The role of the TNFα-mediated astrocyte signaling pathway in epilepsy

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Abstract

Epilepsy is a common disease in the central nervous system. There is growing evidence that epilepsy is associated with glial cells, including astrocytes. Tumor necrosis factor α (TNFα) is a "master regulator" of proinflammatory cytokine production and is secreted by microglia and astrocytes. TNFα secreted by microglia can activate astrocytes. Additionally, TNFα can regulate neuron activity and induce epilepsy by increasing the glutamate release, reducing the expression of γ-aminobutyric acid, inducing neuroinflammation and affecting the synaptic function in astrocytes. This review summarizes the signaling pathways and receptors of TNFα acting on astrocytes that are related to epilepsy and provides insights into the potential therapeutic strategies of epilepsy for clinical practice.

Keywords: Tumor necrosis factor alpha, Astrocytes, Inflammation, Epilepsy

Background

Epilepsy has been identified as a clinical syndrome since the invention of electroencephalogram in 1929 [1], and causes a great burden on patients and their families. Epilepsy is a common disease of the central nervous system, but the pathogenesis of epilepsy has not been fully clarified [2]. It is currently known that the main pathogenesis of epilepsy is the imbalance of excitatory and inhibitory neurotransmission, which involves altered gene expression, inflammation, oxidative stress, among other factors [2–4]. The increased pro-inflammatory factors can lead to excessive neuronal excitation, which in turn affects the glutamate and γ-aminobutyric acid (GABA) receptors. Oxidative stress can affect changes in calcium currents and increase the neuronal excitability [2]. Changes in the blood-brain barrier (BBB) are also associated with altered excitability [4].

Astrocytes account for ~30% of the cells in the human central nervous system (CNS) [5]. Astrocytic inflammation plays a role in numerous neurological diseases,

including Alzheimer's disease, ischemic stroke, and epilepsy [6]. Astrocytes not only have neuroprotective functions, but may also destroy nerve cells [7]. Studies using human brain tissues have found that epilepsy is associated with astrocytic and microglial activation [8]. Activation of astrocytes can be seen in the pilocarpine model of temporal lobe epilepsy (TLE) [9], and astrocytes play critical roles in epilepsy. Astrocytes are sensitive to neuronal activity and extracellular changes, protecting neurons from excessive activity and damage [10]. Astrocytes can mediate neuronal activity through ion channels, transmitter receptors and transporters [6]. In a study using two mouse injury models, the cerebral artery occlusion (MCAO) model of ischemic stroke and a neuroinflammation model induced by intraperitoneal injection of 5mg/kg lipopolysaccharide (LPS), the reactive astrocytes display two phenotypes, A_1 (MCAO phenotype) and A_2 (LPS phenotype). This suggests that the transformation of astrocytes to a particular phenotype depends on the nature of the activating stimulus [11]. A more recent study showed that the classically activated neuroinflammatory microglia can induce the A_1 subtype of reactive astrocytes, through secreting TNFα, IL-1α and C1q. Quantitative polymerase chain reaction also verified that TNFα-, IL-1α- and C1q-knockout mice

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can produce activated microglia, but with a drastically reduced percentage of the A_1 phenotype of astrocytes [7]. The A_1 phenotype of astrocytes destroy synapses, while the A_2 phenotype repairs the BBB and protects the CNS from damage induced by the immune response [11]. The A_1 phenotype has up-regulation of synaptic disruption genes, while the A_2 phenotype has up-regulation of neurotrophic factors. Although the A_1 phenotype possesses neurotoxic properties that destroy neurons, the BBB largely protects the CNS from the neurotoxic damage [5, 7].

Adjacent astrocytes have gap junctions composed of Cx43. The gap junctions enhance cell-to-cell communication with ion coupling and metabolite coupling [12]. In addition, astrocytes can cause neurological damage by changing neurotransmitters, transporters and gap junctions [13]. Extensive experimental evidence has suggested that the gap junctions in astrocytes play a key role in epilepsy. Cx43 has been reported to be upregulated during epilepsy. The Cx43 expression can improve the impaired gap junctions in the kainic acid-induced epilepsy model [14].

In TLE, connexins, potassium channels and water channels are affected [13]. Changes in the levels of astrocyte glutamate transporter-1 (GLT1) and aquaporin-4 have been observed in another kainic acid-induced epilepsy model by real-time polymerase chain reaction, Western blotting, and immunohistochemistry [15].

In astrocytes, the enzyme glutamine synthetase (GS) converts glutamate to glutamine as a precursor of GABA. As a result, defects in GS may increase the neuronal excitability due to the increased extracellular glutamate concentration, which is positively correlated neuronal excitability [13]. At the same time, studies using Genetic Absence Epilepsy Rats from Strasbourg (GAERS), Wistar Albino Glaxo Rats from Rijswijk (WAG/Rij) and control Wistar animals have proven that epilepsy is related to the imbalance between GABA and glutamate and the up-regulation of glial fibrillary acidic protein (GFAP), an astrocytic marker [16].

The astrocytic TNFα participates in the pathological process of epilepsy [17]. TNFα, initially recognized as a target for treatment of tumors, has been confirmed as a target of inflammation [18]. The TNFα-mediated inflammation is a key element in seizures. TNFα not only causes damage to the BBB, but also affects the CNS immune function through multiple pathways to produce inflammatory factors [18, 19]. Hence, it is essential to explore the role of TNFα in epilepsy neuropathology.

TNFα

TNFα is an inflammatory cytokine that plays an important role in the pathogenesis of CNS diseases. TNFα

is a trimer composed of three monomers with a relative molecular mass of 17×10^3 [20, 21]. TNFα gene is located within the major histocompatibility complex class II region, and its expression is maintain at homeostasis [22]. TNFα plays an important role in the CNS (Table 1). Under physiological conditions, TNFα promotes cell proliferation, participates in cognitive processes, and controls ion homeostasis. Under pathological conditions, TNFα causes neurotoxicity, promotes proliferation of glial cells and regulates T cell development [26, 27]. TNFα is involved in epilepsy through releasing glutamate, mediating the downregulation of connexin43 and controlling synaptic transmission [12, 20, 23, 28, 29]. TNFα-treated mice show an increased level of glutaminase protein and improved glutamate production [30]. TNFα also participates in the epileptic process by inducing changes in neuronal excitability via increased glutamate receptors and enhanced GABA receptor endocytosis [31].

There are two types of TNFα receptors in the CNS, namely TNFR1 and TNFR2. They are involved in different signaling pathways and have different functions. TNFR1 plays a role in regulating glutamate receptor transport, while TNFR2 plays a neuroprotective role [32, 33].

The TNFα levels are significantly increased in the hippocampus infected with Theiler's murine encephalomyelitis virus (TMEV). In the TMEV-induced epilepsy, the ratio of TNFR1 to TNFR2 in the hippocampus is increased [19], suggesting that TNFα and TNFR1 are involved in epilepsy. The TNFα-mediated inflammation leads to seizures in mouse epilepsy models *via* increased TNFα and TNFR1 expression [32]. TNFα increases glutamate release after binding to TNFR1, resulting in the increase of excitatory synaptic transmission [19]. In addition, after the activation of the TNFα–TNFR1 signaling pathway, a variety of adaptor proteins can activate transcription factors to affect cell growth, cell death and inflammation [20]. In conclusion, selective inhibition of TNFα and TNFR1 can reduce the susceptibility to epilepsy [19]. TAK1 (transforming growth factor-β-activated kinase 1) and IKKβ (inhibitor of κB (IκB) kinase β) in the

Table 1 Roles of TNFα in epilepsy

Function	Refs
Promote hyperexcitability via purinogenic signaling, glutamate release and modulation of neuronal transmitter release	[23]
Increase ictal discharges	[24]
Modulate glutamate receptor transport	[19]
Induce apoptosis	[25]

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scaffolding ubiquitin network constructed by the TNFR signaling can activate mitogen-activated protein kinase (MAPK) and nuclear factor κB (NF-κB), respectively [18], indicating that MAPK and NFκB also play a role in the TNF–TNFR axis.

Astrocytes and reactive astrocytes
Astrocytes are the most abundant glial cells in the CNS. Astrocytes have long and branched processes. The morphology of an astrocyte is related to the expression of glial proteins and neuronal neuroglobulin. It has been found that astrocytes possess structural plasticity. Glutamate, action potential, mGluRs and IkkB /NF-κB mediate structural remodeling of astrocytes [34]. Astrocyte morphology is responsible for normal brain functions such as neurogenesis, synaptic formation and neurotransmitter transmission [5].

IL-1α, TNFα and C1q secreted by microglia can act on astrocytes and induce their switch to the A_1 reactive phenotype. Reactive astrocytes differ from non-reactive astrocytes in their morphology. The reactive astrocytes are characterized by hypertrophy of cells and processes that is positively correlated with injury severity in reactive astrocytes, and increased GFAP expression [7, 35].

The morphological and structural differences between A1 reactive and non-reactive astrocytes suggest distinct functions of the two types of astrocyte. The A1 reactive astrocytes are neurotoxic. They can impair normal brain functions, they produce pro-inflammatory chemokines and cytokines resulting in neuroinflammation, and impairs synaptic function and phagocytosis. The A1 reactive astrocytes also increase the level of neuroexcitatory glutamate and inhibit the effects of GABA, thereby promoting the development of epilepsy [35].

TNFα signaling pathway in astrocytes
GABA receptors and glutamate receptors
One possible mechanism of epilepsy generation is the imbalance between the excitatory glutamate and the inhibitory GABA neurotransmitter. In excitatory neurons, glutamate released at synapses can promote epilepsy, while in inhibitory neurons, glutamate decarboxylase converts glutamate to GABA and suppresses epilepsy [3].

Astrocytes may be involved in seizure generation *via* their release of TNFα alone or in combination with glutamate. TNFα controls the neurotransmission of glutamate and GABA in post-synaptic membranes. In addition, blocking the release of GABA precursor glutamine from astrocytes, or the loss of glutamine synthetase, can result in increased synaptic glutamate levels, decreased GABA synthesis and excessive neuronal excitement [3, 13].

GABA transporters GAT-1 and GAT-3 can regulate the level of extracellular GABA. Furthermore, in the MCAO model, the participation of GABAergic transmission in post-ischemia epilepsy has been determined by measuring GAT-1 and GAT-3 protein levels at these sites. TNFα released during epilepsy can enhance GAT-1 and GAT-3 expression and reduce GABA levels, which in turn aggravate epilepsy [36].

Studies in the pilocarpine-induced mouse model have found that the epilepsy frequency correlates with the loss of inhibitory GABAergic neurons. Astrocyte dysfunction increases glutamate levels and greatly reduces the Cl⁻ flow into the extracellular space, inducing depolarization. As Cl⁻ gradient has been shown to control the inhibitory effect of $GABA_A$ receptors, $GABA_A$ receptor function may be reduced by the decreased activity of the associated Cl⁻ channel [3, 13].

Interestingly, studies in the GAERS, WAG/Rij and Wistar animal models have shown that the increased GABA uptake by astrocytes significantly promotes the excitatory glutamate levels in somatosensory cortex and thalamic nuclei, leading to excessive neuronal excitement [16].

The balance between glutamate production and elimination ensures the maintenance of a normal level of glutamate. Astrocytes express transporters such as glutamate-aspartate transporter (GLAST) and GLT1, which are essential for glutamate clearance [37]. The uptake of glutamate in the synaptic cleft is important for the maintenance of physiological concentrations of glutamate and thus homeostasis. TNFα up-regulates the glutaminase activity to promote the production while reducing the elimination of glutamic acid, finally leading to glutamic acid accumulation [38]. Moreover, when astrocytes transform into the reactive phenotype, they express a lowered level of GLAST and GLT1, which may also lead to glutamate accumulation [37]. When glutamate reaches pathological levels, it can have toxic effects and cause damage to synapses [38]. Zhou et al. have found that TNFα inhibits glutamate uptake through the NF-κB signaling pathway and enhances the phagocytosis of astrocytes in human NCRM-1 astrocytes [39]. In recent years, autoimmune encephalitis, which is associated with epileptic seizures, has been studied at a greater level. Interestingly, *N*-methyl-*d*-aspartate (NMDA) receptor (NMDAR), the postsynaptic ionotropic glutamate receptor, is one of the pathogenic factors for this autoimmune disease [37].

AMPA receptors
The AMPA receptor is a glutamate-gated ion channel that regulates excitatory synaptic transmission and maintains a balance between excitatory and inhibitory synapses [40]. AMPA receptor ubiquitination maintains a

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balance of excitability and inhibition in neurons [41]. The AMPA receptors dynamically regulate the synaptic plasticity via their altered amount, subunit composition and biological characteristics in the post-synaptic membrane [40]. The AMPA receptor function is mediated by helper proteins and transmembrane AMPA receptor regulatory proteins (TARPs). TARPγ8, located in the forebrain, affects the kinetics of AMPA receptor and prolongs the receptor activation time, which is associated with TLE [42]. The structural abnormalities and dysfunction of AMPA receptors can enhance neuronal excitability, contributing to the generation of epilepsy [43].

AMPA receptors are highly expressed on astrocytes and neurons, and TNFα can enhance excitotoxicity through the AMPA receptors [6, 20]. The AMPA receptors contain four subunits, including GluR1 to GluR4. Enhanced expression of GluR1 flip variants in astrocytes has been detected in the hippocampi of epilepsy animal models. This suggests that astrocytes may induce epilepsy through the AMPA receptors [6]. Abnormal palmitylation of AMPA receptors enhances epilepsy susceptibility, in which the GluA1 subunit plays a key role. Deficiency of GluA1 palmitylation disrupts the balance of excitatory and inhibitory synapses without affecting THE brain structure [44].

One study has confirmed that parallel fibers (PFs) can activate AMPA receptors on Bergmann glia (BG), a type of astrocyte, to induce calcium ion changes, thereby releasing glutamate. Overactivation of AMPA receptors expressed by neurons can also induce neurotoxicity. In addition, studies have found that higher concentrations of TNFα can cause damage by activating the Ca^{2+}-permeable AMPA receptors, altering synapse transmission and producing excitotoxicity [29]. The combination of TNFα and TNFR1 alters AMPA receptors in the synapse, increasing neuronal excitability, while TNFR2 plays a protective role [32]. TNFα readjusts glutamatergic neurotransmission by regulating AMPA receptors, thereby controlling the synaptic function. In conclusion, TNFα performs synaptic regulation and excitability by controlling AMPA receptor trafficking [20].

AMPA receptors act as a target for epilepsy therapy. Currently, AMPA receptor antagonist has been found to have anticonvulsant effects, reducing the excitatory postsynaptic current frequency and inhibiting the excitatory synaptic transmission in neurons, resulting in decreased spontaneous epileptic-like discharge in brain tissue samples from children [45]. Currently known AMPA receptor antagonists that can be used to treat epilepsy are perampanel and talampanel [43]. Perampanel is used for partial-onset and tonic-clonic seizures [46, 47]. However, talampanel is not recommended for use in the clinical treatment of epilepsy due to adverse reactions [48].

NF-κB signaling pathway

The NF-κB signaling pathway is a key pathway of the central inflammatory response. It induces the expression of many pro-inflammatory factors, such as cell adhesion factors, cytokines and iNOS [49]. Astrocytes, after prolonged exposure to glutamic acid, activate the NF-κB signaling pathway *via* NMDA receptors and the activation of I-type voltage-sensitive calcium channel. The activated NF-κB can be involved in structural and synaptic plasticity. One study demonstrated that the activated NF-κB can activate glutamatergic neurons and promote excitatory synaptic formation. In addition, NF-κB regulatory sites have been found on GLT-1 gene [50].

TNFα promotes inflammation through the NF-κB signaling pathway [51]. Previous research demonstrated that NF-κB plays a role in determining the cell fate [52]. TNFα disrupts the BBB, causing excessive neuronal excitement, and thus epilepsy in the pilocarpine-induced status epilepticus rat model. Fluoro-Jade B staining and immunohistochemical analysis of TNFα and NF-κB subunits have demonstrated upregulation of the TNF p55 receptor (TNFR1) and TNF p75 receptor (TNFR2) in astrocytes [53]. Anti-TNF biologics can prevent TNFα from binding to receptors TNFR1 and TNFR2 via the NF-κB signaling, which reduces inflammation. Therefore, TNFα induces apoptosis by NF-κB signaling and aggravates inflammation. Experiments have shown the existence of an NF-κB-independent cell death checkpoint that limits apoptosis by inactivation of the receptor-interacting serine-threonine kinase 1 (RIPK1). Disruption of this checkpoint could cause inflammation *in vivo*. Moreover, blocking RIPK1 can inhibit the TNFα-mediated inflammatory processes [51].

The TNFα-mediated phosphorylation of NF-κB subunits causes angioedema with neutrophil infiltration and astrocyte damage [53]. At the same time, the TNFα–NF-κB signaling also increases the expression of drug transporters, including P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP). There is evidence that P-gp and MRP are present in astrocyte processes and in primary cultures of rat astrocytes. Astrocytes increase P-gp and MRP expression via the TNFα–NF-κB signaling. This may be one of the mechanisms of refractory epilepsy [17].

Moreover, NF-κB has been shown to regulate the expression of COX-2. COX-2 can shorten the interval of seizures, leading to repeated seizures. The peptide κB-decoy binds to free NF-κB and affects its transcription. Experimental results have shown that κB-decoy could inhibit NF-κB activation in epileptic-related brain regions and decrease the mRNA and protein expression of COX-2, thus reducing seizures [54].

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Signal transducer and activator of transcription 3 (STAT3) signaling pathway

STAT3 is a transcription factor that can cause cell proliferation, differentiation and apoptosis. Reactive astrocytes can repair lesions and inhibit inflammation through the STAT3 signaling pathway, in contrast to the NF-κB signaling pathway [35]. However, in the Aβ-oligomer-induced AD model, STAT3 activation can mediate astrocyte activation, resulting in neurotoxicity [55]. Moreover, the STAT3 signaling pathway has also been shown to promote neuronal death, which is associated with epilepsy.

STAT3 polymorphism is associated with the prevalence of epilepsy in children. The levels of STAT3 and p-STAT3 increase in brain tissues of children with epilepsy, as detected by immunohistochemistry. Previous studies have shown high expression of p-STAT3 in the hippocampus in a rat epilepsy model [56]. Another study showed that STAT3 expression is prominent in glial cells and neurons in epilepsy. Moreover, in the kainic acid (KA)-induced TLE epileptic model, the long noncoding RNA urothelial cancer-associated 1 can inhibit astrocyte activation through the JAK/STAT signaling pathway, improving the adverse effects of epileptic episodes. This suggests that the STAT signaling pathway participates in astrocyte activation and plays an important role in the induction of epilepsy [57]. Similarly, miR-21-5p can inhibit STAT3 expression and reduce neuronal apoptosis and damage in the hippocampus, thus exerting protective effects on neurons of epileptic rats [58]. STAT3 phosphorylation is associated with secretion of neuroinflammatory cytokines, such as IL-6, IL-1β and TNF-α. Thus, inhibition of STAT3 is also effective in suppressing neuroinflammation [59].

The STAT3 signaling pathway is also crucial for astrocyte development. TNFα promotes the differentiation of neural progenitor cells into astrocytes through induction of STAT3 activation, as revealed by Western blot analysis of total cell lysates. Immunocytochemical staining also demonstrated that the inhibition of STAT3 can inhibit astrocytic differentiation and increase neuronal differentiation of neural progenitor cells under inflammatory conditions. In addition, the application of STAT3-targeting siRNA (siSTAT3) decreased the TNFα-induced STAT3 activation, inhibiting astrocyte activation [60].

TNFα is also able to enhance the expression of kidney type glutaminase (KGA) via the STAT3 signaling pathway in astrocytes, catalyzing the hydrolysis of glutamine to glutamate and ammonia. The induction of KGA expression by TNFα is inhibited by selective inhibition of STAT3 with a STAT3 inhibitor STA-21. One study demonstrated that the TNFα-mediated KGA expression is associated with increased phosphorylation of STAT3 [28]. Furthermore, the increased KGA expression in astrocytes will increase glutamate content. Astrocytes take up glutamate, thereby reducing GABA synthesis and increasing neuronal excitability, finally decreasing the threshold of seizure induction [31]. The TNFα–STAT3 pathway not only activates astrocytes but also enhances astrocyte uptake of glutamate.

Dasatinib is an FDA-approved drug for the treatment of chronic myeloid leukemia. In an LPS-induced neuroinflammation model, dasatinib inhibits microglial and astrocytic activation *via* the AKT/STAT3 signaling pathway and leads to a decreased level of proinflammatory cytokines [61].

MAPK signaling pathway

MAPK signaling pathways include the extracellular signal regulated kinase (ERK) pathway, the p38 pathway and the C-Jun N-terminal kinases (JNK) pathway. Each pathway plays a different role in epilepsy. The ERK/MAPK signaling pathway up-regulates NMDA receptors and enhances neuronal excitability [62]. There is growing evidence that the p38 MAPK signaling pathway is associated with cognitive impairment in epilepsy. In the rat model of PTZ-induced epilepsy, the p38 MAPK signaling pathway modulates apoptosis and impairs cognitive function [63]. In addition, ferrostatin 1 alleviates the KA-induced cognitive impairment in TLE rats by upregulating the expression of synaptophysin and postsynaptic density protein-95 *via* the p38 MAPK signaling pathway [64]. The p38 MAPK signaling pathway is associated with refractory epilepsy. SB202190, an antagonist of p38 MAPK, inhibits epileptic seizures and increases the concentrations of valproate and lamotrigine in rats [65]. Furthermore, SB203580, which inhibits the p38 MAPK signaling pathway in rats, repairs the damaged hippocampal neurons and reduces the frequency of seizure attack by down-regulating the levels of Adenosine A1 receptor and type 1 equilibrative nucleoside transporter [66]. The activation of JNK/MAPK signaling pathway occurs at both acute and chronic stages of epileptic development. It participates in astrocyte activation, neuronal death and mossy fiber sprouting [67]. The MAPK signaling pathway regulates RNA-binding proteins (RBPs), which are responsible for mRNA translation. Dysregulated RBPs may result in expression of epileptic-related factors. Therefore, the MAPK signaling pathway mediates the translation of synaptic proteins to control synaptic excitability and promote epilepsy development [62].

TNFα can activate the MAPK pathway, which upregulates transcription of pro-inflammatory genes *via* TNFR1 [51]. Consistently, TNFα stimulates astrocytes, leading to activation of the p38 MAPK pathway and the production of nitric oxide as part of the inflammation pathway [68].

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In addition, the TNFα–MAPK signaling is related to gap junctions in astrocytes as well [14]. TNFα inhibits the expression of Cx43 gap junctions in spinal astrocytes and reduces communication between glial cells during inflammation [69]. P38 MAPK participates in Cx43 hemichannel activation and astrocytic inflammatory activation. Application of a p38 MAPK blocker could suppress the ethidium (a fluorescent agent that evaluates cell coupling) signal, which detects hemichannel activity as it enters normal cell cytoplasm through hemichannels, uptake of ethidium by hippocampal astrocytes treated with ethanol, thereby repressing the ethanol-induced channel opening in astrocytes [70]. P38 MAPK activates Cx43 gap junctions in neurons to cause synchrony of firing and participates in the development of epilepsy.
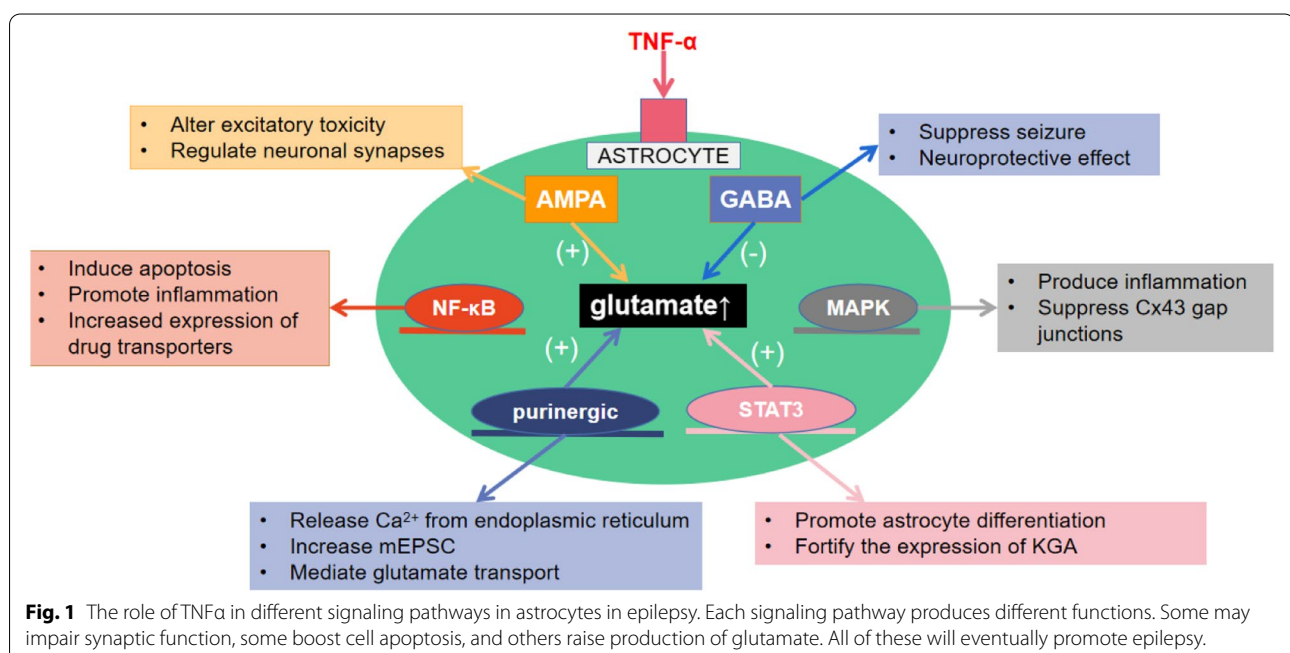
Purinergic signaling

The important role of astrocyte-neuron conduction in the pathogenesis of epileptogenesis is increasingly recognized. Accumulating evidence suggests that there is an enhanced release of glutamate by astrocytes in TLE models [23]. TNFα not only controls the release of glutamate from astrocytes, but also controls the exocytosis of glutamate. Meanwhile, there is evidence that Ca^{2+} and purinergic receptors have a bidirectional cross-talk. On the one hand, astrocytes activated by the release of glutamate, adenosine triphosphate (ATP) and cyclic adenosine monophosphate activate the purinergic receptor through Ca^{2+} waves. On the other hand, ATP released by astrocyte hemichannels activates metabolic P2Y receptors, thereby inducing intracellular Ca^{2+} release from the endoplasmic reticulum. Correlatively, ionic P2X receptors can close gap junctions. Previous studies have demonstrated that inflammatory cytokines such as TNFα are involved in epileptogenesis *via* the ATP-purinergic receptors [14, 20, 23]. However, in the absence of TNFα, ATP-mediated P2Y1 receptors cause elevation of intracellular Ca^{2+}, while glutamate release is reduced. When TNFα concentration increases, TNFR1 causes activation of the P2Y1 receptors and makes astrocyte Ca^{2+} levels change transitorily. Therefore, selective antagonism of P2Y1 receptors prevents TNFα from triggering astrocyte Ca^{2+} transients, and concurrently normalizes the enhanced miniature excitatory postsynaptic current (mEPSC) frequencies. Similarly, the P2Y1 receptor-activated transmitters interact with presynaptic NMDA receptors, boosting the mEPSC frequency, thereby increasing synaptic transmitter release. In summary, TNFα participates in the transport of glutamate neurotransmitters in astrocytes via the Ca^{2+}-induced purinergic signaling [23].

Conclusions

Epilepsy is a chronic brain disease characterized by seizures, transience, repetition and rigidity. The abnormal, highly synchronized discharge of neurons is the pathogenesis factor of epilepsy. Epilepsy is closely related to neuroinflammation. The increase of inflammatory

Fig. 1 The role of TNFα in different signaling pathways in astrocytes in epilepsy. Each signaling pathway produces different functions. Some may impair synaptic function, some boost cell apoptosis, and others raise production of glutamate. All of these will eventually promote epilepsy.

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cytokines and cells is a key factor in epileptic foci. IL-1α, TNFα, and C1q released by microglia transform astrocytes into the A1 reactive phenotype. Astrocytes are one of the targets of TNFα during epilepsy. In this review we describe an important relationship of TNFα with neuronal excitability in the presence of reactive astrocytes. In astrocytes, TNFα affects glutamate uptake and intercellular gap junctions. TNFα can regulate signaling pathways such as NF-κB, and control receptors such as AMPA receptors (Fig. 1). The former could cause apoptosis to produce epilepsy and the latter could lead to neuronal overexcitation. However, TNFα was rarely studied in epilepsy patients, and it remains unclear which pathway or receptor plays a major role in epilepsy. At the same time, further studies on A1 reactive astrocytes and epilepsy-related signaling pathways are needed. In addition, we need to consider whether all inflammatory mediators and signaling pathways can be used as therapeutic targets for epilepsy. The relationship between NF-κB and STAT3 signaling pathways also requires further experimental exploration.

Abbreviations
TNFα: Tumor necrosis factor α; GABA: γ-aminobutyric acid; BBB: Blood-brain barrier; MCAO: Middle cerebral artery occlusion; Cx43: Connexin-43; GLT1: Glutamate transporter-1; GS: Glutamine synthetase; GFAP: Glial fibrillary acidic protein; TMEV: Theiler's murine encephalomyelitis virus; MAPK: Mitogen-activated protein kinase; NF-κB: Nuclear factor κB; GAT: Gamma-aminobutyric acid transporter; GLAST: Glutamate-aspartate transporter; NMDA: N-methyl-d-aspartate; TARPs: Transmembrane AMPA receptor regulatory proteins; PFs: Parallel fibers; BG: Bergmann Glia; iNOS: Inducible nitric oxide synthase; P-gp: P-glycoprotein; MRP: Multidrug resistance-associated protein; COX-2: Cyclooxygenase-2; STAT3: Signal transducer and activator of transcription 3; TLE: Temporal lobe epilepsy; KGA: Kidney type glutaminase; LPS: Lipopolysaccharide; ERK: Extracellular regulated protein kinase; JNK: c-Jun N-terminal kinase; NMDA: N-methyl-D-aspartic acid receptor; RBPs: RNA-binding proteins; ATP: Adenosine triphosphate; mEPSC: Miniature excitatorypostsynaptic current.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
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