glioma [80]. BDNF can inhibit neuronal apoptosis through its functional receptor TRKB. In neurocytoma, BDNF can induce TRKB phosphorylation, activating Ras/ERK signal transduction pathway and promoting cell proliferation [81, 82]. Some studies have shown that the activation of TRKB-BDNF signal pathway can promote nerve cells to synthesize and secrete vascular endothelial growth factor (VEGF), thus promoting the growth of tumor cells [83, 84]. Therefore, some researchers have speculated that SliTrk5 may stimulate the occurrence and proliferation of glioma by modulating TRKB-BDNF signal pathway and other related signal transduction.

SliTrk5 is also associated with tumorigenesis in other organs. SliTrk5 gene presented frequent genetic, epigenetic, and transcriptional alterations in colorectal neoplasia, which has been discovered by a study designed to compare and contrast the molecular profiles of laterally spreading tumors (LSTs) and colorectal cancer (CRC) [51]. Moreover, in thyroid tissue, the expression of SliTrk5 was significantly down-regulated in Trk-T1 transgenic thyroid tumor mice, which was revealed by a global genomic copy number analysis [52]. SliTrk5 also has been identified affecting the molecular or clinical phenotypes of gastric cancer (GC) with TP53 mutation [53] and correlating with the radioresistance of nasopharyngeal carcinoma and the prognosis of lung squamous cell carcinoma (Table 1) [54, 55].

SliTrk5 is not only involved in the tumorigenesis of the central nervous system but also related to tumors of all systems of the whole body, in which there may be some underlying common mechanisms. If we take SliTrk5 as a target for antitumor therapy in the future, there may be some unexpected surprises.

### 4.3. SliTrk5 in Attention Deficit/Hyperactivity Disorder (ADHD)

The main symptoms of ADHD are inattention and/or hyperactivity/impulsivity, and SliTrk5 can regulate the hyperactivity behavior of ADHD by controlling the formation of inhibitory synapses in dopamine neurons [44]. The possible mechanisms are as follows: the neurodevelopmental of the hyperactivity disorder has been discovered to be involved in the dopaminergic neurons system, and SliTrk5 was identified to promote inhibitory synapse formation on midbrain dopaminergic neurons, whereas SliTrk2 has opposite effects. Loss of the function of SliTrk2 results in less excitatory input and hyperactivity, but loss of SliTrk5 function results in less inhibitory input and locomotor activity. Both of them would cause the imbalance of excitatory/inhibitory synaptic, leading to alterations in developing dopaminergic circuits and significantly affecting locomotor activity, which has also been identified to disrupt neurotransmission of dopaminergic neurons and contribute to many neuropsychiatric disorders including hyperactivity disorder (Table 1) [44, 85]. If we can control the balance of

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**Table 1: Possible mechanisms of SliTrk5 in CNS diseases.**

| Disease         | Expression site of SliTrk5 | Possible mechanisms                                                                 | Participants or models | Potential therapeutic target                      |
|-----------------|---------------------------|----------------------------------------------------------------------------------|-----------------------|--------------------------------------------------|
| OCD             | Cortical striatum         | (1) Affecting the anatomy of striatum  
(2) Increasing the orbitofrontal cortex activity  
(3) Modulating region-specific glutamatergic neurotransmission | SliTrk5 knockout mice | SSRI fluoxetine (FLX) can alleviate the OCD-like behaviors in mice, including excessive self-grooming and anxiety-like behaviors. |
| Brain tumor     | Upregulating expression in gliomas | SliTrk5 may stimulate the occurrence and development of glioma by mediating TRKB-BDNF signal pathway and other related signal transduction. | Patients with glioma | SliTrk5 gene targeting therapy may be the trend in the future. |
| ADHD            | Midbrain dopaminergic neurons | SliTrk5 can modulate the hyperactivity behavior of ADHD through promoting inhibitory synapses formation on midbrain dopaminergic neurons. | SliTrk5 knockdown mice | Regulating the expression of SliTrk5 can maintain the balance of excitatory/inhibitory synapse signal transmission. |
| ASDs            | Unknown                   | Genetic factors                                                                  | ASD mice models       | Enhancing interaction of TRKB receptors with SliTrk5 in the absence of exogenous neurotrophic factors or regulating the function of striatal neurons maybe a new potential target for therapeutics of PD. |
| PD              | Unknown                   | Affecting the function of midbrain dopaminergic neuron                           | /                     | /                                                |
excitatory and inhibitory synapses by targeting the relative expression levels of SliTrk5 and Slitrk2, the symptoms of ADHD may be improved to some extent.

4.4. SliTrk5 in Autism-Spectrum-Disorders (ASDs). The autism spectrum disorder (ASD) is characterized as restricted repetitive behavior, including the lower-order stereotypy and self-injury and higher-order indices of circumscribed interests and cognitive rigidity [86]. A genome comparison study designed to identify related genes for autism has found that the SliTrk5 is associated with repeated overgrooming of ASDs (Table 1) [87]. Thus, although no study has identified a specific role of SliTrk5 in the patho-genic mechanism of autism so far, SliTrk5 may be one of the genetic factors of autism from a genetic point of view. Targeting studies of SliTrk5 are needed in autism in the future.

4.5. SliTrk5 in Parkinson’s Disease (PD). SliTrk5 can effectiv-ealy reclaim activated TRKB receptors and promote BDNF-dependent signal pathway, prolonging the intensity and duration of neurotrophic factor signal in striatum neurons in the absence of BDNF supply [33]. Moreover, the SliTrk5 deficiency mice showed that striatum volume and anatomical structure were changed [16]. The striatum is the largest component of the basal ganglia, and the disorder of striatal function has been implicated in neurodegenerative disorders such as Huntington’s disease and Parkinson’s disease [88, 89]. Additionally, SliTrk5 can be regulated by Lmx1a/b, which as a transcription factor involved in each step of midbrain dopaminergic neuron development [90, 91]. The specific inactivation of Lmx1a/b in adult midbrain dopaminergic neuron resulted in dopamine neuron degeneration and parkinsonism [91, 92]. Although no current researches have shown that there is a clear relationship between SliTrk5 and these neurodegenerative diseases, SliTrk5 may affect the function of midbrain dopaminergic neurons through above pathways, leading to neurodegenerative diseases such as Parkinson’s disease. And SliTrk5 has represented a new potential target of therapeutics for these neurodegenerative disorders by enhancing interaction with TRKB receptors in the absence of exogenous neurotrophic factors or regulating the function of striatal neurons (Table 1).

5. Summary

Increasing evidence has revealed that SliTrk5 plays an important role in the physiological and pathological processes of the CNS. The neurite outgrowth, dendritic branching, synaptogenesis, cell differentiation, and signal transmission of neurons are important processes for the development of CNS. SliTrk5 is an important synaptic associated protein that has a crucial regulatory effect on the above response processes of CNS. Furthermore, there have been exhibiting complex interactions between SliTrk5 and many CNS disorders, including obsessive-compulsive disorder, attention deficit/hyperactivity disorder, autism spectrum disorders, and brain gliomas. The role of SliTrk5 in the neurodegenerative diseases, such as Parkinson’s disease, has been starting to come out. Taking SliTrk5 as the target and regulating its expression can alleviate neural function damage and promote the recovery of neural function, suggesting that SliTrk5 may be a promising target molecule for illustrating the mechanisms and the treatment of CNS diseases (Table 1). As the specific pathogenesis and signal pathway of SliTrk5 in CNS diseases are not fully clear, the randomized controlled clinical trials need to take years to conduct. Thus, this review has provided the foundation for subsequent integrated studies into the treatment of CNS disorders by figuring out the various functional roles of SliTrk5 in terms of the development of CNS. The specific relationship and potential mechanism between SliTrk5 and neurodegenerative diseases will also be the focus and trend of future research. Consequently, SliTrk5 very likely plays a crucial role in the development of CNS diseases by regulating synaptogenesis and other physiological mechanisms.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

Yan Liu wrote the manuscript, Linming Zhang searched the literature, Ruijing Pang and Di Xia drew the figure, Mingda Ai edited format, Rong Mei made the table, and Ling Chen and Lianmei Zhong revised the manuscript. All authors read and approved the final manuscript. Yan Liu and Linming Zhang contributed equally to this work.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81760243), the Yunnan Provincial Department of Science and Technology-Kunming Medical University Applied basic Research Joint Special Project (No. 2019FE001-217), the Nature Science Foundation of China (No. 82060252), the Major Science and Technology Special Project of Yunnan Province (202102AA100061), and the Graduate Innovation Fund of Kunming Medical University (No. 2022S040).

References

[1] K. Brose and M. Tessier-Lavigne, “Slit proteins: key regulators of axon guidance, axonal branching, and cell migration,” Current Opinion in Neurobiology, vol. 10, no. 1, pp. 95–102, 2000.
[2] A. Patapoutian and L. F. Reichardt, “Trk receptors: mediators of neurotrophin action,” Current Opinion in Neurobiology, vol. 11, no. 3, pp. 272–280, 2001.
[3] J. Ko, “The leucine-rich repeat superfamily of synaptic adhesion molecules: LRRTMs and Slitrks,” Molecules and Cells, vol. 34, no. 4, pp. 335–340, 2012.
[4] J. Round, B. Ross, M. Angel, K. Shields, and B. Lom, “Slitrk gene duplication and expression in the developing zebrafish nervous system,” Developmental Dynamics, vol. 243, no. 2, pp. 339–349, 2014.
S. V. Shmelkov, J. W. Visser, and A. V. Belyavsky,
"Identification and characterization of Slitrk, a novel neuronal transmembrane protein family controlling neurite outgrowth," Molecular and Cellular Neurosciences, vol. 24, no. 1, pp. 117–129, 2003.

J. Aruga and K. Mikoshiba,
"Human SLITRK family genes: genomic organization and expression profiling in normal brain and brain tumor tissue," Gene, vol. 315, pp. 87–94, 2003.

T. Milde, S. V. Shmelkov, K. K. Jensen, G. Zlotchenko, I. Petit, and S. Rafii, "A novel family of slitrk genes is expressed on hematopoietic stem cells and leukemias," Leukemia, vol. 21, no. 4, pp. 824–827, 2007.

M. A. Meyer, "Highly expressed genes within hippocampal sector CA1: implications for the physiology of memory," Neurology International, vol. 6, no. 2, p. 5388, 2014.

S. V. Shmelkov, J. W. Visser, and A. V. Belyavsky, "Two-dimensional gene expression fingerprinting," Analytical Biochemistry, vol. 290, no. 1, pp. 26–35, 2001.

J. W. Um, K. H. Kim, B. S. Park et al., "Structural basis for LAR-RPTP/Slitrk complex-mediated synaptic adhesion," Nature Communications, vol. 5, no. 1, p. 5423, 2014.

S. Y. Won, P. Lee, and H. M. Kim, "Synaptic organizer: Slitrks and type Ila receptor protein tyrosine phosphatases," Current Opinion in Structural Biology, vol. 54, pp. 95–103, 2019.

H. Kang, K. A. Han, S. Y. Won et al., "Slitrk missense mutations associated with neuropsychiatric disorders distinctively impair Slitrk trafficking and synapse formation," Frontiers in Molecular Neuroscience, vol. 9, p. 104, 2016.

F. Beaubien, R. Raja, T. E. Kennedy, A. E. Fournier, and J. F. Cloutier, "Slitrk1 is localized to excitatory synapses and promotes their development," Scientific Reports, vol. 6, no. 1, article 27343, 2016.

A. Scipio and T. C. Südhof, "LAR receptor phospho-tyrosine phosphatases regulate NMDA-receptor responses," eLife, vol. 9, 2020.

S. V. Shmelkov, A. Hormigo, D. Jing et al., "Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsive-like behaviors in mice," Nature Medicine, vol. 16, no. 5, pp. 598–602, 2010.

T. C. Südhof, "Towards an understanding of synapse formation," Neuron, vol. 100, no. 2, pp. 276–293, 2018.

B. Kobe and J. Deisenhofer, "The leucine-rich repeat: a versatile binding motif," Trends in Biochemical Sciences, vol. 19, no. 10, pp. 415–421, 1994.

B. Kobe and A. V. Kajava, "The leucine-rich repeat as a protein recognition motif," Current Opinion in Structural Biology, vol. 11, no. 6, pp. 725–732, 2001.

K. Brose, K. S. Bland, K. H. Wang et al., "Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance," Cell, vol. 96, no. 6, pp. 795–806, 1999.

A. R. Ypsilanti, Y. Zagar, and A. Chédotal, "Moving away from the midline: new developments for Slit and Robo," Development, vol. 137, no. 12, pp. 1939–1952, 2010.

J. Woo, S. K. Kwon, S. Choi et al., "Trans-synaptic adhesion between NGL-3 and LAR regulates the formation of excitatory synapses," Nature Neuroscience, vol. 12, no. 4, pp. 428–437, 2009.

H. Takahashi, P. Arstikaitis, T. Prasad et al., "Postsynaptic TrkC and presynaptic PTPσ function as a bidirectional excitatory synaptic organizing complex," Neuron, vol. 69, no. 2, pp. 287–303, 2011.

Y. Choi, J. Nam, D. J. Whitcomb et al., "SALM5 trans-synaptically interacts with LAR-RPTPs in a splicing-dependent manner to regulate synapse development," Scientific Reports, vol. 6, no. 1, article 26676, 2016.

J. Ko, M. V. Fuccillo, R. C. Malenka, and T. C. Südhof, "LRRM2 functions as a neurexin ligand in promoting excitatory synapse formation," Neuron, vol. 64, no. 6, pp. 791–798, 2009.

M. L. O’Sullivan, J. de Wit, J. N. Savas et al., "FLRT proteins are endogenous latrophilin ligands and regulate excitatory synapse development," Neuron, vol. 73, no. 5, pp. 903–910, 2012.

D. L. Shattuck, J. K. Miller, M. Laederich et al., "LRIG1 is a novel negative regulator of the Met receptor and opposes Met and Her2 synergy," Molecular and Cellular Biology, vol. 27, no. 5, pp. 1934–1946, 2007.

H. Zhao, K. Tanegashima, H. Ro, and I. B. Dawid, "Lrig3 regulates neural crest formation in Xenopus by modulating Fgf and Wnt signaling pathways," Development, vol. 135, no. 7, pp. 1283–1293, 2008.

Y. S. Yim, Y. Kwon, J. Nam et al., "Slitrks control excitatory and inhibitory synapse formation with LAR receptor protein tyrosine phosphatases," Proceedings of the National Academy of Sciences of the United States of America, vol. 110, no. 10, pp. 4057–4062, 2013.

C. H. Coles, E. Y. Jones, and A. R. Aricescu, "Extracellular regulation of type Ila receptor protein tyrosine phosphatases: mechanistic insights from structural analyses," Seminars in Cell & Developmental Biology, vol. 37, pp. 98–107, 2015.

S. Y. Won and H. M. Kim, "Structural basis for LAR-RPTP-mediated synaptogenesis," Molecules and Cells, vol. 41, no. 7, pp. 622–630, 2018.

A. Yamaqata, Y. Sato, S. Goto-Ito et al., "Structure of Slitrk2-PTPδ complex reveals mechanisms for splicing-dependent trans-synaptic adhesion," Scientific Reports, vol. 5, no. 1, p. 9686, 2015.

M. Song, J. Giza, C. C. Proenca et al., "Slitrk5 mediates BDNF-dependent TrkB receptor trafficking and signaling," Developmental Cell, vol. 33, no. 6, pp. 690–702, 2015.

C. S. Wang, E. T. Kavalali, and L. M. Monteggia, "BDNF signaling in context: from synaptic regulation to psychiatric disorders," Cell, vol. 185, no. 1, pp. 62–76, 2022.

C. C. Proenca, K. P. Gao, S. V. Shmelkov, S. Rafii, and F. S. Lee, "Slitrks as emerging candidate genes involved in neuropsychiatric disorders," Trends in Neurosciences, vol. 34, no. 3, pp. 143–153, 2011.

F. S. Lee, A. H. Kim, G. Khursigara, and M. V. Chao, "The uniqueness of being a neurotrophin receptor," Current Opinion in Neurobiology, vol. 11, no. 3, pp. 281–286, 2001.

A. Obermeier, R. Lammers, K. H. Wiesmüller, G. Jung, J. Schlessinger, and A. Ullrich, "Identification of Trk binding sites for SHC and phosphatidylinositol 3'-kinase and characterisation and cDNA cloning of the human receptor," Nature, vol. 347, no. 6293, pp. 107–111, 1990.

Z. Songyang, B. Margolis, M. Chaudhuri, S. E. Shoelson, and L. C. Cantley, "The phosphorytrosine interaction domain of SHC recognizes tyrosine-phosphorylated NPXY motif," Cell, vol. 70, no. 3, pp. 605–614, 1992.
Journal of Biological Chemistry, vol. 270, no. 25, pp. 14863–14866, 1995.

[39] W. J. Chen, J. L. Goldstein, and M. S. Brown, “NPXY, a sequence often found in cytoplasmic tails, is required for coated pit-mediated internalization of the low density lipoprotein receptor,” The Journal of Biological Chemistry, vol. 265, no. 6, pp. 3116–3123, 1990.

[40] R. M. Stephens, D. M. Loeb, T. D. Copeland, T. Pawson, L. A. Greene, and D. R. Kaplan, “Trk receptors use redundant signal transduction pathways involving SHC and PLC-gamma 1 to mediate NGF responses,” Neuron, vol. 12, no. 3, pp. 691–705, 1994.

[41] E. J. Huang and L. F. Reichardt, “Trk receptors: roles in neuronal signal transduction,” Annual Review of Biochemistry, vol. 72, no. 1, pp. 609–642, 2003.

[42] L. F. Reichardt, “Neurotrophin-regulated signalling pathways,” Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, vol. 361, no. 1473, pp. 1545–1564, 2006.

[43] R. Amanchy, D. E. Kalume, A. Iwahori, J. Zhong, and A. Pandey, “Phosphoproteome analysis of HeLa cells using stable isotope labeling with amino acids in cell culture (SILAC),” Journal of Proteome Research, vol. 4, no. 5, pp. 1661–1671, 2005.

[44] C. Salesse, J. Charest, H. Doucet-Beaupré et al., “Opposite control of excitatory and inhibitory synapse formation by Slitrk2 and Slitrk5 on dopamine neurons modulates hyperactivity behavior,” Cell Reports, vol. 30, no. 7, pp. 2374–2386.e5, 2020.

[45] M. W. Linfoth, J. Laurén, R. M. Cassidy et al., “An unbiased expression screen for synaptogenic proteins identifies the LRRTM protein family as synaptic activators,” Neuron, vol. 61, no. 5, pp. 734–749, 2009.

[46] H. Takahashi, K. Katayama, K. Sohya et al., “Selective control of inhibitory synapse development by Slitrk3-PTPδ trans-synaptic interaction,” Nature Neuroscience, vol. 15, no. 3, pp. 389–398, 2012.

[47] J. Ko and E. Kim, “Leucine-rich repeat proteins of synapses,” Journal of Neuroscience Research, vol. 85, no. 13, pp. 2824–2832, 2007.

[48] L. Menzies, S. R. Chamberlain, A. R. Laird, S. M. Thelen, B. J. Sahakian, and E. T. Bullmore, “Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: the orbitofronto-striatal model revisited,” Neuroscience and Biobehavioral Reviews, vol. 32, no. 3, pp. 525–549, 2008.

[49] S. P. Whiteside, J. D. Port, and J. S. Abramowitz, “A meta-analysis of functional neuroimaging in obsessive-compulsive disorder,” Psychiatry Research, vol. 132, no. 1, pp. 69–79, 2004.

[50] H. Liu and Y. Bi, “The expression level of SliTrk5 and its clinical significance in glioma,” Modern Oncology, vol. 26, no. 11, pp. 1688–1691, 2018.

[51] L. B. Hesson, B. Ng, P. Zarzour et al., “Integrated genetic, epigenetic, and transcriptional profiling identifies molecular pathways in the development of laterally spreading tumors,” Molecular Cancer Research, vol. 14, no. 12, pp. 1217–1228, 2016.

[52] K. J. Heilliger, J. Hess, D. Vitaglione et al., “Novel candidate genes of thyroid tumourigenesis identified in Trk-T1 transgenic mice,” Endocrine-Related Cancer, vol. 19, no. 3, pp. 409–421, 2012.
in the treatment of obsessive-compulsive disorder,” The American Journal of Psychiatry, vol. 156, no. 9, pp. 1409–1416, 1999.

[69] E. Castrén, “Neurotrophins as mediators of drug effects on mood, addiction, and neuroprotection,” Molecular Neurobiology, vol. 29, no. 3, pp. 289–302, 2004.

[70] A. K. Mah, “SLITRK5, a protein that links striatal deficits to OCD-like behaviours in mice,” Clinical Genetics, vol. 78, no. 4, pp. 350–352, 2010.

[71] P. R. Szeszko, S. MacMillan, M. McMeniman et al., “Brain structural abnormalities in psychotropic drug-naïve pediatric patients with obsessive-compulsive disorder,” The American Journal of Psychiatry, vol. 161, no. 6, pp. 1049–1056, 2004.

[72] D. Robinson, H. Wu, R. A. Munne et al., “Reduced caudate nucleus volume in obsessive-compulsive disorder,” Archives of General Psychiatry, vol. 52, no. 5, pp. 393–398, 1995.

[73] D. R. Rosenberg, M. S. Keshavan, K. M. O’Hearn et al., “Frontostriatal measurement in treatment-naïve children with obsessive-compulsive disorder,” Archives of General Psychiatry, vol. 54, no. 9, pp. 824–830, 1997.

[74] C. A. McClung, P. G. Ulery, L. I. Perrotti, V. Zachariou, O. Berton, and E. J. Nestler, “DeltaFosB: a molecular switch for long-term adaptation in the brain,” Brain Research. Molecular Brain Research, vol. 132, no. 2, pp. 146–154, 2004.

[75] S. Saxena and S. L. Rauch, “Functional neuroimaging and the neuroanatomy of obsessive-compulsive disorder,” The Psychiatric Clinics of North America, vol. 23, no. 3, pp. 563–586, 2000.

[76] K. Wu, G. L. Hanna, P. Easter, J. L. Kennedy, D. R. Rosenberg, and P. D. Arnold, “Glutamate system genes and brain volume alterations in pediatric obsessive-compulsive disorder: a preliminary study,” Psychiatry Research, vol. 211, no. 3, pp. 214–220, 2013.

[77] J. Calzà, D. A. Gürsel, B. Schmitz-Koep et al., “Altered Cortico–Striatal Functional Connectivity During Resting State in Obsessive–Compulsive Disorder,” Frontiers in Psychiatry, vol. 10, p. 319, 2019.

[78] B. J. Harrison, C. Soriano-Mas, J. Pujol et al., “Altered corticostriatal functional connectivity in obsessive-compulsive disorder,” Archives of General Psychiatry, vol. 66, no. 11, pp. 1189–1200, 2009.

[79] X. W. Yang and X. H. Lu, “Molecular and cellular basis of obsessive-compulsive disorder-like behaviors: emerging view from mouse models,” Current Opinion in Neurology, vol. 24, no. 2, pp. 114–118, 2011.

[80] K. Morita, M. Itoh, N. Nishibori, S. Her, and M. S. Lee, “Spirulina non-protein components induce BDNF gene transcription via HO-1 activity in C6 glioma cells,” Applied Biochemistry and Biotechnology, vol. 175, no. 2, pp. 892–901, 2015.

[81] T. Sugimoto, H. Kuroda, Y. Horii, H. Moritake, T. Tanaka, and S. Hattori, “Signal transduction pathways through TRK-A and TRK-B receptors in human neuroblastoma cells,” Japanese Journal of Cancer Research, vol. 92, no. 2, pp. 152–160, 2001.

[82] R. D. Almeida, B. J. Manadas, C. V. Melo et al., “Neuroprotection by BDNF against glutamate-induced apoptotic cell death is mediated by ERK and PI3-kinase pathways,” Cell Death and Differentiation, vol. 12, no. 10, pp. 1329–1343, 2005.

[83] K. X. Li, A. M. Li, and J. H. Zhang, “Effects of TrkB-BDNF signal pathway on synthesis and secretion of vascular endothelial growth factor in human neuroblastoma cells,” Zhongguoang Dai En Ke Za Zhi, vol. 13, no. 3, pp. 240–243, 2011.

[84] Q. He, S. Wang, X. Liu et al., “Salvianolate lyophilized injection promotes post-stroke functional recovery via the activation of VEGF and BDNF-TrkB-CREB signaling pathway,” International Journal of Clinical and Experimental Medicine, vol. 8, no. 1, pp. 108–122, 2015.

[85] D. L. Pauls, A. Abramovitch, S. L. Rauch, and D. A. Geller, “Obsessive-compulsive disorder: an integrative genetic and neurobiological perspective,” Nature Reviews. Neuroscience, vol. 15, no. 6, pp. 410–424, 2014.

[86] A. Gmitrowicz and A. Kucharska, “Developmental disorders in the fourth edition of the American classification: diagnostic and statistical manual of mental disorders (DSM IV – optional book),” Psychiatria Polska, vol. 28, no. 5, pp. 509–521, 1994.

[87] S. S. Moy, N. V. Riddick, V. D. Nikolova et al., “Repetitive behavior profile and supersensitivity to amphetamine in the C58/J mouse model of autism,” Behavioural Brain Research, vol. 259, pp. 200–214, 2014.

[88] M. Jodeir, Farshbaf and K. Ghaedi, “Huntington’s disease and mitochondria,” Neurotoxicity Research, vol. 32, no. 3, pp. 518–529, 2017.

[89] D. K. Simon, C. M. Tanner, and P. Brundin, “Parkinson disease epidemiology, pathology, genetics, and pathophysiology,” Clinics in Geriatric Medicine, vol. 36, no. 1, pp. 1–12, 2020.

[90] H. Doucet-Beaupré, S. L. Ang, and M. Lévesque, “Cell fate determination, neuronal maintenance and disease state: the emerging role of transcription factors Lmx1a and Lmx1b,” FEBS Letters, vol. 589, no. 24PartA, pp. 3727–3738, 2015.

[91] H. Doucet-Beaupré, C. Gilbert, M. S. Profes et al., “Lmx1a and Lmx1b regulate mitochondrial functions and survival of adult midbrain dopaminergic neurons,” Proceedings of the National Academy of Sciences of the United States of America, vol. 113, no. 30, pp. E4387–E4396, 2016.

[92] A. Laguna, N. Schintu, A. Nobre et al., “Dopaminergic control of autophagic-lysosomal function implicates Lmx1b in Parkinson’s disease,” Nature Neuroscience, vol. 18, no. 6, pp. 826–835, 2015.