REVIEW

The TWEAK-Fn14 system as a potential drug target

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Fibroblast growth factor-inducible 14 (Fn14) is a member of the tumour necrosis factor (TNF) receptor family that is induced in a variety of cell types in situations of tissue injury. Fn14 becomes activated by TNF-like weak inducer of apoptosis (TWEAK), a typical member of the TNF ligand family. TWEAK is constitutively expressed by monocytes and some tumour cell lines and also shows cytokine inducible expression in various other cell types. Fn14 activation results in stimulation of signalling pathways culminating in the activation of NFκB transcription factors and various MAPKs but might also trigger the PI3K/Akt pathway and GTPases of the Rho family. In accordance with its tissue damage-associated expression pattern and its pleiotropic proinflammatory signalling capabilities, the TWEAK-Fn14 system has been implicated in a huge number of pathologies. The use of TWEAK- and Fn14-knockout mice identified the TWEAK-Fn14 system as a crucial player in muscle atrophy, cerebral ischaemia, kidney injury, atherosclerosis and infarction as well as in various autoimmune scenarios including experimental autoimmune encephalitis, rheumatoid arthritis and inflammatory bowel disease. Moreover, there is increasing preclinical evidence that Fn14 targeting is a useful option in tumour therapy. Based on a discussion of the signalling capabilities of TWEAK and Fn14, this review is focused on two major issues. On the one hand, on the molecular and cellular basis of the TWEAK/Fn14-related pathological outcomes in the aforementioned diseases and on the other hand, on the preclinical experience that have been made so far with TWEAK and Fn14 targeting drugs.

Abbreviations

CIA, collagen-induced arthritis; EAE, experimental autoimmune encephalitis; Fn14, fibroblast growth factor inducible; IBD, inflammatory bowel diseases; IECs, intestinal epithelial cells; MACO, middle cerebral artery occlusion; RA, rheumatoid arthritis; SLE, systemic lupus erythematous; SMCs, smooth muscle cells; TWEAK, tumour necrosis factor (TNF)-like weak inducer of apoptosis

Introduction

Tumour necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) is a typical member of the TNF ligand family and is as such initially expressed as type II class transmembrane glycoprotein from which a soluble ligand form can be released by proteolytic processing (Bodmer *et al.*, 2002). The 249 amino acid-comprising membrane-bound form of TWEAK consists extracellularly of the characteristic C-terminally located TNF homology domain (THD), which is separated from the transmembrane domain by a stalk region of about 55 amino acids (Figure 1A). As in other ligands of the

TNF family, the THD of TWEAK mediates assembly into homotrimeric proteins and receptor binding while the stalk region contains the recognition site for proteases involved in ligand processing, in case of TWEAK, serine proteases of the furin family. The short intracellular N-terminal domain of TWEAK is highly conserved like the rest of the molecule but its functional relevance is still unclear. Although TWEAK expression has been documented at the mRNA level in a variety of cell types and cell lines, cell surface expression has so far only be reported for monocytes, dendritic cells, interferon-γ stimulated NK cells (Nakayama *et al.*, 2000; Felli *et al.*, 2005; Maecker *et al.*, 2005), multiple sclerosis
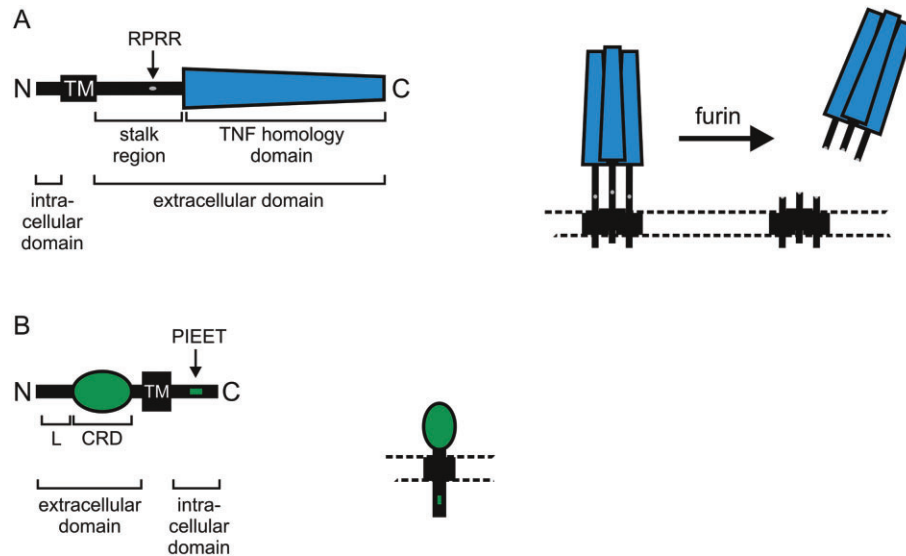
Figure 1

Domain architecture of TWEAK (A) and Fn14 (B). The amino acid sequences of the furin recognition site of TWEAK and the TRAF-binding motif of Fn14 are indicated in the single-letter code.

patient-derived monocytes (Desplat-Jego et al., 2009), a few colonic adenocarcinoma (Kawakita et al., 2005) and hepatocellular carcinoma (Kawakita et al., 2004) cell lines and the MDA-MB-231 breast cancer cell line (Willis et al., 2008). Induction of TWEAK expression has frequently reported in scenarios of tissue damage and hypoxia. In line with this, the hypoxia regulated forkhead transcription factor FOXO3a and interferon-γ have been implicated in TWEAK up-regulation (Baxter et al., 2006). Otherwise the transcriptional regulation of TWEAK has been poorly investigated.

With a molecular weight of about 14 kDa, fibroblast growth factor inducible (Fn14) is an unusual small member of the TNF receptor family (Wiley et al., 2001). Fn14 and B cell maturation, BaffR and the short isoform of transmembrane activator and calcium-modulator and cytophilin ligand interactor are the only TNF receptors which only contain a single cystein-rich domain in their ectodomain while all other TNF receptors have two to six copies of this characteristic motif (Figure 1B, Locksley et al., 2001). Moreover, the cytoplasmic domain of Fn14 only comprises 28 amino acid residues. TNF receptors can be categorized into three classes: (i) death receptors that contain a conserved protein-protein interaction domain, called the death domain, in their cytoplasmic part and which are often able to trigger apoptosis, (ii) TNF receptor associated factor (TRAF)-interacting receptors that harbour one or more binding motifs for adapter proteins of the TRAF family in their cytoplasmic part and (iii) decoy receptors without own signalling capacity that occur either as soluble or membrane-attached proteins. Fn14 is only equipped with a single TRAF binding motif and is thus a typical TRAF-interacting TNF receptor. Fn14 is a single spanning transmembrane type I receptor that has been initially identified as a transcriptional target of fibroblast growth factor-1 in NIH 3T3 fibroblasts (Meighan-Mantha et al., 1999). Only later, it has been recognized as a TNF receptor family member able to transduce TWEAK signals (Wiley et al.,

2001). Fn14 is found to been highly expressed on most tumour cell lines of non-lymphoid origin. Immunohistochemical analysis of murine tissue samples and human biopsy specimens, however, revealed a more differentiated expression pattern of Fn14 in vivo. Low expression of Fn14 has been reported for fibroblasts and endothelial and epithelial cells of healthy homeostatic tissue. In contrast, high Fn14 expression was regularly found on epithelial and mesenchymal progenitor cells and especially in contexts of tissue damage caused by different insults including hypoxia, oxidative stress, chemical and mechanical injuries, inflammation and tumour growth (Winkles, 2008; Burkly et al., 2011). It is therefore not only surprising that induction of Fn14 expression has been reported for a variety of immune diseases, including hepatitis, glomerulonephritis, rheumatic arthritis, multiple sclerosis and bowels disease but also in atherosclerosis and after stroke and heart attack. Furthermore, up-regulation of Fn14 has been found in melanoma, glioma and non-small cell lung, breast, liver and prostate cancer (Feng et al., 2000; Tran et al., 2006; Sanz et al., 2012; Whitsett et al., 2012; Chao et al., 2013; Zhou et al., 2013). In accordance with the tissue damage-associated expression pattern of Fn14, several growth factors, including EGF and VEGF, as well as cytokines, such as IFN-γ, TNF and TGF-β, that are released by immune cells associated with tissue repair processes, have found to be able to induce Fn14 expression (Donohue et al., 2003; Hosokawa et al., 2006; Ebihara et al., 2009; Sanz et al., 2012; Whitsett et al., 2012).

Besides Fn14, the TNF receptor family member DR3 and CD163, a cysteine rich haemoglobin scavenger receptor expressed on monocytes and macrophages, have been claimed to be TWEAK receptors (Marsters et al., 1998; Bover et al., 2007). However, both the TWEAK-DR3 interaction as well as the TWEAK-CD163 interaction failed to be confirmed in other studies (Schneider et al., 1999; Kaptein et al., 2000; Fick et al., 2012).

TWEAK/Fn14-stimulated signalling pathways and resulting cellular responses

In the initial study, describing its cloning and preliminary characterization, TWEAK got its name due to the observation that it induces cell death in IFN-γ sensitized HT29 cells but not in a panel of other cell lines (Chicheportiche *et al.*, 1997). Although it has been found in recent years that TWEAK also efficiently induces cell death in a few other cell lines (e.g. SKOV-3, OVCAR-4, Kym-1), this quality of cellular response remains still the rare exception for the TWEAK-Fn14 system. Other ligands of the TNF family triggering robust cell death responses, such as TNF, TNF-related apoptosis inducing ligand (TRAIL) and CD95L, do this by stimulation of receptors belonging to the above mentioned death receptor subgroup of the TNF receptor family. The characteristic death domain of death receptors either serves directly as the hub for the formation of a complex catalyzing the activation of caspase-8 or triggers the formation of a cytoplasmic caspase-8 activating protein complex (Dickens *et al.*, 2012). Activated caspase-8 then further propagates the apoptotic signal. Death receptors also interact with the death domain-containing serine/threonine kinase RIP1 which allows them to trigger,

under defined circumstances, an alternative necrotic form of programmed cell death (Dickens *et al.*, 2012). Now, Fn14 belongs to the TRAF-interacting subgroup of TNF receptors raising the obvious question how it accomplishes cell death induction without having a death domain. At least for the most sensitive cell lines Kym-1, SKOV-3 and OVCAR-4, the counterintuitive apoptotic signalling capabilities of the TWEAK-Fn14 system could be traced back to an indirect mechanism, namely the induction of TNF and subsequent stimulation of the prototypic death receptor TNFR1 (Schneider *et al.*, 1999; Vince *et al.*, 2008; Figure 2). In experiments where TNFR1 stimulation has been uncoupled from the activity of TWEAK-induced TNF by the use of TNF-specific antibodies to neutralize TWEAK-induced TNF and agonistic TNFR1-specific antibodies to trigger TNFR1, TWEAK treatment enabled per se non-toxic concentrations of the latter to efficiently induce cell death (Schneider *et al.*, 1999). Thus, TWEAK-induced apoptosis not only relies on the induction of TNF but also involves a second per se non-toxic sensitizing mechanism. TNF-induced cell death is also strongly enhanced by TWEAK and Fn14 in per se TWEAK resistant cell lines suggesting that this sensitizing mechanism reflects a common feature of Fn14 activation (Wicovsky *et al.*, 2009). Apoptosis induction by the TNFR1-related TRAIL death receptors is not or only poorly enhanced by TWEAK treatment
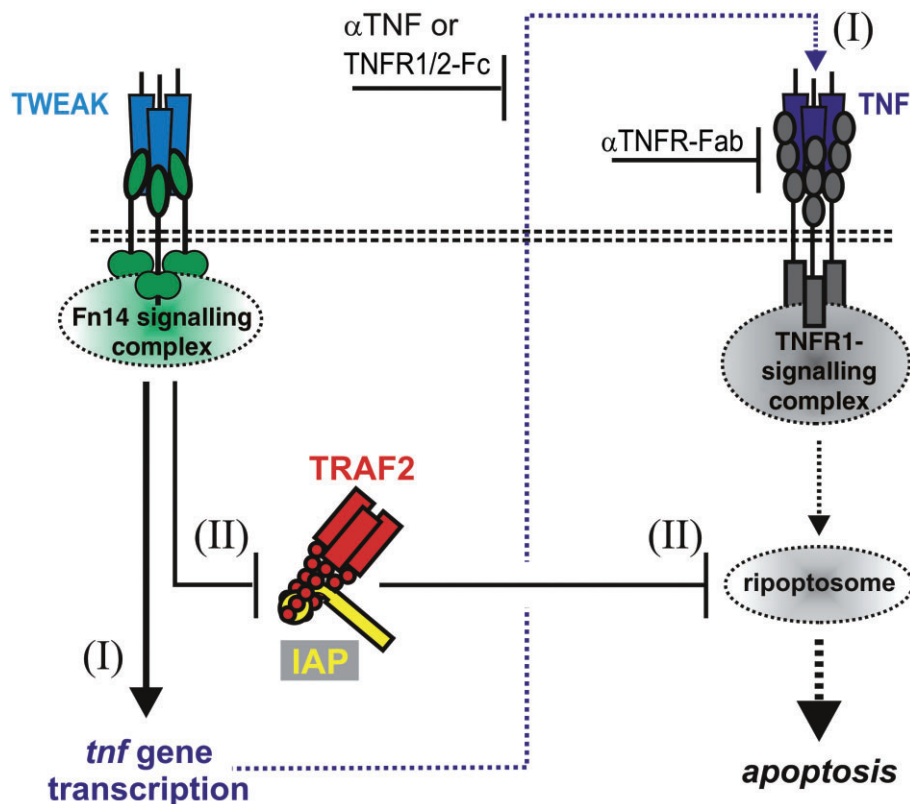

Figure 2

Mechanisms of TWEAK/Fn14-induced apoptosis. Binding of soluble or membrane TWEAK to Fn14 results in the recruitment of TRAF2-cIAP1/2 complexes and their depletion from the cytosol. This triggers two events that in a concerted action stimulate cell death in apoptosis-proficient cells: NFκB-mediated induction of TNF (I) and sensitization for TNFR1-induced ripoptosome activity (II). Thus, TWEAK-induced apoptosis eventually rely on activation of the death receptor TNFR1 and is thus inhibitable by TNF and TNFR1 blocking antibodies.

further indicating that Fn14 signalling affects an apoptosis-relevant issue of special relevance in TNFR1 signalling (Wicovsky *et al.*, 2009). In fact, enhancement of TNFR1-mediated apoptosis by TWEAK has been ascribed to the capability of activated Fn14 to recruit TRAF2-cIAP1/2 complexes. TRAF2 is a typical member of the aforementioned TRAF adapter protein family and possesses a really interesting new gene (RING) domain possibly with E3 ligase activity (Gonzalvez *et al.*, 2012). Cellular inhibitor of apoptosis-1 (cIAP1) and cIAP2 are also RING domain E3 ligases which possess besides the RING domain also a caspase recruitment domain and three baculovirus inhibitor of apoptosis (IAP) repeat domains involved in substrate recognition. Now, TRAF2-cIAP1/2 complexes are efficiently recruited to TNFR1 and are also part of the secondarily formed TNFR1-induced cytosolic caspase-8 activating complex but do not (or only poorly) interact with the caspase-8 activating plasma membrane-associated TRAIL death receptor signalling complex. As the expression levels of TRAF2-cIAP1/2 complexes are rather low compared to Fn14 expression in most cells, TWEAK-induced recruitment of TRAF2-cIAP1/2 complexes to Fn14 results in depletion of the cytosolic pool of these molecules and restricts so their availability for TNFR1 (Vince *et al.*, 2008; Wicovsky *et al.*, 2009). As a consequence, TNFR1 activation leads to enhanced apoptosis induction provided caspase-8 activation is not inhibited by other independently acting anti-apoptotic proteins, for example FLIP or Bcl2. It is worth mentioning that other TRAF2 interacting TNF receptors can substitute for Fn14 in the TNF-Fn14 cross-talk. Indeed, TRAF2 depletion-mediated enhancement of TNFR1-induced cell death has originally been described for TNFR2 and has also been demonstrated for CD30 and CD40 (Duckett and Thompson, 1997; Weiss *et al.*, 1997; 1998; Grell *et al.*, 1999; Fotin-Mleczek *et al.*, 2002). Moreover, SMAC mimetics, a novel group of anticancer drugs currently under investigation in clinical trials trigger proteasomal degradation of cIAP1 and cIAP2 (for review see Fulda and Vucic, 2012) and modulate TNFR1 signalling in a similar fashion to TWEAK and Fn14. SMAC mimetics, however, not only trigger cIAP1/2 degradation but also inhibit the anti-apoptotic xIAP protein (Fulda and Vucic, 2012) and thus let expect a broader apoptosis sensitizing activity than TWEAK. Last but not least, cell death induction by the non-death receptor TNFR2 and SMAC mimetics has also been traced back to the cooperative activity of endogenous TNF and depletion of cIAP1/2 and TRAF2 (Grell *et al.*, 1999; Fulda and Vucic, 2012). Thus, the TRAF2-cIAP1/2 based mechanisms underlying the Fn14-TNFR1 crosstalk are of general nature and are also of relevance for other TRAF2 interacting TNF receptors and SMAC mimetics.

A few studies also describe TNF-independent modes of TWEAK/Fn14-induced cell death in cortical neurons and normal human keratinocytes. The underlying signalling mechanisms, however, have been poorly analysed so far (Haile *et al.*, 2010a; Sabour Alaoui *et al.*, 2012).

Depletion of the cytoplasmic TRAF2-cIAP1/2 pool and enhancement of TNFR1-induced caspase-8 activation are not the only consequences of TWEAK-induced binding of the TRAF2-cIAP1/2 complex to Fn14. TRAF2, cIAP1 and cIAP2 are also crucially involved in the regulation of non-apoptotic signalling pathways triggered by pattern recognition receptors and members of the TNF receptor family. Consequently,
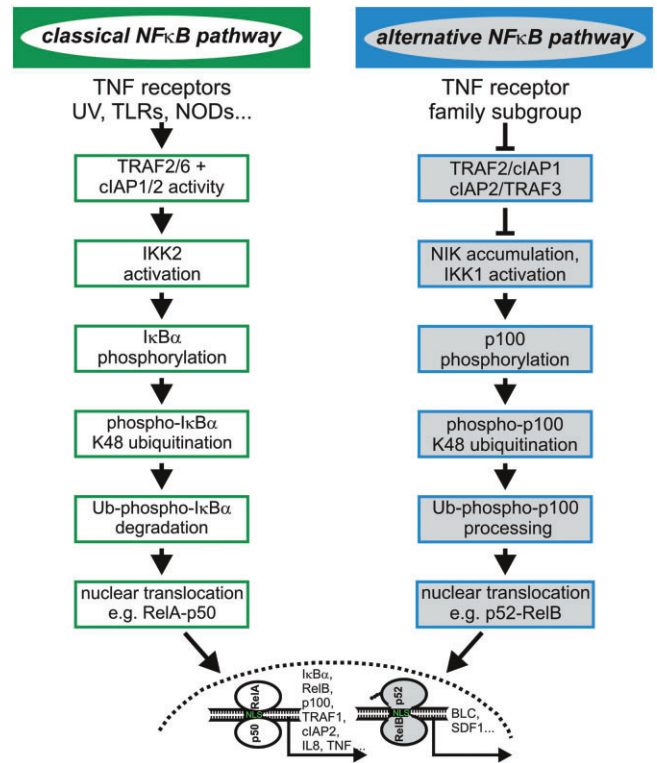

Figure 3

Activation of NFκB transcription factors. Two signalling pathways control nuclear translocation of the dimeric transcription factors of the NFκB family: the classical and alternative NFκB pathway. Noteworthy, the proteins TRAF2, cIAP1 and cIAP2 act as positive mediators in the classical pathway but as inhibitors in the alternative pathway. Please be aware that there can be significant crosstalk between the two pathways in a cell type-specific manner by various mechanisms, including NIK-mediated activation of the IKK complex, inhibition of p65-p50 dimers by p100 and transcriptional upregulation of p100 and RelB via the classical pathway.

these molecules are also of central relevance in TWEAK/Fn14-induced activation of various MAPK family members and transcription factors of the NFκB family (Vince *et al.*, 2008). Fn14-mediated activation of NFκBs is comparably well understood and account for many of the proinflammatory effects that have been described for the TWEAK-Fn14 system (Winkles, 2008; Burkly *et al.*, 2011). The NFκBs comprise homo- and heterodimers formed by the five members of the Rel transcription factor family (Hayden and Ghosh, 2012). NFκBs substantially differ in the way they become activated and also in their target genes. There are two prototypic signalling pathways that lead to activation of distinct NFκB dimers, the classical and the alternative NFκB pathway (Figure 3, (Hayden and Ghosh, 2012). TWEAK and Fn14 are principally able to stimulate both pathways. Central step in the alternative NFκB pathway is the inhibitor of kappaB kinase (IKK)1-mediated phosphorylation of the p100 precursor form of the p52 NFκB subunit. This results in K48-ubiquitination and proteasomal processing of p100 to p52 and nuclear translocation of p52-containing NFκB dimers (Hayden and Ghosh, 2012). Now, IKK1 activation requires

phosphorylation by NIK, a cytosolic MAP3-kinase that is constitutively degraded in non-stimulated cells by a TRAF2-cIAP1/2 complex-dependent mechanism. TRAF2 recruits NIK by interaction with the NIK-binding protein TRAF3 and subjected it so to K48-ubiquitination by the TRAF2-associated IAPs and subsequent proteasomal degradation (Hayden and Ghosh, 2012). Thus, in context of the alternative NFκB pathway cytosolic TRAF2-cIAP1/2 complexes fulfil an inhibitory function that is antagonized by their TWEAK-induced recruitment to membrane-bound Fn14. In contrast, activation of the classical NFκB pathway by TWEAK and various other ligands of the TNF family requires stimulatory activities of TRAF2 and the cIAPs (Hayden and Ghosh, 2012). NFκB dimers activated via the classical NFκB pathway are retained in the cytoplasm of unstimulated cells by forming a ternary complex with inhibitory proteins, the IκBs. Central step in the classical NFκB pathway is the activation of the IKK complex, which contains the scaffolding protein NEMO, IKK2 and IKK1 whereby the latter appears largely dispensable for the function of the IKK complex in classical NFκB signalling. The IKK complex phosphorylates IκB proteins triggering their proteasomal degradation and nuclear translocation of the released NFκB dimers. The TRAF2-cIAP1/2 complex plays a dual role in activation of the classical NFκB pathway. While TRAF2 acts as an adapter protein that recruits the IKK complex to activated TNF receptors, the cIAPs K63-ubiquitinate the IKK subunit NEMO, and in context of TNFR1 signalling also RIP, creating docking sites for a variety of ubiquitin binding proteins. The latter are involved in IKK activation, for example, the IKK2 phosphorylating TAB2-TAB3-TAK1 complex or the linear ubiquitin chain assembly complex (LUBAC), which creates additional docking sites for ubiquitin binding proteins, but also comprises antagonistic factors such as A20 and Cyld, two deubiquitinating enzymes. The relevance of the IKK2, TRAF2, TAK1 and the cIAPs for Fn14-mediated activation of the classical NFκB pathway has been recently demonstrated by help of knockout mice-derived fibroblasts, siRNA experiments, dominant-negative ubiquitin binding domains and IAP antagonists (Saitoh et al., 2003; Kumar et al., 2009; Sims et al., 2012; Varfolomeev et al., 2012). The role of most of the ubiquitin binding proteins interacting with ubiquitinated NEMO or TRAF2, however, has so far only been studied in detail in context of TNFR1 signalling.

Notably, soluble TWEAK only triggers weak and delayed activation of the classical NFκB pathway despite concomitant strong stimulation on the alternative NFκB pathway while membrane TWEAK expressing cells trigger both pathways efficiently. Likewise, artificial immobilization on a cell surface transforms soluble TWEAK from a weak to a strong activator of classical NFκB signalling while having no major effect on the efficacy of activation of the alternative NFκB pathway (Roos et al., 2010). Thus, the enhanced efficacy with which membrane bound TWEAK stimulates classical NFκB signalling seems to be primarily related to the spatially restricted way of how membrane TWEAK is presented to Fn14 and does not reflect a need for sequence information that is only available in membrane TWEAK. Robust activation of the classical NFκB pathway can also be achieved with dimerized or oligomerized trimers of soluble TWEAK. This has, however, no major effect on the dose dependency of TWEAK-induced activation of the

alternative NFκB pathway or on Fn14 occupation by TWEAK (Fick et al., 2012). It is therefore tempting to speculate that the secondary interaction of two or more initially formed TWEAK-Fn14-TRAF2-cIAP1/2 complexes is required for activation of the classical NFκB pathway while the sole recruitment of the TRAF2-cIAP1/2 complex is already fully sufficient for triggering of alternative NFκB signalling. This hypothesis is also in good accordance with the stoichiometry of the TRAF2-cIAP1/2 complex and the mechanisms of activation of the E3 ligase activity of cIAP1 and cIAP2. On the one hand TRAF2 forms homotrimeric molecules that can interact with three Fn14 molecules but only bind a single molecule of cIAP1 or cIAP2 (Zheng et al., 2010). On the other hand, there is evidence that activation of the E3 ligase activity of cIAPs requires trans-activation of two molecules (Dueber et al., 2011; Feltham et al., 2011). Binding of three molecules of Fn14 by soluble TWEAK trimers is therefore sufficient to recruit TRAF2-cIAP1/2 complexes and to keep them from triggering degradation of NIK in the cytosol while Fn14-mediated activation of the classical NFκB pathway needs additionally secondary interaction of two TWEAK-Fn14-TRAF2-cIAP1/2 complexes to ensure trans-activation of two cIAP molecules. Thus, the parallel orientation and the limited mobility of membrane TWEAK in the plasma membrane seem to facilitate by yet poorly understood mechanisms the secondary interaction of TWEAK induced Fn14 signaling complexes and this secondary interaction is required to fully unleash all Fn14 signaling activities (Figure 4).

Activation of the MAPKs ERK, JNK and p38 by TWEAK and Fn14 has also been repeatedly reported and appears to involve, at least in case of JNK and p38 signalling, again TRAF2, cIAPs and TAK1 (Kumar et al., 2009; Varfolomeev et al., 2012). Regulation of the PI3K/Akt pathway by TWEAK has also been described. On the one hand, an inhibitory effect of TWEAK/Fn14 signalling on Akt activity has been shown in C2C12 myotubes (Dogra et al., 2007a,b) and hepatocarcinoma cell lines (Feng et al., 2008); on the other hand, activation of Akt by TWEAK has been reported in renal tubular cells (Sanz et al., 2009), gingival fibroblasts (Hosokawa et al., 2012), glioma cell lines (Fortin et al., 2009) and osteoblastic cells (Ando et al., 2006). The molecular basis of the opposing effects of TWEAK on the PI3K/Akt pathway is currently not understood. Rather unusual for members of the TNF receptor/ligand family, there are reports showing TWEAK/Fn14-induced activation of the Rho GTPases Cdc42 and Rac1 (Tran et al., 2006; Fortin et al., 2012) and stimulation of the JAK2 kinase (Ucero et al., 2013). TWEAK-induced activation of JAK2 in the latter study took place very rapidly within a couple of minutes and in so far clearly differs from another recent report demonstrating a delayed indirect activation of the JAK/STAT pathway by TWEAK-induced upregulation of interferon-β (Chapman et al., 2013). Ligand-induced recruitment to Fn14 has been shown for Rac1 as well as for Cdc42 but epistatic siRNA experiments placed Cdc42 upstream of Rac1. To fulfil their function in Fn14 signal transduction, the two GTPases depend on the support of two guanine nucleotide exchange factors, Ect2 that stimulates Cdc42 and Tio, which activates Rac1 (Fortin et al., 2012). TWEAK-induced activation of Cdc42/Ect2 and Rac1/Tio results in glioma cells in enhanced cellular migration and lamellipodia formation.
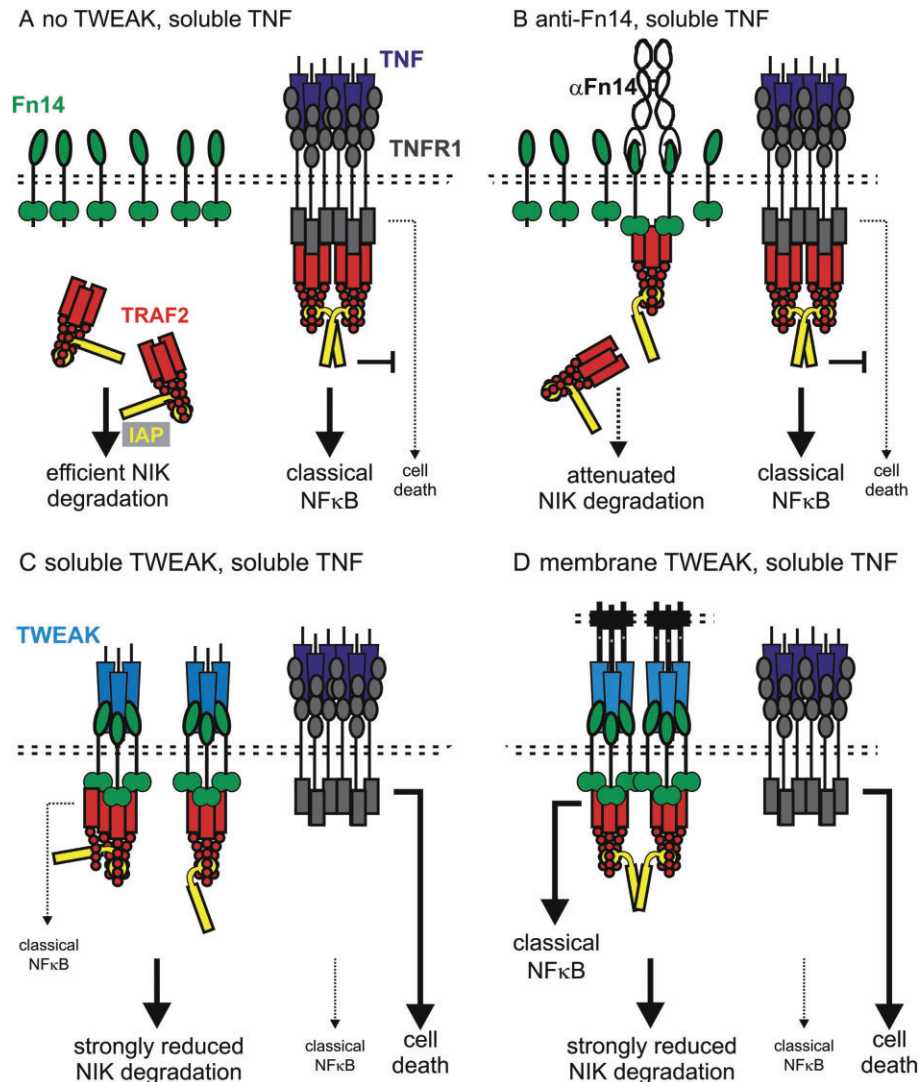
Figure 4

Activation of Fn14-associated signalling pathways. (A) In non-stimulated cells TRAF2-cIAP1/2 are localized in the cytoplasm and trigger NIK degradation. TNFR1 signalling is not affected and dominated by classical NFκB activation. (B) Fn14 antibodies induce Fn14 dimers which poorly recruit TRAF2-cIAP1/2 complexes. This results in weak depletion of the cytosolic TRAF2-cIAP1/2 pool, which can be amplified by an enzymatic cascade leading to p100 processing but is insufficient to relief the ripoptosome from its inhibition by TRAF2-cIAP1/2 complexes. (C) Binding of soluble TWEAK trimers to Fn14 results in recruitment and depletion of cytosolic TRAF2-cIAP1/2 but not in the transactivation of the single inhibitor of apoptosis (IAP) molecules associated with a TRAF2 trimer. Soluble TWEAK thus sensitizes for ripoptosome-dependent cell death signalling and triggers the alternative NFκB pathway, responses otherwise suppressed by cytosolic TRAF2-cIAP1/2. (D) Binding of membrane TWEAK (or hexameric/oligomeric soluble TWEAK or immobilized Fn14 antibodies) to Fn14 not only result in recruitment and depletion of cytosolic TRAF2-cIAP1/2 but also in the transactivation of TRAF2-associated IAP molecules. This triggers the signalling events mentioned under 'C' and in addition in signalling pathways dependent on cIAP activity, such as the classical NFκB pathway.

TWEAK and Fn14 in autoimmune diseases

TWEAK closely resembles TNF in so far that it also stimulates in a variety of cell types proinflammatory pathways. In view of the overwhelming importance of TNF in autoimmune diseases, several groups addressed therefore in animal models a possible similar role of the TWEAK-Fn14 system. Analysis of sera of mice suffering on collagen-induced arthritis and human specimens revealed increased TWEAK levels (Perper *et al.*, 2006; Park *et al.*, 2008). In collagen-induced arthritis (CIA), neutralizing TWEAK antibodies reduced disease score and histological observable defects but failed to interfere with the disease causing immune response to collagen (Kamata *et al.*, 2006; Perper *et al.*, 2006). In accordance with the well-established proangiogenic function of the TWEAK-Fn14 system, TWEAK blockade further inhibited angiogenesis in

the synovium of CIA mice (Kamata *et al.*, 2006; Perper *et al.*, 2006). A disease-driving role of TWEAK and Fn14 in rheumatoid arthritis (RA) is also in line with *in vitro* data showing that TWEAK induces expression of cytokines and adhesion molecules in synovial fibroblasts (Kamijo *et al.*, 2008; Yamana *et al.*, 2012). It is noteworthy that one of the studies reporting TWEAK-induced production of proinflammatory cytokines showed furthermore that TWEAK concomitantly inhibits the much stronger proinflammatory effect of TNF on RA synovial fibroblasts (Yamana *et al.*, 2012). The latter may reflect the antagonizing effect of soluble TWEAK-induced depletion of TRAF2-cIAP1/2 complexes on TNFR1 signalling discussed above and may require attention in the development of TWEAK neutralizing treatment regimes for RA (Yamana *et al.*, 2012).

A pivotal role of the TWEAK-Fn14 system has also been observed in models of inflammatory bowel diseases (IBD). Both TWEAK and Fn14 deficiency protect from trinitrobenzene sulfonic acid-induced colitis and interleukin (IL)10 deficiency-triggered spontaneous colitis (Dohi *et al.*, 2009; Kawashima *et al.*, 2011). These results are in accordance with complementary experiments demonstrating a crucial role of the TWEAK-Fn14 system in immune stimulation and cell death induction in intestinal epithelial cells (IECs) by γ-irradiation and IL13 (Dohi *et al.*, 2009; Kawashima *et al.*, 2011). Cell death induction in IECs represents an early key event in IBD pathogenesis and leads to breakdown of the mucosal epithelial barrier, subsequent activation of immune cells by luminal bacteria-derived pathogen associated molecular patterns and propagation and persistence of inflammatory processes. Now, γ-irradiation induces cell death in IECs and this is inhibited in TWEAK and Fn14-knockout mice (Dohi *et al.*, 2009). In accordance with data showing a pivotal function of IL13 in γ-irradiation-induced IEC cell death, it has furthermore observed that IL13 induces IEC death in primary jejunum cultures by a TWEAK- and Fn14-dependent mechanism (Kawashima *et al.*, 2011). Noteworthy, the cytotoxic effect of IL13 on IECs appears not only to be dependent on TWEAK but also on TNF. IL13-induced IEC apoptosis was blocked by a soluble TNF receptor fusion protein (Table 1) and apoptosis induction in IECs by TNF was reduced in Fn14-knockout cells. Indeed, inhibition of TNF shedding in jejunum tissue reduced IL13-induced caspase activation. Moreover, in the IL10-knockout model of spontaneous colitis, where IL13 plays a major role, TWEAK and Fn14 deficiency not only inhibit colitis pathogenesis but also up-regulation of TNF. It is tempting to speculate that the concerted action of TNF and TWEAK observed in IL13-induced IEC cell death mirrors the *in vitro* data discussed before showing sensitization for TNFR1-induced cell death by Fn14-mediated depletion of protective TRAF2-cIAP1/2 complexes.

Based on *in vitro* data showing induction of proinflammatory genes in astrocytes and microglia (Saas *et al.*, 2000; Desplat-Jego *et al.*, 2002), early on a role of the TWEAK-Fn14 system has been addressed in animal models of demyelinating disorders. Both, in the microglia-driven cuprizone model and the CD4+ T-cell driven myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) model, increased expression of TWEAK and Fn14 has been reported (Desplat-Jego *et al.*, 2002; 2005; Iocca *et al.*, 2008). More important, disease severity in these models

were reduced by various means including TWEAK deficiency, vaccination with recombinant TWEAK or Fn14 and TWEAK-specific antibodies but were enhanced in TWEAK transgenic animals. Similar to the role of the TWEAK-Fn14 system in RA, TWEAK blockade failed in the EAE model to inhibit the disease-inducing anti-MOG T-cell response and rather affected production of proinflammatory proteins involved in immune cell recruitment and control of the permeability of the blood brain barrier (BBB; (Desplat-Jego *et al.*, 2005; Razmara *et al.*, 2009). In accordance with an involvement of TWEAK and Fn14 in multiple sclerosis, increased expression of these molecules has recently also been reported for brain tissue derived from diseased patients and TWEAK-induced leakiness has been observed in an *in vitro* model of the BBB using human cerebral microvascular endothelial cells (Serafini *et al.*, 2008; Stephan *et al.*, 2013).

Morbidity and mortality of patients suffering on systemic lupus erythematous (SLE) often result from the secondary development of kidney disease (lupus nephritis). The TWEAK-Fn14 system has been implicated in several aspects of lupus nephritis that eventually base again on its capability to trigger concerted cellular responses culminating in inflammation, angiogenesis, proliferation, fibrosis and cell death. Higher levels of urinary TWEAK are found in SLE patients suffering on lupus nephritis than in SLE patients without renal involvement (Schwartz *et al.*, 2006; 2009; El-Shehaby *et al.*, 2011). TWEAK expression was also higher in peripheral blood mononuclear cells derived of patients with lupus nephritis compared to patients without renal involvement and, moreover, there is also enhanced TWEAK-induced production of IL-10 and MCP-1 in *in vitro* cultures of lupus nephritis patients (Zhi-Chun *et al.*, 2012). In accordance with this clinical evidence, animal models, where autoantibody-mediated glomerulonephritis was triggered by MHCII mismatched spleen cell-induced chronic graft versus host disease or by treatment with rabbit antisera raised against glomerular basement membrane components (nephrotoxic serum transfer nephritis), revealed reduced disease activity in Fn14-knockout mice (Zhao *et al.*, 2007; Xia *et al.*, 2012). Furthermore, TWEAK neutralizing antibodies reduce renal inflammation and diminish proteinuria in both models without affecting the formation of autoantibodies (Zhao *et al.*, 2007; Xia *et al.*, 2012).

TWEAK and Fn14 in acute kidney injury

The TWEAK-Fn14 system also critical determines various aspects of acute kidney injury. According to its pleiotropic mode of action, practically each cell type of relevance in kidney injury is in some way regulated by the activities of TWEAK and Fn14. This reaches from the up-regulation of proinflammatory gene expression programmes in glomerular mesangial cells, tubular cells and podocytes, over induction of proliferation in tubular and mesangial cells to cell death induction in TNF/IFNγ exposed tubular epithelial cells (Campbell *et al.*, 2006; Justo *et al.*, 2006; Sanz *et al.*, 2008; 2009; Gao *et al.*, 2009). In the model of folic acid overdose-induced acute kidney injury inflammation, histological signs of tissue injury and renal failure are reduced in TWEAK-

Table 1

Therapeutic effects of Fn14 or TWEAK targeting proteins in preclinical models

Reagent	Model	Effect	Reference
Hamster anti-TWEAK	CIA	50% reduction in cumulative disease score Reduced synovial angiogenesis,	(Perper et al., 2006)
Rat anti-TWEAK	CIA	50% reduction in cumulative disease score Reduced synovial angiogenesis,	(Kamata et al., 2006)
anti-TWEAK	TNBS colitis	~40% reduction in mean clinical score	(Dohi et al., 2009)
Fn14-TRAIL	EAE	~50% reduction in mean clinical score, superior to side by side treatment with Fn14-Fc and TRAIL	(Razmara et al., 2009)
anti-TWEAK	EAE	~40–80% reduction in mean clinical score	(Desplat-Jego et al., 2005)
TWEAK vaccination	EAE	~20–60% reduction in mean clinical score	(Mueller et al., 2005)
anti-TWEAK	MACO	~20% reduction in mean infarct size	(Potrovita et al., 2004)
Fn14-Fc	MACO	~33% reduction in mean infarct size	(Yepes et al., 2005)
Fn14-Fc	MACO	~70% inhibition of MACO-induced BBB leakiness	(Polavarapu et al., 2005)
Fn14-Fc	MACO	~75% inhibition of MACO-induced BBB leakiness ~50% reduction in mean infarct size	(Zhang et al., 2007)
TWEAK	MACO	~33% reduction in MACO-induced mean infarct size by TWEAK pretreatment	(Echeverry et al., 2012)
anti-TWEAK	Denervation induced muscle atrophy	Less reduction in muscle fibre cross section area	(Mittal et al., 2010a)
anti-TWEAK	atherosclerosis	Reduced renal damage (~50%) Reduced RANTES and MCP1 expression in atherosclerotic plaques and kidney (>50%)	(Munoz-Garcia et al., 2009)
Fn14-Fc	atherosclerosis	Attenuated plaque progression Reduced collagen deposition in advance plaques	(Schapira et al., 2009)
anti-TWEAK	NTN	>80% reduction in proteinuria Reduced expression of MCP-1, RANTES, IP-10	(Xia et al., 2012)
anti-TWEAK	cGvHD	>50% reduction in proteinuria Reduced expression of MCP-1, RANTES, IP-10	(Zhao et al., 2007)
anti-Fn14	IRI[1]	Reduced inflammation >70% reduction in tubular damage score	(Hotta et al., 2011)
anti-TWEAK	Folic acid-induced nephropathy	Reduced inflammation and T-cell infiltration ~30% reduction in tubular damage score ~50% reduced serum creatinine levels	(Sanz et al., 2008; 2010)

[1]IRI, ischaemia reperfusion injury.

deficient mice or upon treatment with anti-TWEAK (Sanz et al., 2008; 2009). The proinflammatory effects of TWEAK in this model have been traced back to activation of the classical and alternative NFκB pathway whereby the latter is of special relevance for the induction of CCL19 and CCL21 and recruitment of T-cells (Sanz et al., 2010). In the model of unilateral nephrectomy, where compensatory kidney growth occurs independently of inflammation, there was significant less tubular cell proliferation in TWEAK-deficient mice (Sanz et al., 2009). The role of TWEAK and Fn14 has also been addressed in ischaemia reperfusion injury. Here, antagonistic Fn14-specific antibodies not only blocked the up-regulation of proinflammatory cytokines and chemokines and recruitment of inflammatory cells but also reduced apoptosis induction in tubular cell (Hotta et al., 2011). Especially, this translated into reduced chronic fibrosis and prolonged survival.

The TWEAK-Fn14 system in cerebral ischemia

In line with their tissue repair/remodelling-associated expression pattern and their proangiogenic activity, TWEAK and Fn14 have been found to be up-regulated after cerebral ischemia in the middle cerebral artery occlusion (MACO) model (Potrovita et al., 2004; Yepes et al., 2005; Haile et al., 2010a) and Fn14 expression is increased in infarcted and peri-infarct tissue of stroke patients (Inta et al., 2008). Moreover, several lines of evidence suggest that the TWEAK-Fn14 system plays a role in the development of tissue damage secondarily arising from cerebral ischaemia. Fn14-knockout mice displayed significantly reduced infarct size and less degradation of the basement membrane after MACO (Zhang et al., 2007) and various reagents interfering with TWEAK-

Fn14 interaction elicited corresponding therapeutic effects in the MACO model (Table 1). At the cellular level various TWEAK/Fn14-mediated effects may contribute to cerebral ischaemia. Firstly, TWEAK may induce cell death in neurons of the infarct area because oxygen-glucose deprivation experiments, which mimic some aspects of ischemia *in vitro*, revealed TWEAK/Fn14-mediated cell death in stressed cortical neurons (Potrovita *et al.*, 2004; Haile *et al.*, 2010a). Secondly, TWEAK increases the permeability of the BBB *in vitro* and *in vivo* by at least two mechanisms, on the one hand by classical NFκB-mediated induction of the basement degrading MMP9 protease (Polavarapu *et al.*, 2005) and on the other hand by stimulating transcellular transport of neutrophils through the interface of astrocytes and endothelial cells (Haile *et al.*, 2010a). Thirdly, TWEAK-induced activation of microglia and astrocytes results in the production of proinflammatory cytokines and chemokines (Haile *et al.*, 2010b) including TGFα, which can act as an autocrine inducer of astrocyte proliferation via EGFR stimulation (Rousselet *et al.*, 2012). For example, immunohistochemical analysis of the necrotic core of MACO-induced ischaemia revealed a strong increase of activated microglia and this was found to been reduced in animals with intracerebroventricular injection of Fn14-Fc (Yepes *et al.*, 2005). TWEAK and Fn14 appear not only to contribute to the devastating effects induced by ischaemic stroke but also surprisingly elicit a neuroprotective activity. Sublethal exposure to hypoxia protects cortical neurons against a subsequent lethal hypoxic challenge. The protective effect of such a preconditioning treatment has found to be significantly reduced in Fn14- and TWEAK-deficient neurons and is furthermore mimicked by treatment with recombinant TWEAK instead of sublethal hypoxia (Echeverry *et al.*, 2012). Indeed, intraperitoneal injection of recombinant TWEAK 1 day before induction of cerebral ischaemia by MACO resulted in significant reduction in the volume of the infarcted tissue (Echeverry *et al.*, 2012). It is worth mentioning that the protective effect of TWEAK preconditioning on cortical neurons was found to be dependent on TNF induction (Echeverry *et al.*, 2012) while the cytotoxic effect of TWEAK produced in course of oxygen-glucose deprivation was TNF independent (Haile *et al.*, 2010a).

The TWEAK-Fn14 system regulates various aspects of muscle biology

Early on it has been recognized that TWEAK stimulates proliferation of mesenchymal progenitor cells. This aspect of TWEAK-Fn14 biology has been most intensively studied in context of myotube formation from satellite cells during regeneration of skeletal muscle. However, TWEAK and Fn14 have also been implicated in muscle atrophy. Myoblasts express Fn14 and respond *in vitro* to TWEAK dependent on the culture conditions. In growth medium, where myoblasts proliferate and do no undergo differentiation in myotubes, TWEAK enhances proliferation of the mononuclear myoblasts (Girgenrath *et al.*, 2006). In contrast, in differentiation medium TWEAK treatment of murine myoblasts and human mesoangioblasts results in reduced myotube formation and cell death (Dogra *et al.*, 2006; Girgenrath *et al.*, 2006;

Morosetti *et al.*, 2012). From studies with the myoblastic C2C12 cell line, there is further evidence that TWEAK-mediated inhibition of myoblast differentiation is due to NFκB-mediated inhibition of expression of MyoD, a transcription factor crucially involved in myogenic differentiation (Girgenrath *et al.*, 2006). There is further evidence that also endogenous TWEAK contributed to myoblast formation. Lower numbers of myoblasts are generated in cultures derived from Fn14-deficient mice although these cells are still capable to differentiate to myotubes (Girgenrath *et al.*, 2006) and inhibition of TWEAK leads to improved myotube formation in human mesoangioblast cultures (Morosetti *et al.*, 2012). However, there is evidence that the role of TWEAK and Fn14 in myotube formation is more complex. Myotubes derived from Fn14-deficient mice are thicker than their wild type counterparts (Girgenrath *et al.*, 2006) and myotubes derived from C2C12 cells in the presence of TWEAK showed a reduced diameter (Dogra *et al.*, 2007a). Moreover, a recent report even demonstrated that TWEAK induces myotube formation by activation of the alternative NFκB pathway (Enwere *et al.*, 2012). The disparate effects of TWEAK on myoblast differentiation in the various studies cited remain to be solved. However, it is tempting to speculate that the different responsiveness of Fn14 to different agonists together with differences in the dose response of how various cellular signalling pathways are linked to Fn14 activation is here decisive. Indeed, in the study of Enwere *et al.* (2012), low concentrations of trimeric TWEAK, that presumably only activated the alternative NFκB pathway, have been used while in the other studies much higher concentrations or hexameric Fc-TWEAK have been used that activate both NFκB pathways (see above). In line with this idea, recent studies give evidence for an inhibitory role of the classical NFκB pathway on myoblast differentiation and a mitochondrial biogenesis-stimulating function of the alternative NFκB pathway, which involves induction of PPARγ co-activator 1β and support of myotube maintenance (Bakkar *et al.*, 2008; 2012).

The complex effects of TWEAK and Fn14 on myoblasts differentiation observed *in vitro* might contribute to the crucial role of the TWEAK-Fn14 system in muscle regeneration that has been deduced from the cardiotoxin-induced model of muscle injury. Injection of cardiotoxin in the tibialis anterior muscle results in muscle fibre damage and subsequent robust muscle regeneration by activation of quiescent satellite cells, muscle precursor cells of skeletal muscles. While expression of TWEAK and Fn14 is low in healthy skeletal muscles, cardiotoxin injured muscles display high Fn14 expression and induction of TWEAK whereby the latter seems to be primarily expressed from infiltrating macrophages (Girgenrath *et al.*, 2006; Mittal *et al.*, 2010b). Fn14 deficient mice showed significantly delayed muscle regeneration in response to cardiotoxin-induced muscle injury and this delayed regenerative response came along with reduced infiltration of immune cells and lowered cell proliferation in the affected muscle area (Girgenrath *et al.*, 2006). However, as on the one side TWEAK not only stimulates myoblast proliferation but also production of chemotactic cytokines and as on the other side the inflammatory microenvironment contributes to muscle regeneration, it is not unequivocally possible to specify the contribution of direct TWEAK-induced myoblast proliferation to the regenerative response. The

Table 2

Muscle atrophy in genetic models of TWEAK and Fn14

TWEAK/Fn14 model	Atrophy inducer	Effect	Ref.
TWEAK knockout	Denervation	Reduced loss in muscle mass, less reduction in muscle fibre cross section area, reduced fibrosis	(Mittal et al., 2010a)
TWEAK tg	Denervation	Enhanced loss in muscle mass, enhanced reduction in muscle fibre cross section area, more fibrosis	(Mittal et al., 2010a)
TWEAK tg	–	Reduced muscle mass, reduced muscle fibre cross section area	(Dogra et al., 2007a)
TWEAK tg	Cardiotoxin	Enhanced fibrosis	(Mittal et al., 2010b)
TWEAK knockout	Starvation	Less reduction in muscle fibre cross section area	(Paul et al., 2012)

situation becomes even more complex by findings showing essentially the opposite, namely improved muscle regeneration in the cardiotoxin model in TWEAK-deficient mice (Mittal et al., 2010b). Moreover, in various models of skeletal muscle atrophy (Table 2), TWEAK and Fn14 drive the latter by induction of the E3 ubiquitin ligases MuRF1 and MAFbx, which mark myosin heavy chain for degradation by the autophagosome and the proteasome.

TWEAK/Fn14 and heart-related diseases

In view of its multiple functions in immune regulation and tissue remodelling/repair, it is no surprise that the TWEAK-Fn14 system plays also a role in the development of atherosclerosis and some of its most severe possible consequences myocardial infarction and renal damage. TWEAK is expressed on macrophages recruited to atherosclerotic plaques and renal lesions but also by associated smooth muscle cells (SMCs) (Munoz-Garcia et al., 2006; Schapira et al., 2009). In the vascular and renal lesions TWEAK induces production of NFκB-regulated chemokines, such as RANTES and MCP1 (Munoz-Garcia et al., 2009). Furthermore, TWEAK triggers production of thrombosis promoting factors like tissue factor and plasminogen inhibitor-1 in human aortic SMCs in vitro and in the aortic root of ApoE-deficient mice in vivo. Moreover, induction of chemokines, tissue factor and plasminogen inhibitor-1 is reduced when endogenous TWEAK is blocked in ApoE-deficient mice by help of TWEAK neutralizing antibodies or soluble Fn14-Fc (Munoz-Garcia et al., 2009). Especially, Atorvastin, a statin broadly used for the prophylaxis against myocardial infarction and stroke, has been found to inhibit cytokine-induced up-regulation of Fn14 in aortic SMCs by interfering with RhoA and ROCKI activity (Munoz-Garcia et al., 2006). RhoA/ROCKI-mediated up-regulation of Fn14 has also been observed in cultured cardiomyocytes in response to ventricular hypertrophy-stimulating triggers such as stretch-stress, angiotensin II and norepinephrine (Chorianopoulos et al., 2010a). In fact, mice overexpressing soluble TWEAK suffer on dilated cardiomyopathy and die from cardiac dysfunction and Fn14-deficiency completely abrogated this pathology (Jain et al., 2009). Last but not least, like in many other tissue injury-related pathologies, TWEAK

and Fn14 are also of importance for fibrosis in the heart. TWEAK stimulates proliferation and NFκB-mediated collagen synthesis in rat neonatal cardiac fibroblasts in vitro (Chen et al., 2012). Moreover, in an in vivo model of pulmonary arterial hypertension-induced right ventricular failure Fn14-deficiency reduces collagen expression and myofibroblast differentiation (Novoyatleva et al., 2013). Clinical data from patients with acute ST-elevation myocardial infarction or idiopathic dilated cardiomyopathy revealed increased soluble TWEAK levels while patients with stable disease after non-ischaemic heart failure showed reduced levels of soluble TWEAK (Table 3). Noteworthy, in the latter case soluble TWEAK represents an independent adverse prognostic factor (Richter et al., 2010).

TWEAK and Fn14 in tumour development and metastasis

Tumourigenesis is often tightly linked to chronic inflammatory processes and associated with repetitive tissue damage and the corresponding repair processes. The tumour microenvironment therefore typically contains many factors implicated in upregulation of Fn14 expression. Furthermore, tumour infiltrating immune cells represent a bona fide source of TWEAK and expression of the latter might also be induced in activated resident cells of the tumour microenvironment or in the transformed cells itself. Indeed, Fn14 expression is frequently found to be strongly enhanced in tumour tissue compared to non-transformed tissue in specimens of non-small cell lung carcinoma, breast cancer, hepatocellular cancer, pancreatic cancer, malignant melanoma as well as glioblastoma multiforme and is also regularly detectable on tumour cell lines (Feng et al., 2000; Tran et al., 2003; 2006; Willis et al., 2008; Dai et al., 2009; Culp et al., 2010; Kwon et al., 2012; Zhou et al., 2013). TWEAK has been detected at the mRNA level in various tumour entities and tumour cell lines and is also found to be up-regulated in tumour tissue by immunohistochemistry but cell surface expression of membrane TWEAK has only be reported for a very few hepatocellular carcinoma and breast cancer cell lines, so far (Kawakita et al., 2004). With exception of the proapoptotic TWEAK-TNF crosstalk, which appears to be only relevant in rare cases, the huge majority of cellular effects elicited by Fn14 and TWEAK

Table 3

TWEAK in human patients

| Compared samples	TWEAK levels	Remarks	Ref.
SLE with and without LN	16.3 versus 5.5 pg·mg^{-1} creatinine	Urinary TWEAK uTWEAK correlated with renal disease activity index	(Schwartz et al., 2006)
SLE with and without LN SLE with LN and healthy volunteers	12.2 versus 6.6 pg·mg^{-1} creatinine 12.2 versus 5.7 pg·mg^{-1} creatinine	Urinary TWEAK uTWEAK correlated with renal disease activity index	(El-Shehaby et al., 2011)
Peripheral artery disease	134 versus 147 pg·mL^{-1}	161 ± 35 versus 100 ± 6	(Moreno et al., 2010)
Acute ST-elevation myocardial infarction versus healthy or stable coronary artery disease	524 pg·mL^{-1} (healthy) 377 pg·mL^{-1} (CAD) 1393 pg·mL^{-1} (STEMI)	TWEAK increased in patients with acute myocardial infarction Association with adverse short-term outcome	(Chorianopoulos et al., 2010b)
Stable heart failure	217 versus 325 pg·mL^{-1}	sTWEAK reduced in stable heart failure patients TWEAK level below 227 pg·mL^{-1} correlate with significantly higher 4-year mortality rate	(Chorianopoulos et al., 2009)
Non-ischaemic versus ischaemic heart failure	332 versus 362 pg·mL^{-1}	Levels not significantly different between non-ischaemic and ischaemic HF Inverse correlation with mortality in non-ischaemic HF patients	(Richter et al., 2010)
Orthotopic heart transplant recipient versus healthy	229 versus 418 pg·mL^{-1}	Significant reduced sTWEAK levels	(Przybylowski et al., 2011)
Chronic kidney disease (CKD) patients	450 versus 161 (stage 3) to 380 pg·mL^{-1} (stage 1)	sTWEAK levels significantly lower in CKD patients	(Hassan et al., 2012)

are obviously of potential advantage for tumour development. Indeed, TWEAK/Fn14-induced cell migration and invasion have been demonstrated for glioblastoma cells, non-small cell lung cancer, ovarian cancer cells, breast cancer cell lines and androgen-independent prostate cancer cells (Tran et al., 2003; Willis et al., 2008; Dai et al., 2009; Huang et al., 2011). Moreover, Fn14 overexpressing A549 adenoma carcinoma cells display enhanced lung metastasis upon tail vein injection in mice (Whitsett et al., 2012) and TWEAK-expressing matrigel encapsulated Hek293 cells showed enhanced angiogenesis in nude mice (Ho et al., 2004). There is also clinical evidence for protumoural functions of TWEAK and Fn14. High Fn14 expression correlates with clinical indicators of poor prognosis in breast cancer patients (Willis et al., 2008). Likewise, Fn14 expression has been found to be increased in glioma specimens and again correlate with poor prognosis (Tran et al., 2006). High Fn14 level late stage patients suffering on gastric cancer have also a reduced survival rate (Kwon et al., 2012) and strong Fn14 expression is also associated with lower prostate specific antigen-free survival in prostate cancer patients (Huang et al., 2011). It is worth mentioning that high levels of TWEAK and Fn14 might not ever been connected with poor patient outcome. In colorectal cancer, high TWEAK expression correlates with increased overall and disease-free survival and this correspond to an inhibitory effect of TWEAK on the invasiveness of colon cancer cell lines in vitro (Lin et al., 2012).

In view of its broad and high tumour-associated expression and its multiple protumoural functions, Fn14 is an attractive anti-tumour target on two counts. First, blockade of Fn14 or

TWEAK might deprive tumour cells from beneficial Fn14-mediated activities and secondly, Fn14 might serve as a tumour-associated target for delivering of antibodies or antibody drug conjugates, irrespective of its tumour-related functions. In line with the latter, recent reports demonstrated antitumor effects of Fn14 antibodies that based on the activation of antibody effector functions, such as antibody-dependent cellular cytotoxicity (ADCC), or on the activity of antibody conjugated drugs (Culp et al., 2010; Michaelson et al., 2011; Zhou et al., 2011; 2013). In some cases, the apoptotic TWEAK-TNF crosstalk might also be exploited for tumour therapy by triggering tumour cell death using recombinant TWEAK or agonistic Fn14-antibodies. In view of the poor serum half-life of recombinant TNF ligands and the additional Fc effector functions delivered by agonistic antibodies, the latter represents in this respect presumably the more practicable approach (Culp et al., 2010; Michaelson et al., 2011).

TWEAK and Fn14 targeting drugs

Dependent on the concrete pathology considered, blockade and stimulation of Fn14 could be a therapeutic option (Figure 5). Due to their extracellular accessibility, TWEAK and Fn14 are straight forwardly druggable with high selectivity by help of recombinant soluble variants of these molecules or antibodies. Indeed, in a variety of the disease models discussed above, it has been already demonstrated that pronounced therapeutic effects are achievable with soluble Fn14 and TWEAK- and Fn14-specific antibodies (Table 1). It is
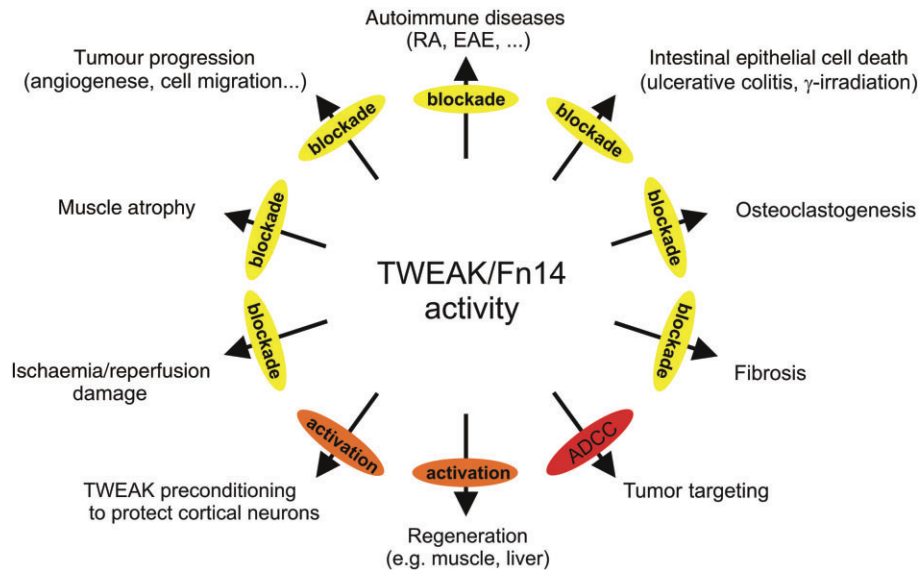
Figure 5

Targeting of TWEAK/Fn14-related pathologies.

thus no surprise that TWEAK- and Fn14-specific antibodies are currently under investigation in phase I studies in patients suffering on RA, lupus or solid tumours (http://clinicaltrials.gov/; NCT00771329, NCT01499355 and NCT00738764).

Recombinant proteins and antibodies targeting Fn14 and TWEAK elicit *in vivo* effects by complex mechanisms going beyond simple blockade or activation of Fn14 and could therefore vary with the type of agonists or antagonist used. The most important TWEAK and Fn14 targeting drug formats and their molecular mode of action are:

Anti-TWEAK antibodies – TWEAK-specific antibodies that block binding to Fn14 have been described (Table 1). Blocking TWEAK antibodies may predominately act as inhibitors of the TWEAK-Fn14 system but as TWEAK is also expressed as a membrane-bound molecule in some cell types, Fc domain-mediated effects, for example, ADCC or complement activation, cannot be completely ruled out.

Fn14-Fc – A fusion protein of the ectodomain of Fn14 with the Fc domain of human IgG1 has been successfully used in various preclinical studies to block TWEAK-mediated effects (Table 1). As in case of blocking TWEAK antibodies, effector function emanating from the Fc domain must be taken into consideration *in vivo* for membrane TWEAK expressing cells.

Anti-Fn14 antibodies – Fn14-specific antibodies can elicit quite different effects dependent on their isotype, their idiotype and the availability of Fcγ-receptor expressing cells. For example, P4A8 and PDL192, two Fn14-specific IgG1 antibodies under investigation in clinical trials (http://clinicaltrials.gov/), strongly differ in their capability to block TWEAK-Fn14 interaction but act both as potent Fn14 agonists upon binding to Fcγ receptors or oligomerization by protein G (Salzmann *et al.*, 2013). Noteworthy, without oligomerization or FcγR-binding, both antibodies significantly trigger p100 processing at moderate concentrations without

showing a relevant effect on other Fn14-mediated cellular effects. In case of the Fn14-blocking P4A8 antibody, this has the strange consequence that it act as agonist for Fn14-mediated p100 processing but concomitantly as an antagonist for other Fn14-mediated responses induced by soluble or membrane TWEAK. Thus, as long not bound to Fcγ receptors Fn14-specific IgG1 antibodies trigger a state of Fn14 activity that neither reflects the activity of soluble TWEAK nor that of membrane TWEAK (Salzmann *et al.*, 2013). Although the molecular mechanisms that constitute the different Fn14 signalling capabilities in response to IgG1 antibodies and TWEAK are yet not fully elucidated, there is evidence for a crucial role of two parameters, the stoichiometry of the antibody/ligand interaction with Fn14 and the differential relevance of amplification mechanisms in distinct Fn14-associated signalling pathways.

Soluble TWEAK – Soluble recombinant trimeric TWEAK mimicking the natural occurring soluble TWEAK generated by furin processing of membrane TWEAK is straight forwardly available by expression of corresponding DNA fragments in *Escherichia coli* or eukaryotic cells. Two major issues must be considered in concepts using soluble TWEAK. First, as discussed before, soluble TWEAK only triggers robust activation of a subset of the Fn14-associated effects that can be induced by membrane TWEAK. Secondly, like other soluble TNF ligands, soluble TWEAK has a very short half-life *in vivo*. However, this limitation can be overcome using fusion proteins of soluble TWEAK with proteins having long half-life *in vivo*, such as serum albumin (Muller *et al.*, 2010).

Fc-TWEAK – Linking soluble TWEAK to the C-terminal end of the Fc domain of human IgG1 results in a fusion protein (Fc-TWEAK) that assembles into hexameric molecules. Fc-TWEAK efficiently activates the full set of Fn14-associated signalling pathways and in so far functionally mimics membrane TWEAK rather than soluble TWEAK (Roos

et al., 2010). Especially, one has to consider *in vivo* that this protein might trigger Fc domain-dependent immune cell responses upon binding to Fn14-expressing cells. As different amino acids of the Fc-domain are involved in dimerization and FcγR binding, it is, however, possible to generate hexameric Fc-TWEAK variants with a mutated Fc domain showing strongly reduced FcγR binding but unchanged Fn14 stimulatory activity.

Conclusions

Inhibition of TWEAK/Fn14 activities has a broad potential for the therapy of tissue damage-associated pathologies and cancer. Indeed, therapeutic concepts that base on the exogenous inhibition of TWEAK or Fn14 haven already been successfully tested in preclinical models and are currently in clinical trials. The latter must now clarify whether the hopes resulting from the impressive preclinical data on TWEAK/Fn14 inhibition become real. Although, the TWEAK-Fn14 system has so far primarily recognized as a 'pathological' factor, it is obvious that its direct cellular effects, such as induction of angiogenic and proinflammatory proteins or stimulation of proliferation might contribute to the control of tissue homeostasis and wound healing in the healthy organism. It is thus tempting to speculate that the disease-associated activities of TWEAK and Fn14 reflect the conversion of initially beneficial functions in the repair of acute tissue damage into an exaggerated and chronic pathological repair response. In view of the potential dichotomous role of the TWEAK-Fn14 system, it appears possible that treatment modalities (e.g. timing of treatment) considerably influence the success of TWEAK/Fn14-targeting therapeutic concepts. Moreover, besides inhibition of the TWEAK-Fn14 system, its activation, at least during a certain stage of a disease, might become a therapeutic option and should be addressed in future preclinical studies.

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Conflict of interest

No conflict of interest.

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