Leucine-rich repeats containing 4 protein (LRRC4) in memory, psychoneurosis, and glioblastoma

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Abstract
Leucine-rich repeats containing 4 (*LRRC4*, also named netrin-G ligand 2 [NGL-2]) is a member of the NetrinGs ligands (NGLs) family. As a gene with relatively high and specific expression in brain, it is a member of the leucine-rich repeat superfamily and has been proven to be a suppressor gene for gliomas, thus being involved in gliomagenesis. LRRC4 is the core of microRNA-dependent multi-phase regulatory loops that inhibit the proliferation and invasion of glioblastoma (GB) cells, including LRRC4/NGL2-activator protein 2 (AP2)-microRNA (miR) 182-LRRC4 and LRRC4-miR185-DNA methyltransferase 1 (DNMT1)-LRRC4/specific protein 1 (SP1)-DNMT1-LRRC4. In this review, we demonstrated LRRC4 as a new member of the partitioning-defective protein (PAR) polarity complex that promotes axon differentiation, mediates the formation and plasticity of synapses, and assists information input to the hippocampus and storage of memory. As an important synapse regulator, aberrant expression of *LRRC4* has been detected in autism, spinal injury and GBs. *LRRC4* is a candidate susceptibility gene for autism and a neuro-protective factor in spinal nerve damage. In GBs, LRRC4 is a novel inhibitor of autophagy, and an inhibitor of protein–protein interactions involving in temozolomide resistance, tumor immune microenvironment, and formation of circular RNA.
Keywords: Netrin G; Excitatory synapses; Long-term potentiation; Autism; Multiple sclerosis; Glioblastoma

Introduction

In 2002, leucine-rich repeats containing 4 (*LRRC4*) was first reported as a gene with relatively high and specific expression in the brain, which is located on human chromosome 7q31–32.[1] In 2003, LRRC4 was found to resemble the NetrinG1 ligand (NGL1/LRRC4C).[2] In 2006, LRRC4 was recognized as the ligand of NetrinG2 and named NGL2, which directly interacts with the neural adhesion molecule NetrinG2 in an isoform-specific manner.[3] Subsequently, the other protein with similar structures to NGL1 and NGL2 was designated as NGL3 (also named LRRC4B).[3] To date, the NGL family is mainly comprised of three members, NGL1 (LRRC4C), NGL2 (LRRC4), and NGL3 (LRRC4B), which are all located in the postsynaptic membrane, they share the same domain structure as a typical type I transmembrane protein, and bind to different neural cell adhesion molecules in the presynaptic membrane to promote the formation and maturation of synapse, such as NetrinG1 binding to NGL1 (LRRC4C), NetrinG2 binding to NGL2 (LRRC4), and the leukocyte common antigen-related protein (LAR) binding to NGL3 (LRRC4B).[3,4] Interestingly, the interaction between NetrinG1 or NetrinG2 located in axons and NGL1 or NGL2 located in dendrites not only promotes the formation of axon-dendritic synaptic structure, but also medicates the localization of NGL1 or NGL2 in specific segments of dendrites via the NetrinG-dependent way. For example, NGL1 is concentrated in dendritic segments corresponding to the termination of netrinG1-positive axons, and NGL2 is concentrated in distinct dendritic segments corresponding to the termination of netrinG2-positive axons. However, NGL1 or NGL2 demonstrated a diffuse dendritic distribution in mice with NetrinG1 or NetrinG2 deletion.[5]

Previous studies have reported *LRRC4* as a suppressor gene of glioma. *LRRC4* expression is negatively correlated with the degree of glioma malignancy. *LRRC4* inhibits the phosphatidylinositol 3-kinase/protein kinase B (AKT)/nuclear transcription factor-κB (NF-κB), K-Ras/c-Raf/extracellular signal-regulated kinase (ERK)/mitogen activated protein kinase (MAPK), c-Jun N-terminal kinase 2

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(JNK2)/c-Jun/mutant P53 (mP53), 70 kDa ribosomal protein S6 kinase (p70S6K)/protein kinase C (PKC), signal transducer and activator of transcription 3 (STAT3), and stromal cell-derived factor 1α (SDF-1α)/C-X-C chemokine receptor type 4 (CXCR4) signaling pathways to prevent glioma cells proliferation and invasion, in which LRRC4 is the core of microRNA (miRNA)-dependent multi-phase regulatory loops, including LRRC4/NGL2-activator protein 2 (AP-2)-microRNA (miR)-182-LRRC4 and LRRC4-miR-185-DNA methyltransferase 1 (DNMT1)-LRRC4/specific protein 1 (SP1)-DNMT1-LRRC4.[6]

In this review, we mainly discuss the effects of *LRRC4* on memory by promoting the formation of excitatory synapses and long-term potentiation (LTP), the effects of *LRRC4* mutation on psychoneurosis, and the new mechanism of *LRRC4* in GB genesis and progression.

LRRC4 and Memory

Brain regions related to learning and memory mainly include the temporal lobe, prefrontal lobe, diencephalon, amygdala, cerebellum, Meynert basal ganglia, and striatal-limbic regions. The hippocampus is now widely considered the memory center of the brain, which mediates learning and memory performance. As early as 1957, the hippocampus was noted to play an important role in memory formation. The study has reported a case of a patient with epilepsy who had anterograde amnesia and retrograde amnesia after undergoing resection of most of the hippocampus, lost a significant amount of old memories, and was unable to form new memories.[7] Another study has reported that LRRC4 is involved in the hippocampal memory function and the molecular mechanism for memory formation and storage.[8] The hippocampus receives information from the cerebral cortex to complete information processing and memory formation. Memory is then stored in the hippocampus through synaptic plasticity. LRRC4/NGL-2 is highly expressed in the cerebellum, cerebral cortex, occipital pole, frontal lobe, temporal lobe and putamen. At present, relevant research on how LRRC4 mediates memory is also focused on the hippocampus. However, the role of LRRC4 in mediating learning and memory in other brain regions has not yet been reported. In this review, we discussed the correlation between LRRC4 and memory function in the hippocampus. A growing body of evidence supports that the deletion of *LRRC4* can impact synaptic plasticity and the information input pathway in the hippocampus.[8,9]

LRRC4 promotes the formation of excitatory synapses

The synapse is an important node of the neuron connection network, which consists of presynaptic membrane, postsynaptic membrane, and synaptic cleft. The presynaptic membrane is mainly formed by axons, and can also be formed by dendrites, such as the dendrodendritic synapses in the olfactory bulb and thalamus. The postsynaptic membrane is generally formed on dendritic spines, dendritic shafts and neuronal soma.[10] Axon can also be assembled as postsynaptic membranes to form axo-axonic synapses. The axo-axonic synapses play an important role in presynaptic inhibition.[11] Most presynaptic neurons release either excitatory neurotransmitters (e.g., glutamate) or inhibitory neurotransmitters (e.g., gamma-aminobutyric acid [GABA]), which functionally subdivide synapses into excitatory and inhibitory synapses. A type of dual glutamatergic-GABAergic neuron has been detected in the lateral habenular area of the mouse brain, which co-releases glutamate and GABA from distinct synaptic vesicles at an independent synapse.[12,13] Generally, when axon is polarized and differentiated into presynaptic structure, synaptic cell adhesion molecules serve as a "glue" to connect the axon with the dendrite or soma of another neuron to complete the assembly of the synaptic structure. Presynaptic and postsynaptic proteins are recruited via the interaction of synaptic cell adhesion molecules, thereby promoting the functional maturation of the synaptic structure.

LRRC4, a typical synaptic cell adhesion molecule, mediates the polarization of axons, synaptic formation, and stabilization of the synaptic structure, especially in the axon-dendrite synapse [Figure 1]. Hippocampal neurons from embryonic day (E) 16 rats cannot form synapses under *in vitro* culture conditions, whereas E18 hippocampal neurons form complete synapses under the same conditions.[14] Interestingly, *LRRC4* is not expressed before E12 during mouse brain embryonic development, and its expression gradually increases after E16, which is consistent with the time when mouse embryonic hippocampal neurons can form synapses.[15] LRRC4 plays a significant role in the formation of excitatory synapses. The deletion of *LRRC4* causes a structural deficit in the first synapse of the spiral ganglion of the auditory nerve in *LRRC4* knockout mice.[16] Then, LRRC4 is enriched in axons at the end of the rat hippocampal neurons, which can mediate axon differentiation.[17] Axon differentiation is not only a milestone in the process of neuronal polarization, but also an important stage to form synapses between neurons.[18] LRRC4 is a new member of the partitioning defective (PAR) polarity complex that is involved in axon differentiation. LRRC4 binds directly to the PDZ domain of PAR6 through the PDZ-binding domain and forms LRRC4-PAR6-PAR3-atypical protein kinase C (aPKC) polar complexes. The polar complexes activate the microtubule affinity regulating kinase 2 (MARK2) signaling pathway, thereby regulating tubulin stability and promoting the axonal differentiation of hippocampal neurons.[17] In the outer plexiform layer of the retina, LRRC4 is selectively located at the tips of horizontal cell axons. The absence of *LRRC4* inhibits the formation of synapses between the axons of horizontal cells and rod photoreceptors, leading to specific developmental disorders of the outer retinal pathways.[19] Furthermore, LRRC4 can mediate the growth of axons in the retinal horizontal cells. The removal of *LRRC4* from horizontal cells in developing or mature retinal neural circuits, results in abnormal axon growth and synaptic reduction.[20] When *LRRC4* is re-expressed in knockout mice, the horizontal cell axon and synapse numbers are restored.[20] These findings demonstrate that LRRC4 mediates the polarization of axons. Furthermore, the postsynaptic scaffold postsynaptic density 95 (PSD95) is a prominent binding partner of the NGL family members, which links NetrinGs–NGLs cross-synaptic

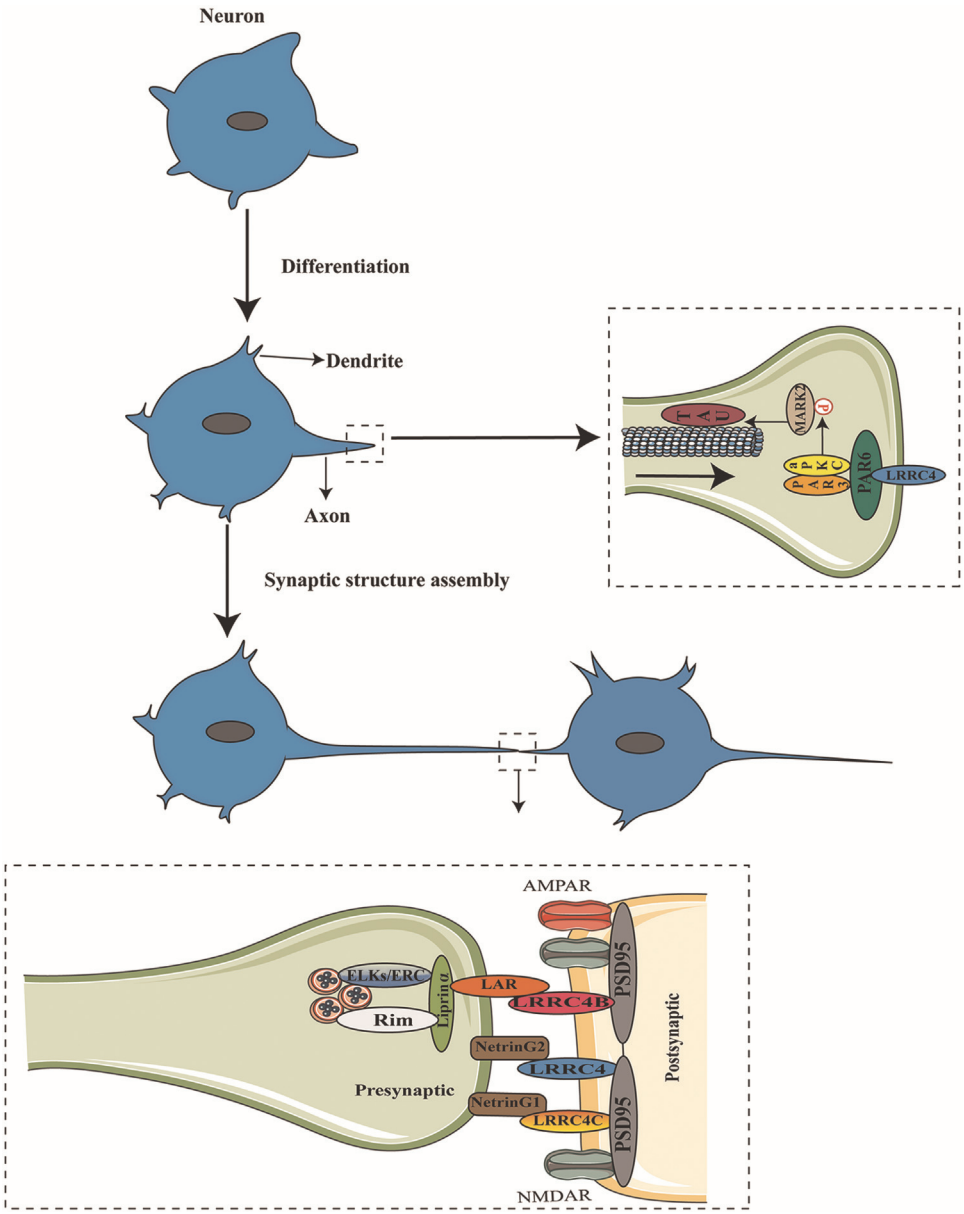

Figure 1: LRRC4 mediates axonal development and synaptic formation. LRRC4 is a new member of the PAR polarity complex, which promotes axon differentiation. The interaction between NetrinGs and NGLs promotes the formation of synaptic structure. The postsynaptic scaffold PSD95 protein completes the recruitment of synapse-associated proteins (such as NMDAR and AMPAR). AMPAR: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; aPKC: Atypical protein kinase C; ELKs/ERC: Glutamine/leucine/lysine/serine-rich protein; LAR: Leukocyte common antigen-related protein; LRRC4: Leucine-rich repeats containing 4; MARK2: Microtubule affinity regulating kinase 2; NGL: NetrinGs ligand; NMDAR: *N*-methyl-D-aspartate receptor; P: Phosphorylation; PAR: Partitioning-defective protein; PSD95: Postsynaptic density 95; Rim: Rab3 interacting molecule.

adhesion events with postsynaptic protein recruitment events. PSD95 has a total of three PDZ domains. The NGL family members located in the postsynaptic membrane bind to the first two PDZ domains of PSD95 through the PDZ-binding domain of the C-terminus. Meanwhile, the last PDZ domain of PSD95 binds to postsynaptic-related proteins (such as N-methyl-D-aspartate receptor [NMDAR] and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor [AMPAR]), thereby recruiting them to the postsynaptic membrane. It is worth noting that NetrinG1 and NetrinG2 are glycosylphosphatidylinositol-anchored proteins lacking cytoplasmic regions, and LAR is a transmembrane protein on the postsynaptic membrane.[21] The interaction between NGL3 and LAR can lead to accumulation of the related presynaptic protein liprin α, which then causes liprin α to link with synaptic vesicles through the glutamine/leucine/lysine/serine-rich protein (ELKs) and Rab3 interacting molecule (Rim), regulating the release of synaptic vesicles.[4] However, information on the mechanisms of NetrinG1 and NetrinG2 regulating presynaptic protein assembly remains scarce. The NetrinG2–NGL2 interaction has been suggested to regulate glutamate release from presynaptic neurons through NMDAR-dependent LTP. In brief, LRRC4 can promote presynaptic and postsynaptic connection and can promote the accumulation of postsynaptic protein NMDAR, resulting in the formation of excitatory synapses.

LRRC4 mediates information input to hippocampus

The hippocampus is mainly divided into three areas: CA1, CA3, and the dentate gyrus. The CA1 area receives various sensory and spatial information from the entorhinal cortex to complete information processing and memory storage through direct and indirect channels. The entorhinal cortex is directly connected to distant dendrites of the stratum lacunosum-moleculare (SLM) in the CA1 area through the temporoammonic axon, which is termed the direct channel. The entorhinal cortex is connected to the dentate gyrus through the perforant pathway. The dentate gyrus is projected to the CA3 through the mossy fiber, and the CA3 is further projected to the proximal dendrites of stratum radiatum (SR) in the CA1 area through the Schaffer collateral axons, which is termed the indirect channel. Destroying the direct channel does not affect the formation of the initial memory, but affects the conversion of short-term memory into long-term memory. The lesion of the indirect channel affects the animal's spatial learning and memory tasks. Studies have reported that temporal ammonia axons mainly express NetrinG1, and Schaffer collateral axons mainly express NetrinG2. LRRC4C (NGL1) is restricted to SLM in the CA1 area and LRRC4 is restricted to SR in the CA1 area.[5,22] The deletion of *LRRC4* significantly reduces the frequency of miniature excitatory postsynaptic currents and synaptic density in the SR region, rather than the SLM region. Thus, LRRC4 regulates only the formation of Schaffer collateral synapses in the SR, thereby mediating the indirect channel of CA1 information input.[8] Thus, LRRC4 regulates the indirect channel of information input to the hippocampus by mediating the formation of Schaffer collateral synapses and affects the formation and storage of memory [Figure 2].

LRRC4 enables LTP of hippocampal CA1 region

Synaptic plasticity is defined as the change in the strength of synaptic connections between neurons, and it has been widely considered an important method of memory storage.[23] LTP and long-term depression are main synaptic plastic processes. The mechanism of LTP production is closely related to the synaptic basis of memory storage.[24] LTP is divided into early-phase LTP and late-phase LTP according to the stimulus intensity. Through a short period of tonic stimulation, the presynaptic membrane in the CA1 area is activated to release glutamate, and some NMDARs on the postsynaptic membrane receive glutamate and are activated. The activated NMDAR causes the influx of $Ca2^{+}$, and the increased intracellular $Ca2^{+}$ combines with calmodulin, which further activates calcium/calmodulin dependent protein kinase II (CaMKII). CaMKII phosphorylates AMPAR and increases their sensitivity to glutamate,[25,26] while promoting the synthesis of nitric oxides (NO, a retrograde messenger), NO diffuses to presynaptic membrane to promote the release of glutamate, thereby enhancing the efficiency of synaptic transmission. However, due to insufficient stimulation intensity, such LTP can only last for 1–3 h, so it is called early phase LTP. With an increase in stimulation intensity, based on early LTP, the continuous Ca^{2+}/calmodulin signal enters the dendrite from the dendritic spine, and activates adenylyl cyclase (AC). And then, AC promotes the production of the cyclic adenosine monophosphate (cAMP), which further phosphorylates protein kinase A (PKA). The phosphorylated PKA further activates MAPK and promotes it to enter the nucleus to phosphorylate cAMP response element binding protein (CREB1), then initiates transcription program, leading to the formation of new synaptic structures.[27] Additionally, the continuous Ca^{2+}/calmodulin signal activates protein kinase Mζ, which promotes the formation of new AMPAR. This process is called late-phase LTP, and it further improves the efficiency of synaptic transmission and can be maintained for a longer period.[28,29] Overall, the Ca^{2+} influx into postsynaptic neurons mediated by NMDAR activation is considered a sufficient and necessary condition, thus NMDAR is considered to be a trigger for the induction of LTP. The NMDAR consists of seven subunits as follows: GluN1, GluN2A/2B/2C/2D, and GluN3A/3B.[30] Notably, the production of LTP requires the activation of the GluN1, GluN2A, and GluN2B subunits, especially GluN2A and GluN2B.[28]

LRRC4 is associated with LTP [Figure 2].[9] LRRC4 can bind to the GluN1, GluN2A, and GluN2B subunits. When GluN1, GluN2A or GluN2B are heterologously expressed in neurons alone, all of them can bind to LRRC4. LRRC4 then recruits NMDAR to the postsynaptic membrane via PSD95, thus forming the LRRC4/NMDAR/PSD95 complex. GluN2A and GluN2B contain PDZ-binding domains, resulting in their binding to LRRC4 through the PDZ domain of PSD95. GluN1 lacks a PDZ-binding domain, and is likely associated with LRRC4 through PSD95-independent mechanisms, but the specific mechanism is unclear. LRRC4 knockout inhibits LTP in the hippocampal CA1 region; however, this inhibitory effect can be neutralized by NMDAR drug activation.[9]

LRRC4 and Psychoneurosis

Abnormalities in synaptic structures and functions are the pathological characteristics of many central nervous system (CNS) diseases.[31] LRRC4 is closely related to the formation of synaptic structures and maintenance of synaptic function. Therefore, the aberrance of *LRRC4* is thought to be associated to the occurrence of some central nervous system diseases, such as autism and spinal cord injury.

LRRC4 is a candidate susceptibility gene for autism

Autism spectrum disorder (ASD) is a neuropsychiatric disorder affecting 1% of the world's population and is characterized by repetitive behaviors and impairments in social affiliative behaviors.[32] An epidemiological investigation has demonstrated that patients with ASD have several genetic characteristics.[33] Genomic analysis has revealed that autism-related genes were mainly enriched in the following three common biological pathways: chromatin remodeling, synaptic cell adhesion, and neuronal development.[32,34] In a genetic analysis of 4504 patients, 23 autism syndrome candidate genes have been identified, including *LRRC4*, neurexins, neuroligins, *synCAM1*, *CHL1*, *ZWILCH*, *MSL2*, and *CAPRIN*.[35,36]

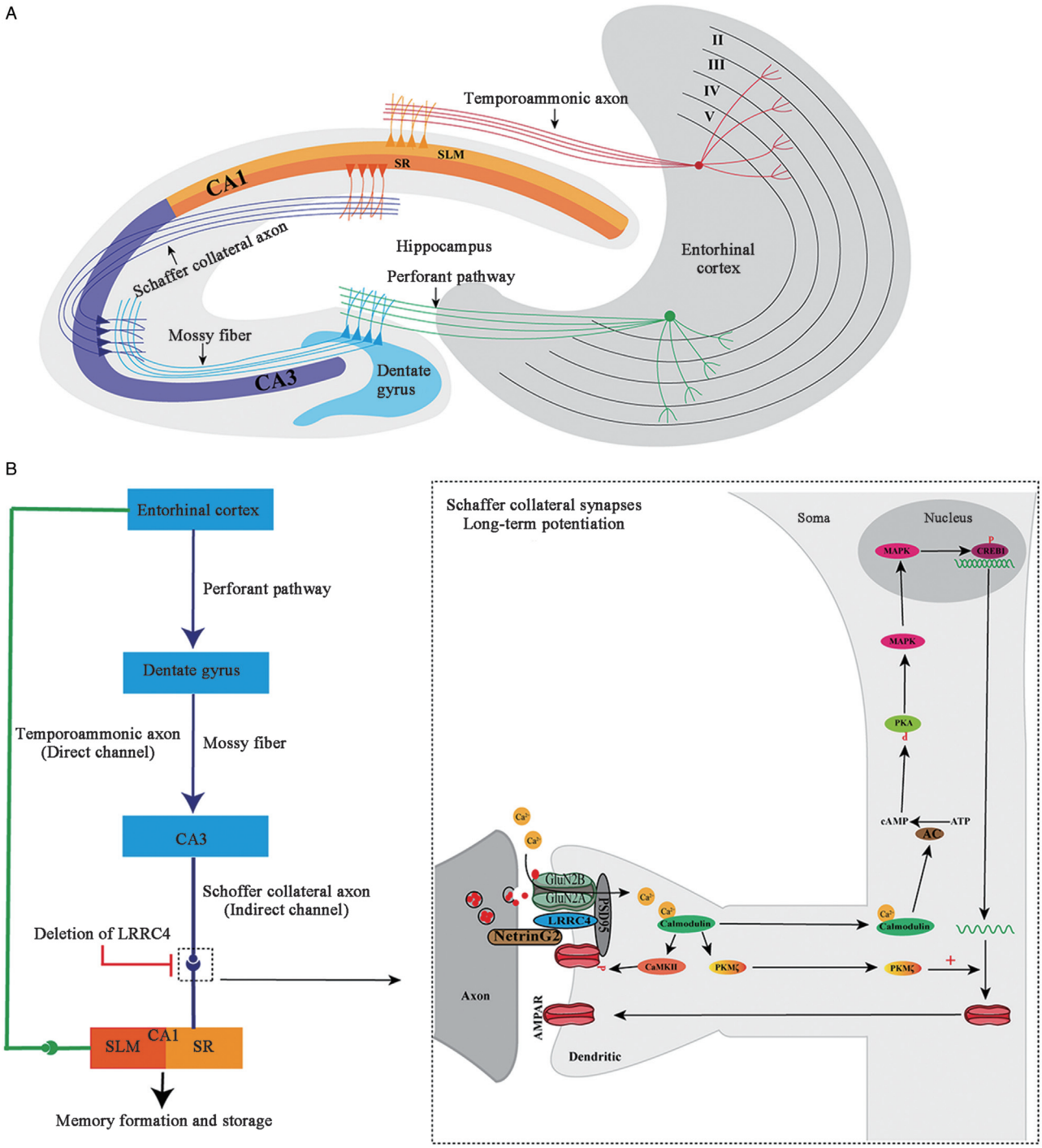

Figure 2: LRRC4 mediates information input pathway and synaptic plasticity in the hippocampal CA1 region. (A) Information input pathway pattern of the hippocampal CA1 region. (B) The deletion of LRRC4 impairs the formation of indirect channel by mediating the formation of Schaffer collateral synapses. LRRC4 mediates NMDAR-dependent synaptic plasticity (LTP) by binding to the subunits of NMDAR (GluN1, GluN2A, and GluN2B). AC: Adenylyl cyclase; AMPAR: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ATP: Adenosine triphosphate; cAMP: Cyclic adenosine monophosphate; CaMKII: Calcium/calmodulin dependent protein kinase kinase II; CREB1; cAMP response element binding protein; LRRC4: Leucine-rich repeats containing 4; LTP: Long-term potentiation; MAPK: Mitogen-activated protein kinase; NMDAR: *N*-methyl-D-aspartate receptor; P: Phosphorylation; PKA: Protein kinase A; PKMζ: Protein kinase Mζ; SLM: Stratum lacunosum-moleculare; SR: Stratum radiatum.

The *LRRC4* gene was detected as a missense mutation of C238G by whole genome sequencing in a patient with ASD.[37] Coincidentally, the deletion mutation of *LRRC4* was also detected in a 4-year-old boy with autistic characteristics.[38] Um*et al*[9] investigated the effect of *LRRC4* deletion ($LRRC4^{-/-}$) on the zoologica behavior of mice and concluded that the $LRRC4^{-/-}$ mice exhibit typical autistic characteristics, such as defects in social interaction and repetitive behaviors. Both *LRRC4* re-expression and NMDAR activation normalize social interaction and self-grooming in $LRRC4^{-/-}$ mice. Further research has revealed that the deletion of *LRRC4* impairs

excitatory transmission by mediating NMDAR dysfunction, which leads to ASD.

LRRC4 is a spinal cord neuron protective factor

Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination the central nervous system. Its clinical symptoms include muscle weakness, abnormal gait, fatigue, vision problems, language barriers, and ataxia, which may eventually lead to paralysis.[39] The known pathogenesis of MS is mainly mediated by T cells. Peripheral T cells, especially $CD4^+$ T cells, stimulated by antigens infiltrate the CNS to activate microglia and macrophages and induce the death of oligodendrocytes and the loss of myelin sheath around nerve fibers, coupled with nerve damage.

Experimental autoimmune encephalomyelitis (EAE) is an internationally recognized animal model for studying MS, and is mainly divided into three stages as follows: induction period, symptom period, and recovery period. The time for mice to experience each period is uncertain and depends on the inducer and individual differences. The mice in the induction period had no evident symptoms, whereas the mice in the symptom period began to exhibit symptoms of limb weakness or paralysis. After the effect period, the mice entered the recovery period and gradually returned to their normal state.

The mice were intra-peritoneally injected with myelin oligodendrocyte glycoprotein to establish an animal model of EAE. The expression of *LRRC4* in spinal cord tissue was significantly downregulated during the symptom period in the EAE mice (the 15th day after injection of myelin oligodendrocyte glycoprotein). The deletion of *LRRC4* exacerbated disease progression and promoted infiltration of leukocytes into the spinal cord, resulting in neuro demyelination in the EAE model constructed using *LRRC4* knockout mice. Meanwhile, the ectopic expression of *LRRC4* alleviated the clinical symptoms of EAE mice and protected the neuron from immune damage, suggesting that *LRRC4* plays a protective role in the pathogenesis of EAE and protects the neuron from immune damage.[40]

Neurorehabilitation, especially appropriate locomotor training, has long been known to contribute to the recovery of motor function after spinal cord injury. However, the clear mechanisms remain poorly understood. To reveal the specific mechanism, rats subjected to spinal cord hemisection injury were placed on a level treadmill, and the treadmill speed began at 9 cm/s and was gradually increased to 21 cm/s. The rats were trained 7 days/week for 35 min/session for 18 days starting at 4 days post-operatively. Compared with untrained rats, 36 synapse-related genes were upregulated in trained rats, with *LRRC4* being the most highly expressed.[41] Further research revealed that training increases the number of *LRRC4*-positive synaptic puncta in the L1 spinal cord after hemisection. Locomotor training promotes the reconstruction of the neuronal network by enhancing the expression of *LRRC4* in injured spinal cord neurons.[41,42]

LRRC4 and GB

GB is the most common malignant brain tumor in the CNS. Despite maximal surgical resection, diffuse invasion of tumor cells into the surrounding brain tissue is responsible for treatment failure or relapse, and the poor prognosis of patients has not improved significantly in the past decade.[43]*LRRC4* has been identified as a tumor suppressor gene in gliomas. *LRRC4* is highly specific in grade I gliomas, and it is reduced in grade II–III gliomas and absent in GB (grade IV gliomas). *LRRC4* expression is significantly lower in recurrent tumors than in primary gliomas. The expression of *LRRC4* is closely related to the malignant degree of gliomas, and the loss of *LRRC4* expression may directly increase the malignant degree and promote the recurrence of gliomas. Thus, the use of *LRRC4* as a marker of degree of malignancy and prognosis in gliomas has been suggested. Hyper-methylation of the promoter region is a frequent event of *LRRC4* low expression in gliomas, and *LRRC4* was also inhibited as a direct target gene of miRNA-182 and miRNA-381,[6] as well as an indirect target gene suppressed by miRNA-101 by reducing the enrichment of *LRRC4* core promoter H3K2me3 by targeting enhancer of zeste homolog 2 (EZH2), embryonic ectoderm development, and DNMT3a [Figure 3].[44]

LRRC4 is a novel inhibitor of autophagy for GB

Autophagy is an evolutionarily conserved catabolic process that involves sequestration and transport of damaged organelles and misfolded and dysfunctional proteins to lysosomes for degradation.[45] Normal autophagy plays an important physiological role in human health, while abnormal autophagy leads to the development of various diseases. Appropriate autophagy acts as a cytoprotective mechanism leading to tumor cell apoptosis resistance and drug resistance;[46] however, excessive autophagy promotes tumor cell death. In *LRRC4* knockdown mice, the expression levels of the autophagy markers Beclin-1 and microtubule associated protein 1 light chain 3B (LC3B) in the brain tissue and spinal cord tissue were significantly higher than those in wild-type mice, and the over-expression of *LRRC4* significantly inhibited GB cell autophagy, suggesting *LRRC4* as a negative regulator of autophagy [Figure 3].[47]Many drugs cannot penetrate intracranial lesions because of the presence of the blood–brain barrier.[48] Therefore, temozolomide (TMZ) is the first-line drug for patients with GB in clinical practice. TMZ resistance is the main reason for poor prognosis in patients with GB.[49] Excitingly, the *LRRC4* can enhance the sensitivity of glioma cells to TMZ by inhibiting autophagy. Combining the re-expression of *LRRC4* and TMZ treatment prolonged the survival of mice with tumor xenografts, indicating the potential of *LRRC4* as a prognostic marker for TMZ sensitivity in patients with GB.[47]

LRRC4 is an inhibitor of protein–protein interactions in GB

LRRC4 is a leucine-rich protein that contains extracellular leucine rich repeat (LRR) domains, immunoglobulin C2 (IgC2) domain, transmembrane domain, and PDZ-binding

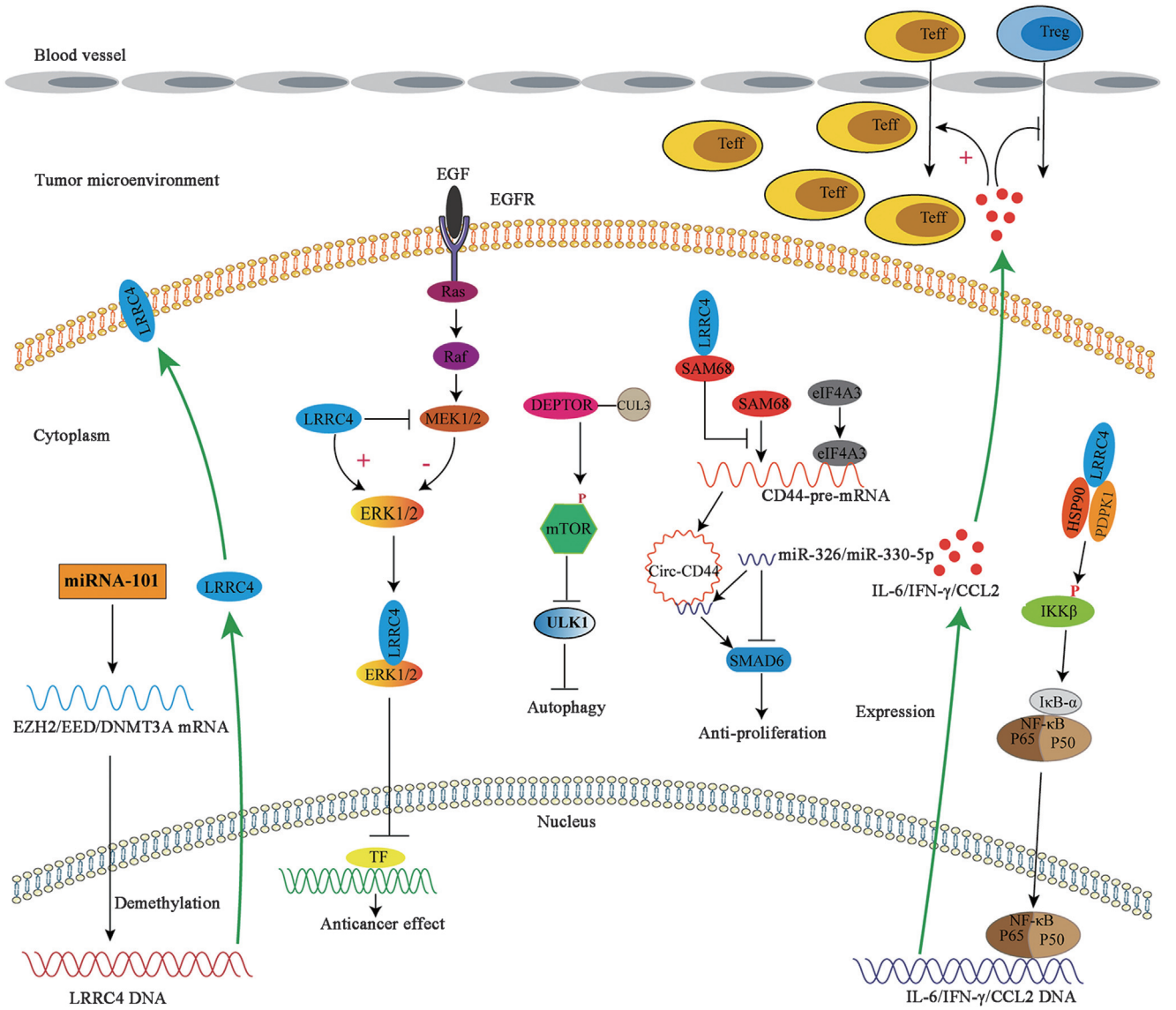

Figure 3: Recent research progress of LRRC4 in glioma. miRNA-101 reverses the hypermethylation modification of the promoter region of LRRC4 by targeting EZH2, EED, and DNMT3a, thereby restoring the expression of LRRC4 in glioma cells. The interaction between LRRC4 and DEPTOR promoted the ubiquitination modification and degradation of DEPTOR, which leads to the phosphorylation of mTOR protein, resulting in inhibiting the activity of downstream protein ULK1, and then inhibiting the occurrence of autophagy. LRRC4 and MEK1/2 competitively bind to ERK1/2, thereby blocking ERK1/2 entry into the nucleus, and inhibiting the transcription of downstream genes. LRRC4 directly interacts with PDPK1 and HSP90 to phosphorylate IKKβSer181, resulting in activating the NF-κB signaling pathway to facilitate cytokine secretion, thereby reprogramming the glioma immune microenvironment. LRRC4 binds to Sam68 to form a complex, which prevents Sam68 from binding to CD44 pre-mRNA, and promotes the binding between eIF4a3 and CD44 pre-mRNA, thereby promoting the formation of circCD44. CCL2: Chemokine (C-C motif) ligand 2; Circ-CD44: CircRNA-CD44; CUL3: Cullin-3; DEPTOR: DEP domain containing mTOR interacting protein; DNMT3A: DNA methyltransferase 3A; EED: Embryonic ectoderm development; EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; eIF4A3: Eukaryotic initiation factor 4A3; ERK: Extracellular-signal-regulated kinases; EZH2: Enhancer of zeste homolog 2; HSP90: Heat shock protein 90; IFN-γ: Interferon-γ; IL-6: Interleukin 6; IκB-α: Inhibitor of NF-κB; IKKβ: Inhibitor of NF-κB kinase subunit beta; LRRC4: Leucine-rich repeats containing 4; MEK1/2: Mitogen-activated protein kinase kinase 1/2; miR: MicroRNA; miRNA: MicroRNA; mRNA: Messenger RNA; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor-κB; P: Phosphorylation; PDPK1: 3-Phosphoinositide dependent protein kinase 1; pre-mRNA: Pre-messenger RNA; SAM68: Signal transduction associated protein 68; SMAD6: Mothers against decapentaplegic homolog 6; Teff: Effector T cell; TF: Transcription factor; Treg: Regulatory T cell; ULK1: Unc-51 like autophagy activating kinase 1.

domain in the cytoplasm. LRRC4 is mainly involved in cell signal transduction through protein–protein interactions. DEP domain containing mammalian target of rapamycin (mTOR) interacting protein (DEPTOR), a pro-autophagy factor and mTOR inhibitor, promotes the phosphorylation of unc-51 like autophagy activating kinase 1 (ULK1) by inhibiting mTOR activity, resulting in autophagy.[50] The PDZ-binding domain of LRRC4 can bind to the C-terminal PDZ domain of DEPTOR, thereby blocking the interaction between DEPTOR and mTOR, activating the activity of mTOR protein, inhibiting GB cell autophagy and increasing the sensitivity to TMZ.[47] The over-expression of *LRRC4* directly interacts with phosphoinositide dependent protein kinase 1(PDPK1) and heat shock protein 90 (HSP90) to phosphorylate IKKβSer181 (inhibitor of NF-κB kinase β), in which the N domain and C domain of HSP90 bind to LRRC4 to stabilize the binding of LRRC4 and PDPK1, activate nuclear factor-κB (NF-κB) signaling pathway to facilitate cytokine secretion, inhibit the infiltration of Treg cells ($CD4^+CD25^+Fxop3^+$ cells), and promote the expansion of Teff cells ($CD4^+CD44^+CD62L^+$ cells).[44]

Additionally, the D domain of LRRC4 competitively binds to the CD domain of ERK1/2 with mitogen-activated protein kinase kinase (MAPKK; MEK1/2) to anchor ERK1/2 in the cytoplasm and inhibits mitogen-activated protein kinase signaling pathway activation.[51] Signal transduction associated protein 68 (Sam68) can bind to the V5 exon of CD44 pre-messenger RNA (pre-mRNA) and mediate the splicing of CD44 pre-mRNA into mature CD44 mRNA.[52] The eukaryotic initiation factor 4A3 (eIF4A3) can combine with pre-mRNA and promote the pre-mRNA cyclization to form circRNA.[53] Both Sam68 and eIF4A3 have binding sites on CD44 pre-mRNA and are close to each other, suggesting that Sam68 and eIF4a3 can competitively bind to CD44 pre-mRNA, whereas LRRC4 can form a complex with Sam68, preventing Sam68 from binding to CD44 pre-mRNA and promoting the combination of eIF4A3 and CD44 pre-mRNA as well as the formation of circRNA-CD44 (circCD44).[54] CircCD44, acts as a competing endogenous RNA, and adsorbs miR-330-5p and miR-326, thereby facilitating the expression of the *SMAD6* gene, and the over-expression of *SMAD6* can inhibit glioma cell proliferation and invasion [Figure 3].

Conclusion and Perspectives

LRRC4 is a key factor for regulating synapse formation, stability and excitatory transmission, and is involved in brain development, memory formation and storage. The absence of LRRC4 not only damages the auditory and optic nerves to a certain extent but also impacts the formation of hippocampal neural circuits and synaptic plasticity, thereby mediating the formation and storage of memory. Deletion or missense mutation of *LRRC4* has been detected in genetic testing of patients with ASD. LRRC4 can act as a neuro-protective factor to promote the recovery of damaged spinal cord neurons and protect them from immune damage, which makes *LRRC4* a promising therapeutic target for spinal cord diseases. LRRC4 is expected to become a potential therapeutic target for spinal cord diseases. In addition, LRRC4 mediates protein–protein interactions, plays an important role in various signaling pathways, and inhibits GB cell proliferation and invasion. LRRC4 not only mediates the biological behavior of GB cells but also influences the GB immune microenvironment. Most importantly, LRRC4 enhances the sensitivity of GB cells to TMZ by inhibiting autophagy. Furthermore, LRRC4 also functions as a tumor suppressor in other tumors. LRRC4 has been confirmed to be missing in nasopharyngeal carcinoma and thus may be a tumor suppressor gene in nasopharyngeal carcinoma.[55] In ovarian cancer, LRRC4 is also considered a tumor suppressor gene, and its expression is down-regulated.[56] Subsequently, the hypermethylation events of the LRRC4 promoter region were not only observed in GB but also in hepatocellular carcinoma, and promoted the occurrence of hepatocellular carcinoma.[57] Considering that LRRC4 plays an important role in brain development, mental diseases, and tumorigenesis, in-depth exploration is necessary. In conclusion, LRRC4 has potential as a diagnostic marker of mental diseases and tumors, and up-regulating the expression of *LRRC4* may be a method for treating mental diseases and tumors in the future.

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Conflicts of interest

None.

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