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TWEAK and the Central Nervous System

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Abstract Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a member of the tumor necrosis factor superfamily that acts on responsive cells via binding to a cell surface receptor named fibroblast growth factor-inducible 14 (Fn14). TWEAK can regulate numerous cellular responses in vitro and in vivo. Recent studies have indicated that TWEAK and Fn14 are expressed in the central nervous system (CNS), and that in response to a variety of stimuli, including cerebral ischemia, there is an increase in TWEAK and Fn14 expression in perivascular astrocytes, microglia, endothelial cells, and neurons with subsequent increase in the permeability of the blood–brain barrier (BBB) and cell death. Furthermore, there is a growing body of evidence indicating that TWEAK induces the activation of the NF-κB in the CNS with release of proinflammatory cytokines and matrix metalloproteinases. In addition, inhibition of TWEAK activity by either treatment with a Fn14-Fc fusion protein or neutralizing anti-TWEAK antibodies has shown therapeutic efficacy in animal models of ischemic stroke, cerebral edema, and multiple sclerosis.

Keywords TWEAK · Fn14 · Cytokines · Cerebral ischemia · Neurovascular unit · Blood–brain barrier · TNF

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Introduction

The tumor necrosis factor superfamily (TNFSF) of cytokines comprises 19 ligands and 28 receptors [1]. Most of the ligands are synthesized as type II transmembrane proteins with an extracellular C terminus TNF homology domain that facilitates their assembly into homotrimers, which allows receptor cross-linking and activation of signal transduction. The receptors are characterized by one or more cysteine-rich domains in the extracellular region, and by the presence in the intracellular region of either a "death domain" (DD) or a binding site for a group of adaptor molecules known as TNFR-associated factors (TRAFs). Ligand-mediated aggregation of TNFSF receptors plays a role in a number of biological events including immune system development and function [2], as well as in the pathogenesis of many central nervous system (CNS) diseases such as multiple sclerosis [3] and cerebral ischemia [4]. This review will focus on the structure, biological function and therapeutic importance of the TNFSF member tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK; TNFSF12) and its receptor, fibroblast growth factor-inducible 14 (Fn14; TNFRSF 12a).

TWEAK and Fn14 Structure and Expression

TWEAK

TWEAK is initially synthesized as a 249-amino acid (aa) type II transmembrane glycoprotein containing a C-terminal 206-amino acid extracellular domain and an N-terminal 18-aa intracellular domain. Most cells express both full-length, membrane-anchored TWEAK and a soluble, 156-aa TWEAK extracellular domain fragment [2]. TWEAK gene

expression has been detected in a variety of tissues and cell types including brain, heart, skeletal muscle, and pancreas in vivo [2, 5–10] as well as spleen, lymph nodes, thymus, lymphocytes, mouse peritoneal macrophages, fibroblasts, and human smooth muscle cells cultured in vitro [11–13]. TWEAK mRNA levels do not always reflect TWEAK protein abundance [13, 14], suggesting the possibility that TWEAK synthesis is translationally controlled. Furthermore, the TWEAK mRNA 3′-untranslated region has an AU-rich sequence element, and a similar motif is implicated in TNF-α mRNA stability [2, 15]. APRIL is a member of the TNFSF that promotes tumor growth and is important for immunomodulation [13]. The gene for APRIL is located 1 Kb downstream from the gene encoding for TWEAK in chromosome 17 in humans. A transmembrane protein consisting of the intracellular and transmembrane region of TWEAK fused to the APRIL receptor binding site has been described as TWEPRIL in human lymphocytes and monocytes [13, 16]. The biological function of this fusion protein is still unknown.

Fn14

TWEAK activity is mediated via binding to a cell surface receptor named Fn14 , which was initially described as a growth factor-inducible gene encoding a small type I transmembrane protein of unknown function [17]. Subsequent studies by Wiley et al. [18] identified Fn14 as a TWEAK-binding cell surface receptor. Mature Fn14 is only 102-aa in length, making it the smallest TNF receptor superfamily (TNFRSF) member identified to date. Indeed, the Fn14 extracellular domain containing the TWEAK-binding site is only 53-aa in length [19], and the Fn14 cytoplasmic tail, essential for signal transduction [20, 21], is only 28-aa in length. Most of the TNFRSF members have two or more cysteine-rich domains in their extracellular ligand-binding region. In contrast, Fn14 has a single cysteine-rich domain [2, 18]. Another characteristic of the TNFRSF is the presence of either a ~80-aa DD sequence in their cytoplasmic region that promotes caspase activation and cell death [22], or one or more TRAF-binding sites. The cytoplasmic domain of Fn14 is too short to have a DD but instead, as will be discussed below, contains a binding site for TRAFs [2]. TWEAK is the only TNFSF member that binds Fn14, and TWEAK does not bind to any other TNFRSF members [23].

The human Fn14 gene is located at chromosome 16p13.3 [24]. Fn14 mRNA expression is detected in the mouse embryo as early as 7.5 days post coitum [17], and the highest levels in the adult mice are found in the heart, ovary, kidney, lung, and skin [17]. Fn14 protein is expressed in a variety of cells and tissue types with relatively high levels of expression in the skeletal muscle, heart, kidney, lung, and placenta [2, 6, 8–10, 25, 26]. In addition, Fn14 gene expression is upregulated after injury to the vessel wall [18], partial hepatectomy [24], sciatic nerve transection [27], and in response to cerebral ischemia [28, 29]. It has also been shown that Fn14 is expressed at high levels in advanced brain [30], breast [31], and liver [24, 32] tumor specimens.

The use of mice deficient in either TWEAK ($TWEAK^{-/-}$) or Fn14 ($Fn14^{-/-}$) will expand the understanding of the biology and function of this cytokine and its receptor. Both $TWEAK^{-/-}$ and $Fn14^{-/-}$ mice are viable and fertile. However, $TWEAK^{-/-}$ mice have elevated natural killer (NK) cell production [8] and Fn14-KO mice show reduced progenitor cell expansion after liver injury [32].

The TWEAK-Fn14 Signaling Pathway

TNFRSF members have no intrinsic protein kinase activity but instead, as discussed above, they contain sequence motifs that bind adaptor proteins, which activate downstream signal transduction pathways. Indeed, TRAF 1, 2, 3, and 5 can bind to the Fn14 cytoplasmic tail [18, 20, 33]. Binding of the TNFSF ligands to TNFRSF that associate with TRAF adaptor proteins has been shown to induce the activation of several intracellular signal transduction cascades, including the NF-κB pathway and mitogen-activated protein kinases pathways [34]. This is the case for TWEAK, which has been demonstrated to activate the NF-κB, ERK and JNK signal transduction pathways in several cell types [6, 20, 21, 25, 26, 33, 35–41].

TWEAK Biological Activity

Cell Death

Several of the members of the TNFSF can directly induce cell death. This property is shared by TWEAK, although as its name suggests, with reduced potency relative to TNF-α [42]. Indeed, the ability of TWEAK to induce cell death in tumor cell lines is relatively weak and in many instances this effect requires incubation with agents such as gamma-interferon. This is true in the case of TWEAK-induced apoptosis in HT29 [11], gastric adenocarcinoma KATO-III, and oral squamous carcinoma HSC3 cells [43]. Furthermore, CD4+ T cell-induced apoptosis of monocytes and macrophages is also mediated by TWEAK [14, 43]. The mechanism by which TWEAK induces cell death is not completely understood. It was initially thought that TWEAK induces apoptosis via endogenously produced TNF-α and TNFR1 activation [44], a mechanism described for other members of the TNFSF without a DD [44].

However, it was subsequently demonstrated that TWEAK could mediate cell death by TNF-α-independent mechanisms involving both caspase-induced apoptosis and cathepsin B-dependent necrosis [43, 45].

Angiogenesis

Angiogenesis is a tightly regulated process that is critical for several physiologic and pathological events [46, 47]. TWEAK has a proliferative effect in vitro on aortic smooth muscle cells, and on umbilical vein, dermal microvasculature and brain microvascular endothelial cells (EC), and in vivo in the rat cornea [48]. In addition, TWEAK can enhance bFGF and VEGF-stimulated proliferation of human umbilical vein endothelial cells (HUVEC) and induce endothelial cell migration [35]. Furthermore, TWEAK stimulates the formation of lumen-containing structures as well as the invasion of endothelial cells into a fibrin matrix [35, 49].

Inflammation

Inflammation is a key event in the pathogenesis of CNS diseases [50–52]. TWEAK has been shown to be a pro-inflammatory factor. Indeed, TWEAK gene expression has been detected in monocytes/macrophages [6, 53] and the intravenous administration of TWEAK upregulates CCL2/MCP-1 and CXCL10/IP-10 gene expression in mouse kidney in vivo [26]. A role for TWEAK in the genesis of an inflammatory response in the CNS is supported by results indicating that TWEAK and Fn14 are found in astrocytes and microglia [29, 54, 55], and that treatment of astrocytes with TWEAK induces a dose-dependent increase in IL-6 and IL-8 secretion as well as in ICAM-1 expression [55].

Immune Regulation

A recent report suggests that TWEAK plays a role in the modulation of the immune response [8]. TWEAK regulates the production of IFNγ in mice by decreasing the number of natural killer (NK) cells and TWEAK suppresses macrophage IL-12 secretion. Finally, TWEAK$^{-/-}$ mice have an increased sensitivity to lipopolysaccaride (LPS).

Inhibition of TWEAK Activity

TWEAK activity may contribute to the pathophysiology of a number of human CNS diseases including ischemic stroke, cerebral edema, and multiple sclerosis; therefore, inhibition of TWEAK binding to Fn14 may be a potential therapeutic strategy for these patients. To this date, two approaches have been utilized to inhibit TWEAK activity in in vitro and in vivo systems: a Fn14-Fc fusion protein and anti-TWEAK monoclonal antibodies.

The Fn14-Fc fusion protein Inhibition of TWEAK activity with an Fn14-Fc fusion protein was first described in 2003 by Donohue et al. [35]. This protein consist of an N-terminal murine Ig kappa chain signal peptide (21-aa), most of the murine Fn14 extracellular domain (46-aa), the Fc portion and hinge region of the murine IgGl heavy chain (228-aa), a c-*myc* epitope tag (10-aa), and a C-terminal polyhistidine tract (6-aa). ELISA [35] and surface plasmon resonance (BIAcore) assays [19] have demonstrated that human TWEAK binds the Fn14-Fc protein with an affinity constant (K_d) of ~1.0 nM. This Fn14-Fc protein has also been shown to inhibit TWEAK promitogenic activity on human EC cultured in vitro [19, 35]. Fn14-Fc has been shown to inhibit TWEAK biological activity on other cell types as well, including mouse astrocytes [55], mouse glomerular mesangial cells [26], mouse Eph4 mammary epithelial cells [31], rat vascular smooth muscle cells [33], and human glioma cells [21, 30].

Anti-TWEAK monoclonal antibodies Murine anti-TWEAK monoclonal antibodies have been generated in Armenian hamsters after immunization with soluble human TWEAK protein [56]. The blocking properties and efficacy of these antibodies have been demonstrated in mouse models of cerebral ischemia [28], multiple sclerosis [57], and rheumatoid arthritis [58].

TWEAK and FN14 in the Normal CNS

TWEAK and Fn14 Expression in the Brain

TWEAK and Fn14 expression has been detected in monoculture systems of astrocytes, microglia, EC, and neurons [29, 55]. In vivo, TWEAK and Fn14 are detected in EC of medium and small caliber blood vessels, and in perivascular astrocytes, neurons, and microglia, mainly throughout the cerebral cortex and in the caudate nucleus, putamen, substantia nigra, cerebellar Purkinje cells, and spinal cord. However, whereas TWEAK immunostaining is observed predominantly in EC and astrocytes, Fn14 immunostaining is most prominent in EC, neurons, and microglia [29]. Together, these observations suggest a “cross-talk” between astrocytes, neurons and microglial cells in the non-ischemic

CNS where TWEAK released from astrocytes and EC could bind to Fn14 receptors on neurons, EC, and microglia.

TWEAK and the NF-κB Pathway

The NF-κB family includes five structurally-related proteins that bind to a specific DNA motif and regulate gene expression [59, 60]. These factors form homodimers or heterodimers that together control the transcription of over 150 target genes encoding a diverse set of proteins involved in the immune response, inflammation, apoptosis, and tumorigenesis [59–61]. NF-κB complexes can be activated by many divergent stimuli (e.g., cytokines, chemotherapeutic drugs, bacterial lipopolysaccharide) via two distinct pathways [59–61]. In the classic or canonical pathway, NF-κB complexes are present in the cytoplasm as inactive, latent transcription factors as a consequence of their association with the repressor protein IκBα. Stimulation of cells induces IκBα phosphorylation and proteosome-mediated degradation, resulting in the liberation of NF-κB, allowing nuclear translocation and binding to DNA. In the non-canonical NF-κB signaling pathway, IκBα is not involved but instead extracellular stimuli induce phosphorylation and proteolytic processing of the NF-κB/Rel family member p100, which promotes nuclear translocation of p52 homodimers or RelB/p52 heterodimers. In vitro studies have demonstrated that incubation of EC, and primary neuronal, astrocytic and microglial cultures with TWEAK results in NF-κB pathway activation in each cell type [40]. In vivo studies have shown that the intracerebral injection of recombinant TWEAK into non-ischemic brains induces IκBα phosphorylation as early as 1 h post middle cerebral artery occlusion with a progressive decrease of total IκBα levels, mainly in neurons and perivascular astrocytes [40]. Together, these observations demonstrate that TWEAK induces NF-κB activation in vitro and in vivo in the normal CNS.

TWEAK and Fn14 in CNS Disease

There is a growing body of evidence indicating that TWEAK and Fn14 are involved in the pathogenesis of several human neurological diseases including ischemic stroke [28, 29, 41], cerebral edema [40, 41], and multiple sclerosis [54, 55, 57] and that the inhibition of TWEAK activity may have a therapeutic impact on these conditions.

Ischemic Stroke

Ischemic stroke is a leading cause of disability and a second cause of mortality in the world [62, 63]. After the onset of the ischemic insult, there is a densely ischemic area where cerebral blood flow (CBF) is decreased to <15% of normal values and brain tissue is irreversibly damaged (ischemic core). This zone is surrounded by a second area where CBF is reduced to 15–40% of the normal value but the brain tissue is still viable (ischemic penumbra) [64]. However, with time and in the absence of any effective treatment, this potentially salvageable area of penumbra or reversible ischemia becomes infarcted.

Effect of cerebral ischemia on TWEAK and Fn14 expression It has been demonstrated in an animal model of permanent focal cerebral ischemia that TWEAK and Fn14 mRNA increases in the ischemic hemisphere as early as 3 and 4.5 h after the onset of the ischemic insult, respectively [28]. Moreover, whereas TWEAK mRNA returns to normal levels 24 h later, Fn14 mRNA peaks at 24–48 h, and decreases at 72 h after the onset of the ischemic insult [29]. Immunohistochemical analysis of ischemic brain sections demonstrate that TWEAK and Fn14 protein expression is significantly increased in the area of ischemic penumbra as early as 24 h after MCAO, mainly in endothelial cells, perivascular astrocytes, microglial cells, and neurons [29].

Inhibition of TWEAK activity is protective during cerebral ischemia A deleterious role for TWEAK during cerebral ischemia was underscored by the finding that inhibition of TWEAK activity by treatment with either Fn14-Fc fusion protein [29] or anti-TWEAK monoclonal antibodies [28] after the onset of the ischemic insult results in a 20–40% decrease in the volume of the ischemic lesion. This observation was supported by the demonstration that $Fn14^{-/-}$ mice have an approximately 60% reduction in the volume of the ischemic lesion after MCAO when compared to their littermate wild-type controls [41] (Fig. 1). Likewise, either treatment with Fn14-Fc decoy or genetic deficiency of Fn14

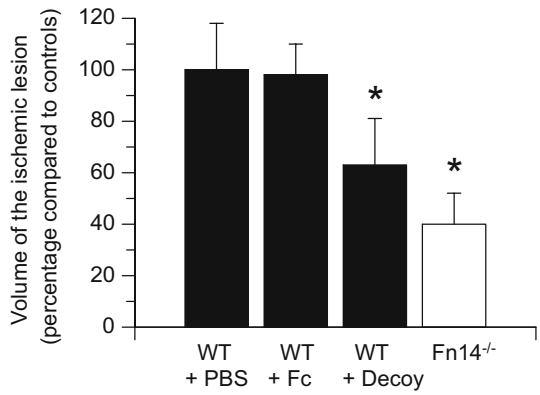

Fig. 1 Effect of Inhibition of TWEAK activity on the volume of the ischemic lesion. Wild-type (*WT*; *black bars*) and Fn14 deficient ($Fn14^{-/-}$; *white bar*) underwent middle cerebral artery occlusion. WT mice were treated with either PBS (control), Fc protein (control) or Fn14-Fc decoy. n=5 per group. *p<0.05 compared to WT treated with PBS (control) or Fc protein (control)

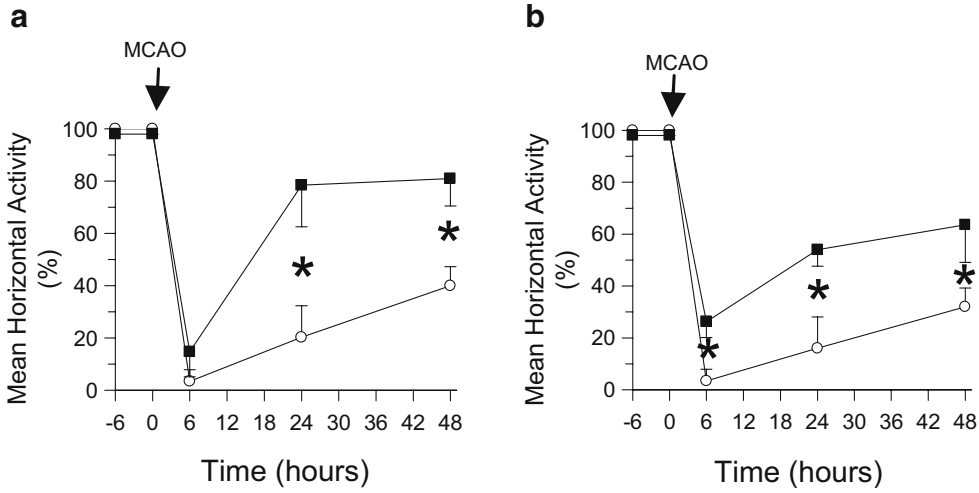

Fig. 2 Effect of inhibition of TWEAK activity on motor activity after MCAO. Mean horizontal motor activity at 6, 24 and 48 h after MCAO in wild-type (*WT*) mice treated with either Fc protein (*white circles*) or Fn14-Fc decoy (*black squares*) either immediately after (**a**) or 1 hour following MCAO (**b**). *Lines* denote SEM. $n=4$, $^*p<0.05$

results in a better clinical outcome at 24 and 48 h after MCAO [41] (Fig. 2). Together, these observations demonstrate that the interaction between TWEAK and Fn14 is deleterious during cerebral ischemia and that the inhibition of TWEAK activity has a potential therapeutic role for acute ischemic stroke.

Cerebral Edema

TWEAK and the neurovascular unit (NVU) The NVU is assembled from EC, the extracellular matrix of the basal lamina, astrocytic end-feet processes that surround the microvessels, perivascular microglia, and adjacent neurons [65]. One of the main functions of the NVU is the regulation of the supply of nutrients to the brain and prevention of the passage of harmful substances from the intravascular space into the CNS.

Early after MCAO, there is disruption of the architecture of the NVU with increase in the permeability of the blood–brain barrier and development of cerebral edema [66, 67]. A role for TWEAK and Fn14 in the regulation of the function of the NVU is suggested by the finding that this cytokine and its receptor are preferentially expressed in EC, perivascular astrocytes and neighboring neurons and microglia [29, 40]. In addition, subsequent studies have demonstrated that the intracerebral injection of recombinant TWEAK into the non-ischemic brain induces a dose-dependent increase in the permeability of the NVU [40]. This effect is not observed after the intracerebral injection of TWEAK in animals deficient in the NF-κB family member p50, indicating that TWEAK-induced increase in the permeability of the blood–brain barrier is mediated by activation of the NF-κB pathway [40].

Electron microscopy studies have shown that the intracerebral injection of TWEAK induces edema of perivascular astrocytes with detachment of astrocytic end-feet processes from the basement membrane and development of areas of perivascular edema [40] (Fig. 3). This effect of TWEAK on the ultrastructure of the NVU is significantly decreased by genetic deficiency of either Fn14 [41] or p50 [40]. The

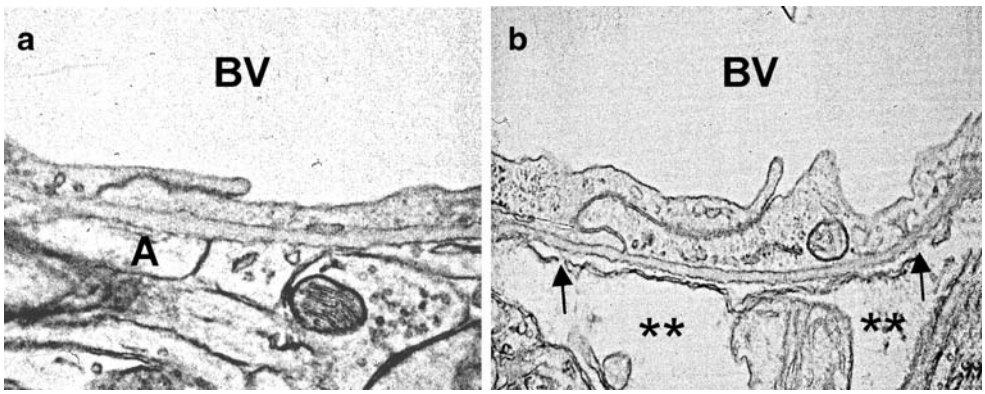

Fig. 3 TWEAK disrupts the structure of the NVU. Electron microscopy of cerebral arterioles in the left striatum of mouse brains injected with either PBS (**a**) or TWEAK (**b**). The *asterisks* show fluid-filled spaces indicative of developing edema in the TWEAK-treated brain (**b**). The *arrows* indicate places in the neurovascular unit with disruption of the *glia limitans* and detachment of the astrocytic processes. *BV* blood vessel, *A* astrocytic processes. Magnification ×30,000. (Reprinted with permission from Polavarapu et al. [40])

permeability of the NVU is determined not only by the integrity of the interendothelial tight junctions but also by the composition of the basal lamina [65] and the interaction between astrocytes, endothelial cells and the extracellular matrix (ECM) [68]. Therefore, it is plausible to propose that in response to the ischemic insult, there is the release of TWEAK from perivascular astrocytes, that may interact with Fn14 on astrocytes, EC, and microglia. This may induce the release of proinflammatory cytokines and metalloproteinases with resultant increase in the permeability of the NVU and development of cerebral edema. Indeed, it has been reported that proinflammatory cytokines released in response to the ischemic signal act directly on elements of the NVU with resultant increases in BBB permeability [69].

This proposed role for TWEAK and Fn14 in the regulation of the permeability of the NVU during cerebral ischemia was supported by the demonstration that either

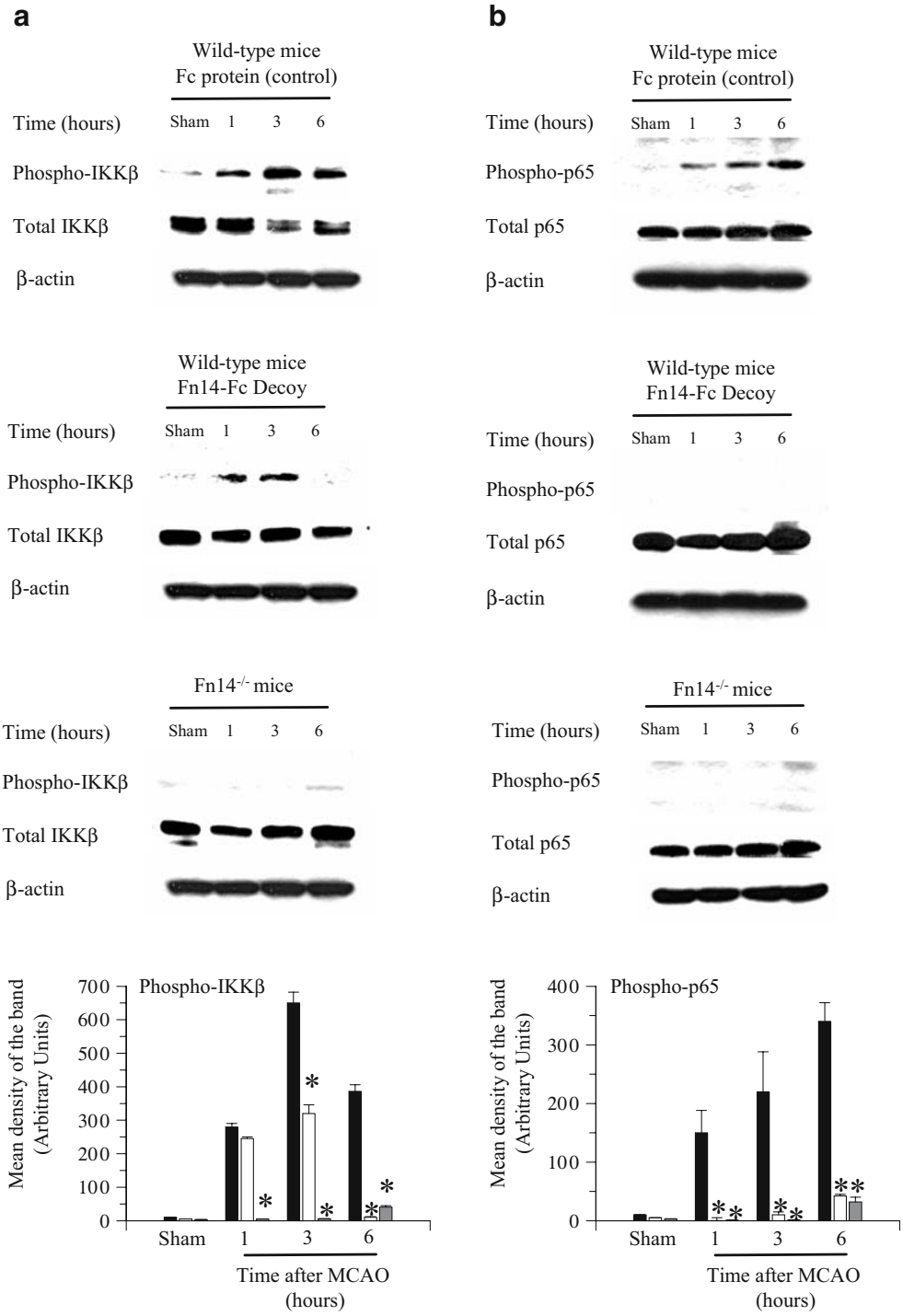

Fig. 4 TWEAK contributes to NF-κB pathway activation after MCAO. Western blot analysis of phospho-IKKβ and total IKK (**a**) and phospho-p65 and total p65 (**b**), in brain extracts of wild-type mice treated either with Fc protein or Fn14-Fc decoy immediately after MCAO, and Fn14$^{-/-}$ mice, at 0, 1, 3 and 6 h after the onset of the ischemic insult. Actin expression levels were assayed as a control for protein loading. Each experiment was repeated three times. *Graphs* describe the mean density of the band for a total of five observations for each time point. *Lines* depict SEM. *$p<0.05$. (Reprinted with permission from Zhang et al. [41])

treatment with Fn14-Fc decoy or genetic deficiency of Fn14 result in a 66–87% decrease in MCAO-induced Evans blue dye extravasation, a marker for BBB permeability [41], and in preservation of the interaction between astrocytic end-feet processes and the basement membrane in the ischemic area [41].

TWEAK and cerebral ischemia-induced NF-κB pathway activation NF-κB activity is significantly increased in animal models of ischemic stroke and data obtained from mice deficient in the NF-κB p50 protein indicates that NF-κB activation enhances ischemic neuronal death [70, 71]. Also, NF-κB inhibition using a recombinant adenovirus expressing a dominant negative form of IκBα reduced ischemic lesion volume in a murine model of cerebral ischemia [72]. However, whereas activation of the NF-κB pathway in microglial cells promotes neuronal degeneration, activation of this same pathway in neurons after ischemic insult appears to be neuroprotective [70, 72–74].

As described above, TWEAK induces activation of the NF-κB pathway in in vitro and in vivo systems, including neurons and perivascular astrocytes [40]. Based on these observations, it was proposed that TWEAK has a direct effect on NF-κB pathway activation during cerebral ischemia. This hypothesis was confirmed by the demonstration that inhibition of TWEAK activity after MCAO by either Fn14-Fc decoy administration or genetic deficiency of Fn14 inhibits the nuclear translocation of p65 and the phosphorylation of IKKβ and IκBα as early as 6 h after the onset of the ischemic insult [41] (Fig. 4). This effect of TWEAK on NF-κB pathway activation during cerebral ischemia has been confirmed by electrophoretic mobility shift assay (EMSA) using nuclear extracts from wild-type and Fn14$^{-/-}$ mice [41].

TWEAK and matrix metalloproteinase-9 activity Metalloproteinases are zinc-dependent endopeptidases that regulate cellular activity at multiple levels. Matrix metalloproteinases (MMPs) are a subfamily of metalloproteinases recognized as matrix-degrading enzymes and involved in physiological and pathological processes such as tissue remodeling during development, hemostasis, atherosclerosis, arthritis, cancer, and cerebral ischemia [75–79]. MMP-9 is an NF-κB-regulated gene [80]. TWEAK can induce MMP-9 activation when added to macrophages, Eph4 cells, and astrocytes cultured in vitro [6, 40]. Furthermore, intracerebral injection of TWEAK into the non-ischemic brain results in a significant increase in MMP-9 activity, and this effect is not observed in p50$^{-/-}$ mice [40]. In summary, TWEAK induces MMP-9 activation in the CNS through NF-κB pathway activation.

Early after the onset of the ischemic insult there is a progressive increase in MMP-9 activity [75–77] and there is a strong evidence indicating that MMP-9 plays a role in the pathological process leading to the increase in BBB permeability during cerebral ischemia [81, 82]. Indeed, MMP-9 deficient (MMP-9$^{-/-}$) mice have a significant decrease in the volume of the ischemic lesion and preservation of the integrity of the tight junction protein ZO-1 after the onset of the ischemic insult [81, 82]. A role for TWEAK in MCAO-induced MMP-9 activation has been demonstrated by the finding that inhibition of TWEAK by either treatment with Fn14-Fc decoy receptor or genetic deficiency of Fn14 results in a significant attenuation of cerebral ischemia-induced MMP-9 activation [41] (Fig. 5). The effect of Fn14-Fc decoy on MMP-9 activation is dose-dependent and parallels a progressive decrease in MCAO-induced Evans blue dye extravasation [41].

TWEAK and the basement membrane The interaction between astrocytes and the basement membrane is one of the most important determinants of the permeability of the NVU [83, 84]. The main components of the basement membrane are laminins, collagen type IV, fibronectin and proteoglycans [65]. Early after the onset of the ischemic insult, there is a rapid degradation of laminin in the vascular basement membrane [85, 86], and it has been proposed that

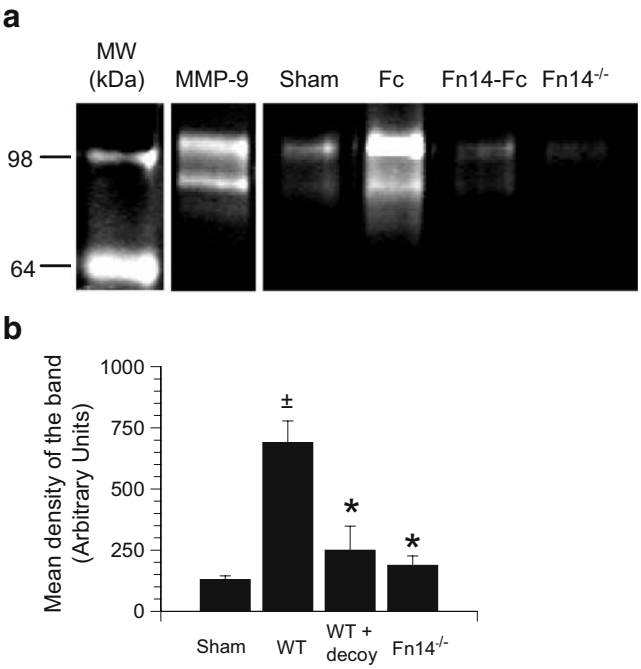

Fig. 5 TWEAK contributes to MMP-9 activation after MCAO. **a** Gelatin zymography assay for MMP-9 activity 6 h after MCAO in sham-operated wild-type mice (*Sham*), wild-type mice treated with Fc protein (*Fc*), wild-type treated with Fn14-Fc decoy (*Fn14-Fc*), and Fn14$^{-/-}$ mice. Each experiment was repeated four times. **b** Quantification of the mean density of the bands (n=4). *Bars* represent the mean density of the bands in sham, wild-type treated with Fc (*WT*), wild-type treated with Fn14-Fc decoy (*WT+decoy*), and Fn14 deficient (*Fn14*$^{-/-}$) mice. *Error bars* represent SEM. *p<0.005 compared with wild-type brains. **p<0.005 compared with sham animals. (Reprinted with permission from Zhang et al. [41])

laminin is a substrate for MMP-9 [87]. As MMP-9 is an NF-κB-regulated gene [88] and TWEAK induces NF-κB pathway activation, it was postulated that during cerebral ischemia TWEAK induces laminin degradation through induction of MMP-9 activation. This hypothesis is supported by the demonstration that inhibition of TWEAK activity by either Fn14-Fc decoy receptor or genetic deficiency of Fn14 results in a significant inhibition of MCAO-induced laminin degradation [41].

In conclusion, in consideration of the information available to this date, we can propose a model where in response to the ischemic insult the interaction between TWEAK and Fn14 in perivascular astrocytes results in the activation of the NF-κB pathway. This induces the release of proinflammatory cytokines and metalloproteinases with resultant increase in the permeability of the NVU and cell death. A role for TWEAK and Fn14 in neurons and EC is still under investigation.

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic progressive disease characterized by loss of myelin in the CNS with subsequent neuronal damage and marked physical disability [89, 90]. This disease affects 0.05–0.15% of the Caucasian population and affects women more frequently than men [91]. Experimental autoimmune encephalomyelitis (EAE) is a research model for multiple sclerosis [92]. There is a growing body of evidence indicating a strong relation between MS and members of the TNFSF. Indeed, the presence of TNF-α in the CNS correlates with disability in patients with MS [93], and expression of this cytokine precedes clinical relapses by few days [68, 94]. It is thought that the induction of cell adhesion molecules on cerebral endothelial cells and increase in the permeability of the BBB are some of the main mechanisms whereby members of the TNFSF play a role in the pathogenesis of MS [68, 94].

A role for TWEAK in the pathogenesis of MS has been proposed. Indeed, TWEAK mRNA increases in the spinal cord during EAE and the clinical severity of EAE is significantly enhanced in TWEAK-overexpressing transgenic mice [55]. In addition, TWEAK stimulates the monocyte chemotactic protein-1 (MCP-1) expression by astrocytes and EC [54], and induction of specific inhibitory antibodies by treatment with either TWEAK or Fn14 resulted in amelioration of the development of inflammatory infiltrates in the spinal cord and in a better clinical outcome in a rat model of EAE [95]. A potential application for inhibition of TWEAK activity in multiple sclerosis was further supported by the demonstration that treatment with neutralizing anti-TWEAK antibodies in a model of EAE results in a reduction in the severity of the disease and leukocyte infiltration when mice were treated after the priming phase [57].

Conclusions

TWEAK and Fn14 play a role in the pathogenesis of several neurological diseases associated with inflammation, edema, and cell death. The interaction between TWEAK and Fn14 induces activation of the NF-κB pathway in EC, astrocytes, microglia, and neurons. This results in the induction and release of proinflammatory cytokines known to have a direct effect on the permeability of the NVU and cell survival. Thus, inhibition of the TWEAK-FN14 interaction may have a therapeutic role for CNS diseases characterized by inflammatory infiltration and edema such as cerebral ischemia, multiple sclerosis, head trauma, and autoimmune disorders involving the brain.

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