Published in final edited form as:
Nat Rev Drug Discov. 2008 May ; 7(5): 411–425. doi:10.1038/nrd2488.

The TWEAK–Fn14 cytokine–receptor axis: discovery, biology and therapeutic targeting

Jeffrey A. Winkles
Departments of Surgery and Physiology, Center for Vascular and Inflammatory Diseases, and the Marlene and Stewart Greenebaum Cancer Center, 800 West Baltimore Street, University of Maryland School of Medicine, Baltimore, Maryland 21201, USA. jwinkles@som.umaryland.edu

Abstract

TWEAK is a multifunctional cytokine that controls many cellular activities including proliferation, migration, differentiation, apoptosis, angiogenesis and inflammation. TWEAK acts by binding to Fn14, a highly inducible cell-surface receptor that is linked to several intracellular signalling pathways, including the nuclear factor-κB (NF-κB) pathway. The TWEAK–Fn14 axis normally regulates various physiological processes, in particular it seems to play an important, beneficial role in tissue repair following acute injury. Furthermore, recent studies have indicated that TWEAK–Fn14 axis signalling may contribute to cancer, chronic autoimmune diseases and acute ischaemic stroke. This Review provides an overview of TWEAK–Fn14 axis biology and summarizes the available data supporting the proposal that both TWEAK and Fn14 should be considered as potential targets for the development of novel therapeutics.

Cytokines are a large and diverse group of plasma-membrane-associated or secreted proteins that bind cell-surface receptors and thereby regulate many important biological processes. These processes include development, haematopoesis, inflammation, immune responses and tissue repair1. The tumour necrosis factor (TNF) ligand superfamily, and the receptors that mediate their effects, is a cytokine-receptor subgroup that has attracted considerable interest as a potential source of therapeutic targets for the management of complex human diseases. TNF superfamily ligands are primarily expressed as type II transmembrane proteins, but in some cases they are processed into smaller, secreted proteins that retain biological activity2,3. Both the anchored and soluble cytokines contain a C-terminal TNF homology domain that mediates self-trimerization and receptor binding. TNF superfamily members bind to one or more members of the TNF receptor (TNFR) superfamily, most of which are type I or type III transmembrane proteins2,3. These receptors are characterized by the presence of an extracellular, ligand-binding region containing one to four cysteine-rich domains and a cytoplasmic tail containing one or more adaptor-protein binding sites.

Competing interests statement
The author declares competing financial interests: see web version for details.

DATABASES
OMIM:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Ischaemic stroke | multiple sclerosis | rheumatoid arthritis | systemic lupus erythematosus
UniProtKB: http://ca.expasy.org/sprot
Fn14 | TWEAK

FURTHER INFORMATION
Jeffrey A. Winkle's homepage: http://medschool.umaryland. edu/facultyresearchprofile/viewprofile.aspx?id=8019
All LINKS ARE ACTIVE IN THE ONLINE PDF

TWEAK (also known as TNFSF12, APO3L, CD255) and its cognate receptor Fn14 (also known as TNFRSF12A, TWEAKR, CD266) are members of the TNF and TNFR superfamilies, respectively, and the discovery of this cytokine–receptor axis was in itself an interesting scientific journey. In 1997, Chicheportiche *et al.*[4] reported the identification of a novel TNF-like protein that had pro-apoptotic activity on interferon-α (IFN-γ)-treated human HT-29 colon carcinoma cells. Consequently, they named this cytokine TNF-like weak inducer of apoptosis (abbreviated to TWEAK). These investigators were recipients of unexpected good fortune, as they were not originally aiming to identify new TNF superfamily members; indeed, the TWEAK cDNA was cloned during a screen for erythropoietin-related mRNAs in mouse macrophages. In 1998, Marsters *et al.*[5] reported the identification of the same protein by searching an expressed sequence tag database for TNF-α-related sequences. As they found that it could bind to a previously discovered TNFR superfamily member named APO3 (DR3), they named the protein APO3 ligand (abbreviated APO3L). TWEAK (APO3L) did have some capacity to induce cell death, and DR3 was considered to be a so-called 'death receptor', so this interaction seemed plausible. However, when studies were published in 1999 and 2000 indicating that TWEAK could act on DR3-negative cells6,7 and did not always induce cell death when added to cells in culture7,8, it became clear that there was probably another TWEAK receptor. In 2001, Wiley *et al.*[9] reported that they had successfully used a cDNA expression library screening approach to isolate a cDNA clone that encoded a TWEAK-binding protein. They named this protein TWEAK receptor (abbreviated TWEAKR). The predicted amino-acid sequence of Tweak R was identical to that of a type I transmembrane protein that our group had discovered in the late 1990s. We had named this protein fibroblast growth factor-inducible 14 (abbreviated to Fn14) because its expression was upregulated when quiescent cells were exposed to either fibroblast growth factor 1 (FGF1) or FGF2 and because its predicted molecular mass before signal peptide cleavage was ~14 kilodaltons10,11. The discovery that TWEAK was a ligand for Fn14 was very fortunate for our group, because up to that point we had concluded that Fn14 was just a small cell-surface protein that might modulate cell–extracellular matrix interactions10. Indeed, we now recognize that we were unable to classify Fn14 as a member of the TNFR superfamily by sequence database searches because individual family members have low primary sequence similarity. It has now been convincingly demonstrated that TWEAK binds to Fn14 but not to any other TNFR superfamily member, including DR3 (REF. 12). Therefore, previous studies that investigated TWEAK function using soluble DR3-Fc decoy proteins should be interpreted with this in mind13–15.

The number of TWEAK–Fn14 axis papers that have been published annually has increased during the past decade (FIG. 1), a trend which suggests that more research groups are encountering one or both of these molecules during the course of their studies. This Review will summarize some of the most unique and also puzzling aspects of TWEAK and Fn14 biology. Particular emphasis will be on recent findings supporting the notion that this ligand–receptor axis may contribute to tissue repair after injury and the pathogenesis of several diseases that together are responsible for significant morbidity and mortality worldwide. Strategies that could be useful for therapeutic targeting of this axis will also be discussed. The reader is referred to recent review articles by Vince and Silke[16] and Burkly *et al.*[17] for additional information on the TWEAK–Fn14 axis and other perspectives on its likely function in normal and diseased tissues.

TWEAK: not just a weak inducer of apoptosis

TWEAK structure

TWEAK is initially synthesized as a 249-amino-acid type II transmembrane protein. TWEAK has a C-terminal extracellular region (206 amino acids; comprised of a stalk region

and a prototypical TNF-homology domain containing one potential N-glycosylation site); a transmembrane domain (25 amino acids); and an N-terminal intracellular domain (18 amino acids; containing a potential protein kinase C phosphorylation site)4,5. It has been reported that TWEAK contains several putative nuclear localization sequences and that endogenously expressed TWEAK may enter the cell nucleus18,19. However, more studies are necessary to assess both the importance of these sequence motifs (for example, using site-directed mutagenesis) and determine whether TWEAK nuclear localization occurs in other cell types. Full-length TWEAK is proteolytically processed in the C-terminal region, most probably at one or both of the furin endoprotease consensus recognition motifs in the stalk portion, and the 156-amino-acid proteolytic product can function as a soluble cytokine[4]. Recombinant soluble TWEAK purified from human embryonic kidney (HEK) 293 cells is a homotrimeric, N-glycosylated molecule6. Cells can co-express both full-length, plasma-membrane-anchored TWEAK and soluble TWEAK4,20,21, but the mechanism controlling the relative production of these two forms is not known, nor is it known if full-length TWEAK can bind and activate the Fn14 receptor.

TWEAK gene expression

TWEAK, in contrast to other TNF superfamily members (for example, TNF-α5), is a widely expressed cytokine. Indeed, *TWEAK* mRNA and/or protein expression has been detected in many different tissues[4,5,19,22–25] and tumour specimens[20,23,26,27]. TWEAK is also expressed in primary murine neurons[28] and astrocytes[28,29], in monocytes/macrophages[4,21,22,30,31] and in various human tumour cell lines[4,5,20,26,27]. It has been reported that IFN-γ30,32 or phorbol myristate acetate[30] treatment of human primary monocytes can increase TWEAK levels, as determined by fluorescence-activated cell-sorter analysis. Also, lipopolysaccharide (LPS) treatment of human THP-1 monocytic cells induces *TWEAK* mRNA expression[25]; curiously, LPS treatment of murine peritoneal macrophages has the opposite effect[22]. Finally, *TWEAK* expression is upregulated in three murine models of acute injury; specifically, in the brain after focal cerebral ischaemia[28,33], in the kidney after intraperitoneal injection of folic acid[34] and in skeletal muscle after cardiotoxin injection[31]. These results suggest that under these conditions TWEAK could play a role in either tissue injury or repair.

TWEAK biological activities

In the past decade, it has become apparent that TWEAK is a multifunctional cytokine, and one issue that is constantly raised is whether all TWEAK-mediated cellular responses occur by binding to a single cell-surface receptor; specifically, the TNFR superfamily member Fn14. In 2003, Polek *et al.*[35] reported that TWEAK-stimulated murine RAW264.7 cell differentiation occurred in an Fn14-independent manner and proposed that these cells expressed a second TWEAK receptor, but there have been no subsequent reports by this or any other group describing this putative receptor. More recently, Bover *et al.*[36] reported that TWEAK can bind to CD163, a cysteine-rich scavenger receptor that is expressed exclusively on the monocyte/macrophage lineage. This is an interesting finding as a TWEAK–CD163 interaction could have biological relevance during the inflammatory response and in the tumour microenvironment (see below). In summary, although one cannot rule out that TWEAK may act on some cells via CD163 or other, as yet unidentified, cell-surface molecules, at the present time Fn14 is the only known signalling-competent receptor. Therefore, throughout this Review, it will be assumed that TWEAK activity on all cell types is mediated by this receptor.

TWEAK activity in cell-culture-based assays

TWEAK has been shown to regulate cell proliferation, migration, survival, differentiation and death when added to either human, mouse or rat primary cells or immortalized cell lines

cultured *in vitro* (TABLE 1). In most of these studies the investigators used recombinant human soluble TWEAK produced in bacteria, which indicates that TWEAK glycosylation is not required for biological activity and that human TWEAK can bind to the mouse and rat Fn14 receptors. TWEAK–Fn14 human and mouse cross reactivity has been confirmed biochemically12, and this is not unexpected as the TWEAK and Fn14 amino-acid sequences are 4،37،38. In general, soluble TWEAK promotes cellular responses when added to culture medium at a final concentration of 10–1,000 ng per ml (0.6–60 nM). At the present time, how TWEAK binding to Fn14 can have such diverse cell-type-specific biological effects is not understood, but the differential activation of intracellular signalling cascades is likely to be one explanation (see below). It is particularly puzzling how the TWEAK–Fn14 interaction can induce cell death, although it should be emphasized that in most cases this TWEAK effect is relatively weak, requires long incubation periods, and can only be detected when the target cells are sensitized by either pre-treatment with cycloheximide or co-incubation with other cytokines. Also, it has been reported that TWEAK-stimulated Kym-1 cell death is an indirect effect, mediated by TNF-α–TNFR1 interaction6،39, and it is possible that TWEAK induces the production of TNF-α (or other death cytokines such as FASL) when added to other cell lines. The Fn14 cytoplasmic tail does not contain a canonical death domain, so it is unlikely that TWEAK binding to Fn14 can trigger the extrinsic apoptotic pathway that is characterized by formation of the death-inducing signalling complex and caspase 8 (or caspase 10) activation40. There have been some studies investigating whether TWEAK-induced cell death is caspase-dependent, but this is still an unresolved issue. This is primarily because in most of these studies TWEAK was just one component of a death-inducing cytokine cocktail34،39،41،42, and in one of the studies the target cells were stably transfected cell lines expressing artificially high levels of the Fn14 receptor[42]. Finally, it has been reported that TWEAK-triggered neuronal cell death requires nuclear factor-κB (NF-κB) activation[33], so this signalling pathway may contribute to the apoptotic effects that have been noted in other cells as well.

Studies testing the effects of TWEAK on cells cultured *in vitro* have helped investigators to formulate specific hypotheses regarding the potential role of TWEAK *in vivo.* For example, the observation that TWEAK can stimulate endothelial cell growth and migration *in vitro*8،38،42–44 led our group and others to test whether it could induce blood-vessel formation, a process referred to as angiogenesis (see below). Also, two groups have reported that TWEAK promotes myoblast proliferation and thereby inhibits myoblast differentiation *in vitro*[31]،45،46. These findings led to additional research investigating whether TWEAK can regulate skeletal-muscle formation in mice. Finally, the finding that TWEAK can both stimulate osteoclast differentiation and inhibit osteoblast differentiation *in vitro*35،47 suggests that it may have a role in pathological bone remodelling. For example, breast cancer cells frequently metastasize to bone and in most cases this results in osteoclast-mediated bone resorption (osteolysis), which can lead to bone pain and fractures[48]. As breast cancer cells express TWEAK (J. Winkles, unpublished observations), they could deliver this cytokine into the bone microenvironment, where it could act in concert with other cytokines (for example, the TNF superfamily member RANKL) and hormones (for example, parathyroid hormone-related protein; PTHR) to promote bone destruction. This is a testable hypothesis as well.

TWEAK activity in rats and mice

In 1999, Lynch *et al.*[8] reported that soluble TWEAK could stimulate angiogenesis when delivered into the rat cornea (50–200 ng dosage per eye) and they noted that this pro-angiogenic activity was comparable to that exhibited by the well-known angiogenic polypeptides vascular endothelial growth factor A (VEGFA) and FGF2. This activity has been confirmed using other angiogenesis assays, including the chick embryo chorioallantoic

membrane (N. Boudreau and J. Winkles, unpublished observations); rat fatpad (T. Rosengart and J. Winkles, unpublished observations); and rat aortic ring (J. Winkles, unpublished observations) assays. These findings identify TWEAK as a potential regulator of physiological and/or pathological angiogenesis *in vivo*.

Several research groups have studied TWEAK function using transgenic and/or gene knockout mice, and these studies have revealed that elevated TWEAK levels can have physiological consequences *in vivo* and that TWEAK can have either positive (beneficial) or negative (detrimental) biological effects, depending on the tissue injury or disease model that is used (TABLE 2).

Both the *Tweak*-null mice (derived at Genentech) and the *Fn14*-null mice (derived at Biogen Idec) described to date are viable and fertile with no gross abnormalities; thus, TWEAK–Fn14 function is not required for embryonic development or postnatal growth[30,49]. In addition, although it has been reported that the *Tweak*-null mice exhibit spleen and lymph node enlargement, increased natural killer cell numbers and activity, an enhanced innate inflammatory response and stronger adaptive antitumour immunity[30], independently generated *Tweak*-null mice do not exhibit all of these immune system abnormalities[17,50]. The basis for this apparent phenotypic difference between the two *Tweak*-null mouse lines is unknown at this time.

Studies using genetically engineered mice have indicated that TWEAK–Fn14 signalling could have a role in the pathogenesis of autoimmune inflammatory diseases. Specifically, transgenic mice engineered to produce soluble TWEAK in the liver have elevated levels of circulating TWEAK and develop a more severe form of experimental autoimmune encephalitis, a model for multiple sclerosis[29]. Also, Zhao *et al.*[50] reported recently that, when compared with wild-type littermates, *Fn14*-null mice have decreased kidney inflammation and damage in a model of systemic lupus erythematosus (SLE).

TWEAK also appears to function in a deleterious manner in the brain. TWEAK increases blood–brain barrier permeability when injected into the non-ischaemic mouse brain[51], and when compared with wild-type littermates, *Fn14*-null mice have less brain tissue damage, less oedema and faster motor recovery in a permanent middle cerebral artery occlusion ischaemic stroke model[52].

However, under certain experimental conditions, TWEAK activity can be beneficial, not detrimental. Jakubowski *et al.*[49] used TWEAK transgenic mice, adenovirus-mediated *TWEAK* overexpression and *Fn14*-null mice to test whether TWEAK was a physiological regulator of liver regeneration. They found that TWEAK was a mitogen for liver progenitor (oval) cells and that endogenous TWEAK contributed to liver progenitor expansion after chemical injury.

Finally, genetically engineered mice have also been instrumental in revealing that TWEAK can have beneficial and detrimental functions in the same tissue — in this case, skeletal muscle. Adult mammalian skeletal muscle undergoes a rapid and extensive repair process after injury, which is characterized by necrosis of the damaged tissue, activation of an inflammatory response and muscle regeneration. The activation of satellite cells, a population of undifferentiated myogenic cells within adult muscle, is a key element in muscle repair[53]. Girgenrath *et al.*[31] reported that compared with wild-type mice, *Fn14*-null mice had a reduced inflammatory response and delayed muscle-fibre regeneration following intramuscular injection of the snake-venom peptide cardiotoxin, a known trigger for satellite-cell-mediated skeletal muscle repair. This result identifies the TWEAK cytokine as a novel positive regulator of adult regenerative myogenesis *in vivo*. Remarkably, TWEAK can also function as a negative regulator of the musculature. Dogra *et al.*[54] found that

intraperitoneal TWEAK injection (100 μg per kg body weight; one injection per week for 4 weeks) decreased overall body weight as well as hindlimb-muscle weight and fibre diameter. This is consistent with their results demonstrating that TWEAK can promote C2C12 myotube atrophy *in vitro.* Furthermore, skeletal-muscle-specific overexpression of full-length TWEAK in transgenic mice results in reduced body weight and loss of muscle mass[54]. Thus, TWEAK–Fn14 axis function can both contribute to muscle repair after injury and stimulate skeletal muscle atrophy, or wasting, a process that is associated with numerous chronic diseases.

The Fn14 receptor: small but powerful

Fn14 structure

The Fn14 receptor is the smallest TNFR superfamily member described so far. It is initially synthesized as a 129-amino-acid type I transmembrane protein that is then proteolytically processed by signal peptidase into a 102-amino-acid cell-surface receptor[9–11].

TWEAK binds to Fn14 with an interaction affinity constant (K_d) of ~0.8–2.4 nM[9,38,43], and it is the only TNF superfamily member that can bind this receptor[12]. The Fn14 extracellular ligand-binding region, only 53 amino acids in length, contains a single cysteine-rich domain (FIG. 2a). Site-specific mutagenesis has revealed that TWEAK binding to the Fn14 cysteine-rich domain requires all three of the predicted disulphide bonds and several charged, evolutionarily conserved amino-acid residues[38] (FIG. 2b). The Fn14 cytoplasmic tail, only 28 amino acids in length, contains a single TNFR-associated factor (TRAF) consensus binding motif (FIG. 2a), and TRAF1, TRAF2, TRAF3 and TRAF5 are able to bind this site[55,56]. Interestingly, it has been reported that immunoprecipitates of Fn14 contain RAC1, a Rho family GTPase[46,57,58]. This Fn14–RAC1 interaction does not occur if the Fn14 TRAF-binding site is deleted[58]; therefore, RAC1 may either bind directly to this site or, more likely, its presence in Fn14 immunoprecipitates is due to its association with one or more TRAF proteins.

Fn14 gene expression

TWEAK has biological effects on many cell types, so one would predict that Fn14 would be expressed to at least some degree in most cell and tissue types. This seems to be the case[17,59]; indeed, the only Fn14-negative cells described so far are primary T and B cells[17,30,39] and their corresponding immortalized cell lines (for example, Jurkat cells39,42). One interesting aspect of Fn14 biology, which is not shared by any other member of the TNFR superfamily, is that *Fn14* gene expression is highly regulated both *in vitro* and *in vivo*. As mentioned earlier, the *Fn14* gene was initially described by our group in 1999 as a polypeptide growth-factor-inducible, immediate-early response gene in murine NIH3T3 fibroblasts[10]. We also found that fetal bovine serum, a complex mixture of polypeptide mitogens and peptide hormones, and phorbol myristate acetate, an activator of protein kinase C, induced *Fn14* expression in murine[10] and human[11] fibroblasts. Research conducted since then has revealed that several other growth factors, cytokines, hormones and compounds can increase *Fn14* expression *in vitro* (TABLE 3). Recently, Tran *et al.*[58] reported that TWEAK itself can induce *Fn14* expression when added to glioma cell lines, and it will be of interest to investigate whether this occurs in other cell types as well. If so, this could be a mechanism to amplify TWEAK-stimulated cellular responses under certain conditions *in vivo.* The human *Fn14* promoter region contains several potential transcription factor binding sites, including AP-1 sites and an NF-κB site[58], but the transcription factors and promoter regulatory elements involved in *Fn14* gene activation *in vitro* have not yet been reported.

Our early observation that serum, the soluble fraction of coagulated blood that is encountered *in vivo* only at sites of tissue injury and remodelling, was a potent inducer of *Fn14* expression *in vitro*[10,11], suggested that *Fn14* expression might be increased after tissue injury. This possibility was confirmed using the murine liver partial hepatectomy[11] and the rat artery balloon injury[9] models. Subsequent studies have demonstrated that *Fn14* gene activation also occurs in response to other tissue insults (FIG. 3). These findings, together with two independent studies demonstrating *Fn14* induction during nerve regeneration[57,60] and the results obtained using genetically engineered mice, are consistent with the proposal that the TWEAK–Fn14 axis contributes to adult tissue repair and remodelling.

Another intriguing aspect of Fn14 biology that could have future clinical applications is that *Fn14* is overexpressed in certain solid tumours relative to control non-tumorous tissue (FIG. 3). Indeed, high *Fn14* mRNA expression levels in glioblastoma multiforme tumours correlates with poor patient outcome[58]. Also, a recent transcriptional profiling study identified Fn14 as one of the 12 best candidate biomarkers to diagnose oesophageal adenocarcinoma at its early stages[61]. Tumour cells that express high levels of *Fn14* might be more sensitive to TWEAK compared with non-neoplastic cells; alternatively, they may not even require TWEAK for effective Fn14 signalling (see below).

It is presently unknown why *Fn14* expression is elevated in human tumours, but taking into consideration the results described above identifying *Fn14* as a tissue injury-responsive gene, it is of interest to recall Harold Dvorak's proposal made over 20 years ago that solid tumours are essentially "wounds that do not heal"62. Recent global gene expression profiling studies demonstrating that many of the serum/growth factor-responsive genes expressed in fibroblasts encode proteins involved in the physiology of wound repair63 and that many of these same genes are constitutively expressed in the tumour microenvironment[64,65], are consistent with this proposal. Remarkably, the expression of a selected set of the entire serum/growth factor-responsive gene repertoire (the "wound-response signature") in a primary tumour is associated with an increased risk of metastasis and reduced overall survival[64,65]. The possibility that Fn14 could be a tumour biomarker and/or a molecular target for cancer therapy is discussed below.

TWEAK-dependent Fn14 signalling

Studies focused on other members of the TNF superfamily that signal via interaction with TNFR superfamily members devoid of death domains have revealed that ligand binding to the receptor extracellular domain induces receptor trimerization, TRAF association with the cytoplasmic tail and activation of intracellular signalling cascades[2,3]. It is likely that TWEAK engagement of the Fn14 receptor initiates the same series of events (FIG. 4a). Indeed, it is known that TWEAK activation of intracellular signalling pathways requires the Fn14 TRAF-binding motif[66] and TRAF2 and TRAF5 function[5,44,56,66]. Here, the focus will be on the molecular events that occur following Fn14–TRAF association.

TWEAK treatment of Fn14-positive cells has been shown to activate several different signalling cascades, but NF-κB pathway activation seems to be a universal cellular response. The NF-κB/Rel family of transcription factors includes five structurally related proteins that bind to DNA and regulate gene expression[67]. These factors form homodimers or heterodimers that together control the transcription of target genes encoding a diverse set of proteins involved in the immune response, inflammation, apoptosis and tumorigenesis[68,69]. NF-κB complexes can be activated by many divergent stimuli by two major pathways[67,70]. In the canonical or classic 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, and this results in NF-κB nuclear translocation and binding to DNA. In the

non-canonical or alternative NF-κB signalling pathway, IκBα is not involved but instead extracellular stimuli induce proteolytic processing of NF-κB2 (p100), which promotes nuclear translocation of p52/RelB heterodimers. Numerous groups using a variety of cell types have reported that TWEAK–Fn14 binding activates the canonical NF-κB signalling pathway[5,24,26,31,33,43–46,54–56,58,66,71–76]. TWEAK can also activate the canonical pathway when injected into the mouse brain[51]. One group has reported that TWEAK activates both the canonical and non-canonical NF-κB pathways when added to certain rat and mouse fibroblast lines66. However, non-canonical NF-κB pathway activation does not occur in all cells54. Finally, TWEAK treatment of several cell types has been shown to activate multiple signalling pathways. Specifically, in human endothelial cells, the canonical NF-κB, ERK and JNK pathways are activated43; in murine C2C12 myoblasts, the canonical NF-κB and ERK pathways are activated[46]; and in murine MC3T3-E1 osteoblast progenitor cells, the canonical NF-κB, ERK and phosphatidylinositol 3-kinase (PI3K)/AKT pathways are activated[73]. Thus, TWEAK regulation of vastly different cellular responses is probably due, at least in part, to cell type-specific differences in Fn14-mediated intracellular signalling events.

As binding of TWEAK to Fn14 activates the NF-κB pathway, it is not surprising that treatment of various cell types *in vitro* with TWEAK has been shown to induce the expression of known NF-κB target genes, including the secreted matrix metalloproteinase *MMP9* (REFS 47,51,76,77), the anti-apoptotic proteins A20, cIAP2, BCL-2 and BCL-XL31,71, TRAF1 and TRAF3 (REF. 31), and, as mentioned earlier, Fn14 itself58. Most of the other TWEAK-responsive genes identified to date encode inflammation-associated proteins, which is consistent with the central role of the NF-κB signalling pathway in controlling gene transcription during inflammation, a complex, dynamic process that occurs in tissues following traumatic, infectious, toxic or autoimmune injury78,79. Examples of TWEAK-inducible pro-inflammatory molecules include cytokines (for example, interleukin 6 (IL6)80,81; granulocyte macrophage-colony stimulating factor (GM-CSF))75; chemokines (for example, IL84,44,75,80–82, monocyte chemotactic protein 1 (MCP1)[31,44,72,83,84] and RANTES31,72–74,81); and cell–cell adhesion molecules (for example, intercellular adhesion molecule 1 (ICAM1)31,44,80,82, vascular cell adhesion molecule 1 (VCAM1)31,72,82). Recently, Campbell *et al.*[72] reported that intravenous TWEAK injection (200 μg) induces *MCP1* and *IP10* mRNA expression in kidney. This gene-expression data, in combination with additional findings demonstrating that anti-TWEAK neutralizing monoclonal antibodies reduce inflammatory cell infiltration in mouse models of rheumatoid arthritis47,83 and multiple sclerosis[85] (see below) and that *Fn14*-null mice display a deficient inflammatory response following tissue injury[31], is consistent with the proposal that TWEAK is in fact a pro-inflammatory cytokine. This property, as well as its ability to regulate cell proliferation and angiogenesis, supports the postulated role for TWEAK as an important regulator of tissue regeneration and repair *in vivo.*

TWEAK-independent Fn14 signalling

Several groups have reported that Fn14 may be able to signal in a ligand-independent manner when it is ectopically overexpressed *in vitro*. Specifically, ectopic *Fn14* expression has been shown to activate the NF-κB signalling pathway[55,56] and induce cellular responses, including rat PC12 cell neurite extension and growth cone formation[57], rat aortic smooth muscle cell migration[56], human glioma cell resistance to pro-apoptotic stimuli[71], glioma cell migration and invasion[58,86] and oesophageal adenocarcinoma cell invasion[87]. These Fn14-triggered effects require an intact TRAF binding site in the Fn14 cytoplasmic tail[55–58,71,86]. Interestingly, Fn14-triggered PC12 neurite extension and glioma cell migration/invasion require RAC1 function[57,58]. In all of the experiments described above, recombinant soluble TWEAK was not added to the cell culture medium; therefore, this effect is likely to be due

to TWEAK-independent Fn14 signalling. However, it should be noted that if the fetal bovine serum in the cell culture medium contains TWEAK, or the cells under investigation produce soluble TWEAK, there could be some TWEAK–Fn14 binding under these experimental conditions. Also, at least in theory, the medium could contain an unidentified Fn14 ligand.

It has been shown that Fn14-triggered PC12 neurite extension cannot be inhibited with a soluble Fn14-Fc decoy protein[57], so at least in this experimental system TWEAK-dependent signalling is not mediating the observed cellular response. In consideration of this finding, as well as previous results demonstrating that other TNFR superfamily members can signal on their own when overexpressed in cells[88–90], it is possible that TWEAK-independent Fn14 signalling can occur *in vitro* when intracellular Fn14 levels reach a certain threshold level. The likely explanation for this effect is that Fn14 overexpression on the cell surface induces spontaneous trimerization and multimerization, and this 'receptor clustering' promotes TRAF association and intracellular signalling cascade activation (FIG. 4b).

Could TWEAK-independent Fn14 signalling occur *in vivo*? Fn14 is expressed at relatively high levels in injured tissues and in solid tumours, but these levels of expression may or may not be high enough to trigger ligand-independent signalling. If these Fn14 expression levels are sufficient, then the TWEAK expression level in these tissues/tumours could be the critical factor that controls whether ligand-dependent or -independent Fn14 signalling predominates (FIG. 5). In this model, if both TWEAK and Fn14 expression is elevated, which seems to be the case in ischaemic brain tissue[28,33,91] and in injured kidney[34] and skeletal muscle[31], then TWEAK-dependent Fn14 signalling would be the primary mechanism triggering cellular responses. Conversely, if TWEAK expression is low, but Fn14 expression is high, for example, as has been reported in advanced brain tumours[27,58], then TWEAK-independent Fn14 signalling may predominate.

TWEAK and Fn14 as therapeutic targets

The studies summarized above indicate that TWEAK-dependent Fn14 signalling, and possibly also TWEAK-independent Fn14 signalling, might have an important role in wound repair — a coordinated, complex process involving blood clotting, cell proliferation, cell migration, inflammation and angiogenesis[92,93]. In those instances of acute injury, Fn14 signalling would be transient in nature and beneficial. However, in conditions of chronic tissue injury and inflammation, TWEAK and/or Fn14 levels may be elevated, which could result in persistent activation of Fn14-coupled intracellular signalling cascades. This would probably produce harmful, pathological effects. This section will first review data indicating that TWEAK–Fn14 axis signalling could have a detrimental role in several different diseases and will then discuss possible therapeutic targeting strategies for inhibiting TWEAK-dependent and -independent Fn14 signalling in the clinical setting.

Cancer

Cancer initiation and progression is a multistep process that converts normal human cells into aggressive, malignant derivatives that cannot be effectively eliminated by the body's immune system[94,95]. As discussed earlier, TWEAK and Fn14 are expressed in tumour tissue and TWEAK activates several cellular processes associated with tumour progression (for example, proliferation, invasion, angiogenesis and inflammation). The possibility that TWEAK–Fn14 axis function could contribute to tumour growth and metastasis has been discussed in two previous review articles[86,96]. Here, evidence supporting the concept that TWEAK and Fn14 might play a part in several different aspects of tumour biology will be highlighted.

TWEAK-dependent Fn14 signalling may regulate tumour growth

Solid tumours are complex tissues containing both neoplastic tumour cells and non-neoplastic cell types (for example, vascular endothelial cells, fibroblasts and macrophages) that together comprise the tumour microenvironment[95]. TWEAK expression has been detected in primary tumours, and it is probably produced, to varying degrees, by both tumour cells and non-neoplastic cells (discussed above). TWEAK that is present within solid tumour tissue could act on the tumour cells themselves, stimulating growth, survival, migration or even death (TABLE 1). Two recent reports indicate that immune cell-derived TWEAK can have either a positive[30] or negative[97] effect on tumour outgrowth. In one study, *Tweak*-null mice and wild-type littermate controls were injected subcutaneously with either moderately aggressive (B16.F10) or highly aggressive (B16.BL6) murine B16 melanoma subclones and tumour growth was monitored[30]. TWEAK deficiency completely inhibited B16.F10 tumour establishment and growth, and significantly attenuated B16. BL6 tumour growth. Additional research led the authors to conclude that TWEAK produced by cells of the innate immune system might normally act to suppress both innate and adaptive antitumour immunity. In the second study, Fn14-positive murine tumour cell lines were implanted subcutaneously into severe combined immunodeficient mice or syngeneic, immunocompetent DBA/2 mice and then either an anti-mouse TWEAK monoclonal antibody (mAb) or control immunoglobulin G (IgG) was administered intraperitoneally97. TWEAK neutralization promoted tumour growth, and this effect could be suppressed if macrophage infiltration into the tumour site was inhibited, suggesting that TWEAK contributes to the antitumour effect of tumour-associated macrophages. More studies are necessary in order to establish whether TWEAK, like several other cytokines98, can in fact exert either pro-tumorigenic or antitumorigenic effects depending on the particular microenvironmental conditions.

TWEAK-dependent Fn14 signalling may promote tumour angiogenesis and inflammation

TWEAK produced in the tumour microenvironment could also act on vascular endothelial cells and stimulate both angiogenesis, which is crucial for primary tumour growth and metastasis[99,100], and inflammatory cell infiltration, which has both pro-and antitumorigenic effects[95,101]. It is important to note that the processes of angiogenesis and inflammation are closely linked. Indeed, in the context of cancer, macrophage-derived TWEAK may contribute to inflammatory angiogenesis, an important force in tumour growth and expansion[102].

A potential role for TWEAK in tumour angiogenesis is supported by our findings showing that HEK293 cells that overexpress TWEAK form large, highly vascularized tumours when injected subcutaneously into mice[23], but this possibility still needs to be vigorously tested in cancer models using *Fn14*-null mice or inhibitors of TWEAK–Fn14 binding. Of course, tumours produce multiple angiogenic factors, including VEGFA and FGF2; so, is it really important to add TWEAK to the ever-expanding list of tumour angiogenesis factors? Several interesting findings are relevant to this issue. First, VEGFA and FGF2 treatment of endothelial cells can induce *Fn14* expression[43]. Second, TWEAK can act in concert with VEGFA or FGF2 to stimulate a synergistic endothelial cell growth response *in vitro*[43,103]. Third, FGF2 angiogenic activity *in vitro* is potentiated by TWEAK[103], and FGF2 angiogenic activity *in vivo* can be partially inhibited by a TWEAK antagonist[9]. Given these findings, VEGFA, FGF2 and TWEAK might cooperate during tumour angiogenesis. Specifically, VEGFA, FGF2 and TWEAK binding to their respective receptors on the endothelial cell surface may not only activate the endothelium and initiate the angiogenic process, but the first two factors, and possibly also TWEAK itself[58], might act as TWEAK sensitizers by increasing *Fn14* expression levels (FIG. 6). In this scenario, TWEAK angiogenic activity could represent a significant fraction of the total angiogenic stimulus, and TWEAK could

act both alone and in an additive or synergistic manner with VEGFA and FGF2 to promote vessel sprouting. If this is the case, then one could argue that TWEAK would be an attractive target for anti-angiogenesis therapy for patients with cancer.

TWEAK-independent Fn14 signalling may promote tumour cell invasion and metastasis

Tumour cells frequently travel through the blood or lymphatic vessels to distant organs and this metastatic spread is responsible for the majority of cancer deaths[104]. Effective tumour cell metastasis requires the sequential completion of a complex, multistep process, which includes invasion through the tumour stroma, intravasation through the endothelium, survival in the circulatory system and extravasation into a distant site[105,106]. In certain tumours — for example, brain tumours — cell invasion itself, not metastatic spread, is the predominant cause of tumour lethality[107]. In previous sections of this Review it was noted that Fn14 is overexpressed in certain solid tumours relative to control non-tumorous tissue and that ectopic *Fn14* expression can activate intracellular signalling pathways and induce tumour cell responses. These findings provide the rationale for the hypothesis that TWEAK-independent Fn14 signalling may occur in tumour cells *in vivo* if Fn14 levels reach a certain threshold value. What would be the probable outcome? Recent studies using glioma and oesophageal adenocarcinoma cell lines have revealed that the invasive capacity of tumour cells can be altered by manipulating Fn14 levels; specifically, inhibiting endogenous *Fn14* expression using siRNAs can decrease invasive activity, and increasing *Fn14* expression levels using adenoviral vectors can increase invasive activity[58,87]. Therefore, sustained TWEAK-independent Fn14 signalling in tumour cells may increase tumour cell invasiveness, and in certain tumour types, this could act in concert with other cytokine/chemokine-triggered signalling pathways to promote metastatic spread. Taken together, these findings suggest that Fn14 might not only be a tumour biomarker, but also a pro-invasive/metastatic molecule that warrants consideration as a novel therapeutic target for cancer.

Chronic autoimmune diseases

TWEAK is a proinflammatory cytokine, and as such, persistent TWEAK–Fn14 axis signalling could in theory play a role in human pathologies caused by excessive or abnormal inflammatory responses[78,79]. Recent reports using rodent models of human disease have indicated that TWEAK-dependent Fn14 signalling can indeed contribute to the clinical severity of three autoimmune, inflammatory diseases: rheumatoid arthritis, SLE and multiple sclerosis.

Rheumatoid arthritis is a chronic inflammatory autoimmune disorder of the synovial joints that leads to cartilage and bone destruction. Angiogenesis, discussed earlier, is one of the key processes associated with disease progression[108]. Numerous cytokines, including TNF-α, are present in synovial tissues and contribute to the pathogenesis of rheumatoid arthritis[109]. Two independent groups, both using the mouse collagen-induced arthritis model, have recently reported that TWEAK is a potent arthritogenic cytokine[47,83]. In this model, serum TWEAK levels increase during disease development[47]. These investigators found that intraperitoneal injection of an anti-TWEAK neutralizing mAb reduced overall disease severity[47,83] and diminished several specific disease-associated processes including synovial hyperplasia[83], inflammatory cell infiltration[47,83], synovial angiogenesis[47,83] and cartilage/bone loss[47]. It will be important to confirm these results using *Tweak*- or *Fn14*-null mice backcrossed into the DBA/1 strain. In any case, these studies indicate that TWEAK–Fn14 axis signalling could be a novel therapeutic target for human rheumatoid arthritis.

SLE is an autoimmune, inflammatory disease characterized by autoantibody production and deposition of immune complexes in multiple organs, including the kidney (lupus

nephritis)[110]. The possibility that TWEAK could contribute to the inflammatory damage that is associated with this disease has been reviewed111. Initial work revealed that patients with SLE and nephritis had higher urinary TWEAK levels than lupus patients with no history of renal involvement or inactive nephritis112. In a pivotal paper, Zhao *et al.*[50] studied TWEAK–Fn14 axis function in a chronic graft-versus-host SLE mouse model, with particular focus on renal disease severity. These investigators found that when mice with induced lupus were treated with an anti-TWEAK neutralizing mAb (intraperitoneal injection), both kidney cytokine/chemokine production and urinary total protein levels (an indictor of renal damage) were significantly reduced. Furthermore, when they compared disease development in wild-type versus *Fn14*-null mice, they found that Fn14 deficiency reduced proteinuria as well as kidney IgG deposition, cytokine/chemokine production and macrophage infiltration. These findings indicate that TWEAK blockade might be a novel therapeutic approach to treat patients with SLE and with active kidney disease.

Multiple sclerosis is the most common demyelinating disease of the central nervous system[113]. Experimental autoimmune encephalitis (EAE), perhaps the best characterized animal model of human autoimmune disease, recapitulates many, but not all, aspects of multiple sclerosis[113,114]. *TWEAK* mRNA expression in brain and spinal cord is increased during the development of myelin-oligodendrocyte glycoprotein (MOG)-induced EAE and, as mentioned earlier, soluble TWEAK-overexpressing transgenic mice develop a more severe form of EAE[29] (TABLE 2). Consistent with this finding, Mueller *et al.*[84] found that intramuscular injection of a rat full-length *TWEAK* expression plasmid increased spinal cord inflammation and disease severity in a rat model of MOG-induced EAE. Additionally, two independent approaches have indicated that TWEAK–Fn14 axis blockade is efficacious in EAE models. In one study, rats or mice were injected intraperitoneally with recombinant rat TWEAK or Fn14 extracellular domain proteins to generate TWEAK-specific or Fn14-specific antibodies that were capable of neutralizing TWEAK biological activity *in vitro*[84]. Vaccination with these proteins reduced spinal-cord inflammation and disease severity in both the rat and mouse EAE models. In another study, Desplat-Jego *et al.*[85] found that intraperitoneal injection of an anti-TWEAK neutralizing mAb reduced spinal-cord inflammatory cell infiltration and disease severity in the mouse MOG-induced EAE model. These findings support the possibility that TWEAK–Fn14 axis blockade could be a promising therapeutic approach for multiple sclerosis.

Acute ischaemic stroke

Stroke is the second most common cause of death and the leading cause of long-term disability in the world. In most cases, this disease is initiated by occlusion of a cerebral blood vessel, which results in irreversible ischaemic brain injury. Brain ischaemia triggers a cascade of molecular and cellular events, including energy depletion, excitotoxicity, an inflammatory response, an increase in blood–brain barrier permeability, the development of cerebral oedema and neuronal cell death[115–117]. Experimental evidence implicating the TWEAK cytokine in the pathogenesis of acute ischaemic stroke has been reviewed previously[118–120]; therefore, this section will provide a brief overview of the available data supporting the notion that TWEAK–Fn14 axis targeting might be a novel strategy for stroke therapy.

The first report indicating that TWEAK was involved in cerebral ischaemia-induced cell death was published in 2004. In this study, Potrovita *et al.*[33] found that TWEAK could induce murine cortical neuron apoptosis *in vitro*, and that this response was mediated, at least in part, by NF-κB pathway activation. They also reported that *TWEAK* and *Fn14* expression increased after cerebral ischaemia in mice, and that intraperitoneal administration of an anti-TWEAK neutralizing mAb could reduce cerebral infarct size in a permanent middle cerebral artery occlusion stroke model. Subsequently, Yepes and colleagues reported

that intracerebroventricular injection of a soluble Fn14-Fc decoy receptor significantly reduced both infarct volume[28] and cerebral oedema[51] using a similar murine stroke model. The possibility that TWEAK–Fn14 axis signalling within the ischaemic brain contributes to both cell death and an increase in the permeability of the blood–brain barrier was recently confirmed using *Fn14*-null mice[52] (TABLE 2). Finally, TWEAK can activate the NF-κB pathway in brain tissue[51,52], and it probably participates in the post-ischaemic inflammatory reaction (M. Yepes and J. Winkles, unpublished observations). These findings implicate TWEAK-dependent Fn14 signalling in the pathogenesis of ischemic cerebral damage; accordingly, the TWEAK–Fn14 axis might also be a good target for ischaemic stroke therapy.

Therapeutic strategies for inhibition of Fn14 signalling

The studies summarized above indicate that TWEAK and Fn14 might have a role in tumour progression and that TWEAK–Fn14 axis signalling can increase disease severity in rodent models of autoimmune disease and stroke. Hyperactive or persistent TWEAK-dependent or-independent Fn14 signalling may trigger pathological effects in humans, and there are numerous approaches that one could consider for inhibiting Fn14 signalling in diseased tissues (FIG. 7). In regard to the conventional TWEAK-dependent Fn14 signalling pathway, either an anti-TWEAK neutralizing mAb or soluble Fn14-Fc decoy protein could be used to prevent TWEAK binding to Fn14 cell-surface receptors, and in fact these two agents have shown therapeutic efficacy *in vivo*. Indeed, similar protein-based drugs targeting the TNFα–TNFR2 axis are some of the most successful marketed biologics to date[121]. Other strategies include the use of anti-Fn14 extracellular domain mAbs that prevent TWEAK–Fn14 binding or Fn14 trimerization. Alternatively, one could administer small-molecule antagonists that prevent TWEAK trimerization or TWEAK–Fn14 association. Compounds that penetrate the plasma membrane and block Fn14 trimerization, TRAF–Fn14 association, or signalling pathway activation (for example, NF-κB inhibitors) could also be considered. In regard to the postulated TWEAK-independent Fn14 signalling pathway that may be activated when Fn14 expression is elevated and TWEAK levels are low, one strategy would be to administer an anti-Fn14 mAb that interferes with Fn14 multimerization or disassociates preformed multimers.

Alternatively, one could deliver proteins consisting of either anti-Fn14 mAb Fv fragments or the TWEAK-receptor binding domain fused, linked or chemically conjugated to either a plant or bacterial toxin. These types of agents have been developed by various groups to target and destroy cells that overexpress particular cell-surface receptors[122]. One of the several issues that must be considered when developing these types of agents for clinical use is that patients will develop neutralizing antibodies to the toxin moiety. Finally, TWEAK-independent Fn14 signalling could also be targeted using cell-permeable agents as described above.

Concluding remarks and future studies

Research conducted in numerous laboratories during the past decade has revealed that TWEAK, acting via the Fn14 cell-surface receptor, is a multifunctional proinflammatory/pro-angiogenic cytokine that might normally participate in the process of wound repair and tissue regeneration. Furthermore, these studies suggest that dysregulated (that is, sustained) Fn14 signalling may contribute to the pathogenesis of cancer, inflammatory diseases and ischaemic stroke. Although much progress has been made, there are many unanswered questions. Can membrane-anchored TWEAK function in a juxtacrine manner to bind and activate Fn14 receptors on neighbouring cells? Is membrane-anchored TWEAK capable of reverse signalling, as demonstrated for several other TNF-like cytokines[123]? How does TWEAK binding to the Fn14 receptor induce neuronal cell apoptosis? Could the TWEAK–

Fn14 axis have a role in the pathogenesis of an even greater spectrum of human diseases? Atherosclerosis, the cause of myocardial infarction, is primarily an inflammatory disease, and atherosclerotic lesions contain both inflammatory/immune cells and vascular smooth muscle cells (SMC)124. As TWEAK and Fn14 are co-expressed in atherosclerotic lesions76·125 and TWEAK is a smooth muscle cell mitogen8 and chemotactic factor[56], could TWEAK activity contribute to intimal smooth muscle cell accumulation and local inflammation in this setting? Also, TWEAK and Fn14 expression has been detected in the heart[4,5,10,11], but there have been no reports investigating TWEAK–Fn14 axis function in this critical organ. Does TWEAK exert beneficial or detrimental effects after cardiac injury? Could TWEAK, like TNF-α [126], have a role in chronic heart failure? It is anticipated that future basic and translational studies focused on the TWEAK–Fn14 axis will undoubtedly reveal more unexpected and unique functions for this axis and further clarify if TWEAK and Fn14 are indeed suitable molecular targets for the development of novel therapeutics.

Acknowledgments

I thank my laboratory manager S. Brown and all of my other laboratory colleagues for their important contributions to our TWEAK–Fn14 studies. I also thank my external TWEAK–Fn14 research collaborators (in particular, M. Berens of the Translational Genomics Research Institute and M. Yepes of Emory University School of Medicine) for involving my laboratory in their various projects. I am also grateful to A. Keegan, M. Williams and three anonymous referees for their helpful comments on this article. Research in the author's laboratory is supported by NIH grants R01-HL39727 and R01-NS55126 and Susan G. Komen for the Cure grant BCTR0503968.

Glossary

Type II transmembrane protein	An integral membrane protein that is composed of an amino-terminal intracellular region, a transmembrane domain and a carboxy-terminal extracellular region (for example, Ly49).
Type I transmembrane protein	An integral membrane protein that is composed of an amino-terminal extracellular region, a transmembrane domain and a carboxy-terminal intracellular region (for example, T-cell receptors).
Type III transmembrane protein	An integral membrane protein with a similar orientation as a Type I protein but lacking a signal peptide (for example, B-cell maturation antigen (BCMA)).

References

1. Foster D, Parrish-Novak J, Fox B, Xu W. Cytokine–receptor pairing: accelerating discovery of cytokine function. Nature Rev. Drug Discov 2004;3:160–170. [PubMed: 15040579]
2. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell 2001;104:487–501. [PubMed: 11239407]
3. Bodmer J, Schneider P, Tschopp J. The molecular architecture of the TNF superfamily. Trends Biochem. Sci 2002;27:19–26. [PubMed: 11796220]
4. Chicheportiche Y, et al. TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. J. Biol. Chem 1997;272:32401–32410. [PubMed: 9405449] References 4 and 5 describe the initial cloning and characterization of the TWEAK cytokine.
5. Marsters SA, et al. Identification of a ligand for the death-domain-containing receptor Apo3. Curr. Biol 1998;8:525–528. [PubMed: 9560343]
6. Schneider P, et al. TWEAK can induce cell death via endogenous TNF and TNF receptor 1. Eur. J. Immunol 1999;29:1785–1792. [PubMed: 10382740]
7. Kaptein A, et al. Studies on the interaction between TWEAK and the death receptor WSL-1/TRAMP (DR3). FEBS Lett 2000;485:135–141. [PubMed: 11094155]

8. Lynch CN, et al. TWEAK induces angiogenesis and proliferation of endothelial cells. J. Biol. Chem 1999;274:8455–8459. [PubMed: 10085077]
9. Wiley SR, et al. A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis. Immunity 2001;15:837–846. [PubMed: 11728344] A pivotal paper describing the successful cloning of a TWEAK-binding cell surface protein (TweakR, Fn14) from an endothelial cell cDNA expression library.
10. Meighan-Mantha RL, et al. The mitogen-inducible *Fn14* gene encodes a type I transmembrane protein that modulates fibroblast adhesion and migration. J. Biol. Chem 1999;274:33166–33176. [PubMed: 10551889]
11. Feng SY, et al. The *Fn14* immediate-early response gene is induced during liver regeneration and highly expressed in both human and murine hepatocellular carcinomas. Am. J. Pathol 2000;156:1253–1261. [PubMed: 10751351] An initial report demonstrating *Fn14* overexpression in human tumour specimens.
12. Bossen C, et al. Interactions of tumor necrosis factor (TNF) and TNF receptor family members in the mouse and human. J. Biol. Chem 2006;281:13964–13971. [PubMed: 16547002]
13. Inman GJ, Allday MJ. Apoptosis induced by TGF-β1 in Burkitt's lymphoma cells is caspase 8 dependent but is death receptor independent. J. Immunol 2000;165:2500–2510. [PubMed: 10946276]
14. Kaplan MJ, Ray D, Mo RR, Yung RL, Richardson BC. TRAIL (Apo2 ligand) and TWEAK (Apo3 ligand) mediate $CD4^+$ T cell killing of antigen-presenting macrophages. J. Immunol 2000;164:2897–2904. [PubMed: 10706675]
15. Borysenko CW, et al. Death receptor-3 mediates apoptosis in human osteoblasts under narrowly regulated conditions. J. Cell. Physiol 2006;209:1021–1028. [PubMed: 16986165]
16. Vince JE, Silke J. TWEAK shall inherit the earth. Cell Death Differ 2006;13:1842–1844. [PubMed: 16932755]
17. Burkly LC, Michaelson JS, Hahm K, Jakubowski A, Zheng TS. TWEAKing tissue remodeling by a multifunctional cytokine: Role of TWEAK/Fn14 pathway in health and disease. Cytokine 2007;40:1–16. [PubMed: 17981048] An excellent review article on the TWEAK–Fn14 pathway.
18. De KA, et al. Involvement of GSK-3β in TWEAK-mediated NF-κB activation. FEBS Lett 2004;566:60–64. [PubMed: 15147869]
19. Baxter FO, et al. IKKβ/2 induces TWEAK and apoptosis in mammary epithelial cells. Development 2006;133:3385–3394.
20. Kawakita T, et al. Functional expression of TWEAK in human colonic adenocarcinoma cells. Int. J. Oncol 2005;26:87–93. [PubMed: 15586228]
21. Nakayama M, Harada N, Okumura K, Yagita H. Characterization of murine TWEAK and its receptor (Fn14) by monoclonal antibodies. Biochem. Biophys. Res. Commun 2003;306:819–825. [PubMed: 12821115]
22. Chicheportiche Y, Fossati-Jimack L, Moll S, Ibnou-Zekri N, Izui S. Down-regulated expression of TWEAK mRNA in acute and chronic inflammatory pathologies. Biochem. Biophys. Res. Commun 2000;279:162–165. [PubMed: 11112433]
23. Ho DH, et al. Soluble tumor necrosis factor-like weak inducer of apoptosis overexpression in HEK293 cells promotes tumor growth and angiogenesis in athymic nude mice. Cancer Res 2004;64:8968–8972. [PubMed: 15604260] An initial report indicating that TWEAK could contribute to tumour angiogenesis.
24. De A, et al. Intraovarian tumor necrosis factor-related weak inducer of apoptosis/fibroblast growth factor-inducible-14 ligand-receptor system limits ovarian preovulatory follicles from excessive luteinization. Mol. Endocrinol 2006;20:2528–2538. [PubMed: 16762976]
25. Chacon MR, et al. Expression of TWEAK and its receptor Fn14 in human subcutaneous adipose tissue. Relationship with other inflammatory cytokines in obesity. Cytokine 2006;33:129–137. [PubMed: 16503147]
26. Kawakita T, et al. Functional expression of TWEAK in human hepatocellular carcinoma: possible implication in cell proliferation and tumor angiogenesis. Biochem. Biophys. Res. Commun 2004;318:726–733. [PubMed: 15144899]

27. Tran NL, et al. The human Fn14 receptor gene is up-regulated in migrating glioma cells *in vitro* and overexpressed in advanced glial tumors. Am. J. Pathol 2003;162:1313–1321. [PubMed: 12651623]
28. Yepes M, et al. A soluble Fn14-Fc decoy receptor reduces infarct volume in a murine model of cerebral ischemia. Am. J. Pathol 2005;166:511–520. [PubMed: 15681834]
29. Desplat-Jego S, et al. TWEAK is expressed by glial cells, induces astrocyte proliferation and increases EAE severity. J. Neuroimmunol 2002;133:116–123. [PubMed: 12446014]
30. Maecker H, et al. TWEAK attenuates the transition from innate to adaptive immunity. Cell 2005;123:931–944. [PubMed: 16325585] The first publication that used *Tweak*-null mice.
31. Girgenrath M, et al. TWEAK, via its receptor Fn14, is a novel regulator of mesenchymal progenitor cells and skeletal muscle regeneration. EMBO J 2006;25:5826–5839. [PubMed: 17124496]
32. Nakayama M, Kayagaki N, Yamaguchi N, Okumura K, Yagita H. Involvement of TWEAK in interferon γ-stimulated monocyte cytotoxicity. J. Exp. Med 2000;192:1373–1380. [PubMed: 11067885]
33. Potrovita I, et al. Tumor necrosis factor-like weak inducer of apoptosis-induced neurodegeneration. J. Neurosci 2004;24:8237–8244. [PubMed: 15385607] An initial report linking TWEAK activity to ischaemia-induced cerebral tissue damage in a murine model of stroke.
34. Justo P, et al. Cytokine cooperation in renal tubular cell injury: the role of TWEAK. Kidney Int 2006;70:1750–1758. [PubMed: 17003819]
35. Polek TC, Talpaz M, Darnay BG, Spivak-Kroizman T. TWEAK mediates signal transduction and differentiation of RAW264.7 cells in the absence of Fn14/TweakR. Evidence for a second TWEAK receptor. J. Biol. Chem 2003;278:32317–32323. [PubMed: 12794080]
36. Bover LC, et al. A previously unrecognized protein–protein interaction between TWEAK and CD163: potential biological implications. J. Immunol 2007;178:8183–8194. [PubMed: 17548657]
37. Glenney GW, Wiens GD. Early diversification of the TNF superfamily in teleosts: genomic characterization and expression analysis. J. Immunol 2007;178:7955–7973. [PubMed: 17548633]
38. Brown SAN, Hanscom HN, Vu H, Brew SA, Winkles JA. TWEAK binding to the Fn14 cysteine-rich domain depends on charged residues located in both the A1 and D2 modules. Biochem. J 2006;397:297–304. [PubMed: 16526941]
39. Nakayama M, et al. Multiple pathways of TWEAK-induced cell death. J. Immunol 2002;168:734–743. [PubMed: 11777967]
40. Peter ME, Krammer PH. The CD95(APO-1/Fas) DISC and beyond. Cell Death Differ 2003;10:26–35. [PubMed: 12655293]
41. Wilson CA, Browning JL. Death of HT29 adenocarcinoma cells induced by TNF family receptor activation is caspase-independent and displays features of both apoptosis and necrosis. Cell Death Differ 2002;9:1321–1333. [PubMed: 12478469]
42. Nakayama M, et al. Fibroblast growth factor-inducible 14 mediates multiple pathways of TWEAK-induced cell death. J. Immunol 2003;170:341–348. [PubMed: 12496418]
43. Donohue PJ, et al. TWEAK is an endothelial cell growth and chemotactic factor that also potentiates FGF-2 and VEGF-A mitogenic activity. Arterioscler. Thromb. Vasc. Biol 2003;23:594–600. [PubMed: 12615668]
44. Harada N, et al. Pro-inflammatory effect of TWEAK/Fn14 interaction on human umbilical vein endothelial cells. Biochem. Biophys. Res. Commun 2002;299:488–493. [PubMed: 12445828]
45. Dogra C, Changotra H, Mohan S, Kumar A. Tumor necrosis factor-like weak inducer of apoptosis inhibits skeletal myogenesis through sustained activation of nuclear factor-κB and degradation of myoD protein. J. Biol. Chem 2006;281:10327–10336. [PubMed: 16461349]
46. Dogra C, Hall SL, Wedhas N, Linkhart TA, Kumar A. Fibroblast growth factor inducible 14 (Fn14) is required for the expression of myogenic regulatory factors and differentiation of myoblasts into myotubes. Evidence for TWEAK-independent functions of Fn14 during myogenesis. J. Biol. Chem 2007;282:15000–15010. [PubMed: 17383968]
47. Perper SJ, et al. TWEAK is a novel arthritogenic mediator. J. Immunol 2006;177:2610–2620. [PubMed: 16888023] An initial report linking TWEAK activity to tissue inflammation, angiogenesis and damage in a murine model of rheumatoid arthritis.

48. Kingsley LA, Fournier PG, Chirgwin JM, Guise TA. Molecular biology of bone metastasis. Mol. Cancer Ther 2007;6:2609–2617. [PubMed: 17938257]
49. Jakubowski A, et al. TWEAK induces liver progenitor cell proliferation. J. Clin. Invest 2005;115:2330–2340. [PubMed: 16110324] The first publication that used *Fn14*-null mice.
50. Zhao Z, et al. TWEAK/Fn14 interactions are instrumental in the pathogenesis of nephritis in the chronic graft-versus-host model of systemic lupus erythematosus. J. Immunol 2007;179:7949–7958. [PubMed: 18025243]
51. Polavarapu R, Gongora MC, Winkles JA, Yepes M. Tumor necrosis factor-like weak inducer of apoptosis increases the permeability of the neurovascular unit through nuclear factor-κB pathway activation. J. Neurosci 2005;25:10094–10100. [PubMed: 16267216]
52. Zhang X, et al. TWEAK–Fn14 pathway inhibition protects the integrity of the neurovascular unit during cerebral ischemia. J. Cereb. Blood Flow Metab 2007;27:534–544. [PubMed: 16835630]
53. Charge SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. Physiol. Rev 2004;84:209–238. [PubMed: 14715915]
54. Dogra C, et al. TNF-related weak inducer of apoptosis (TWEAK) is a potent skeletal muscle-wasting cytokine. FASEB J 2007;21:1857–1869. [PubMed: 17314137]
55. Brown SAN, Richards CM, Hanscom HN, Feng SL, Winkles JA. The Fn14 cytoplasmic tail binds tumour-necrosis-factor-receptor-associated factors 1, 2, 3 and 5 and mediates nuclear factor-κB activation. Biochem. J 2003;371:395–403. [PubMed: 12529173]
56. Han S, et al. TNF-related weak inducer of apoptosis receptor, a TNF receptor superfamily member, activates NF-κB through TNF receptor-associated factors. Biochem. Biophys. Res. Commun 2003;305:789–796. [PubMed: 12767899]
57. Tanabe K, Bonilla I, Winkles JA, Strittmatter SM. Fibroblast growth factor-inducible-14 is induced in axotomized neurons and promotes neurite outgrowth. J. Neurosci 2003;23:9675–9686. [PubMed: 14573547]
58. Tran NL, et al. Increased fibroblast growth factor-inducible 14 expression levels promote glioma cell invasion via Rac1 and nuclear factor-κB and correlate with poor patient outcome. Cancer Res 2006;66:9535–9542. [PubMed: 17018610] An important paper linking high Fn14 levels in advanced brain tumours to poor patient outcome.
59. Wiley SR, Winkles JA. TWEAK, a member of the TNF superfamily, is a multifunctional cytokine that binds the TweakR/Fn14 receptor. Cytokine Growth Factor Rev 2003;14:241–249. [PubMed: 12787562]
60. Fischer D, Petkova V, Thanos S, Benowitz LI. Switching mature retinal ganglion cells to a robust growth state *in vivo*: gene expression and synergy with RhoA inactivation. J. Neurosci 2004;24:8726–8740. [PubMed: 15470139]
61. Wang S, et al. Transcriptional profiling suggests that Barrett's metaplasia is an early intermediate stage in esophageal adenocarcinogenesis. Oncogene 2006;25:3346–3356. [PubMed: 16449976]
62. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N. Engl. J. Med 1986;315:1650–1659. [PubMed: 3537791]
63. Iyer VR, et al. The transcriptional program in the response of human fibroblasts to serum. Science 1999;283:83–87. [PubMed: 9872747]
64. Chang HY, et al. Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumors and wounds. PLoS Biol 2004;2:e7. [PubMed: 14737219]
65. Chang HY, et al. Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. Proc. Natl Acad. Sci. USA 2005;102:3738–3743. [PubMed: 15701700]
66. Saitoh T, et al. TWEAK induces NF-κB2 p100 processing and long lasting NF-κB activation. J. Biol. Chem 2003;278:36005–36012. [PubMed: 12840022]
67. Perkins ND. Integrating cell-signalling pathways with NF-κB and IKK function. Nature Rev. Mol. Cell Biol 2007;8:49–62. [PubMed: 17183360]
68. Hayden MS, West AP, Ghosh S. NF-κB and the immune response. Oncogene 2006;25:6758–6780. [PubMed: 17072327]
69. Karin M. Nuclear factor-kappaB in cancer development and progression. Nature 2006;441:431–436. [PubMed: 16724054]

70. Neumann M, Naumann M. Beyond IκBs: alternative regulation of NF-κB activity. FASEB J 2007;21:2642–2654. [PubMed: 17431096]
71. Tran NL, et al. The tumor necrosis factor-like weak inducer of apoptosis (TWEAK)-fibroblast growth factor-inducible 14 (Fn14) signaling system regulates glioma cell survival via NF-κB pathway activation and BCL-XL/BCL-W expression. J. Biol. Chem 2005;280:3483–3492. [PubMed: 15611130]
72. Campbell S, et al. Proinflammatory effects of TWEAK/Fn14 interactions in glomerular mesangial cells. J. Immunol 2006;176:1889–1898. [PubMed: 16424220]
73. Ando T, et al. TWEAK/Fn14 interaction regulates RANTES production, BMP-2-induced differentiation, and RANKL expression in mouse osteoblastic MC3T3-E1 cells. Arthritis Res. Ther 2006;8:R146. [PubMed: 16945157]
74. Jin L, et al. Induction of RANTES by TWEAK/Fn14 interaction in human keratinocytes. J. Invest. Dermatol 2004;122:1175–1179. [PubMed: 15140220]
75. Xu H, et al. TWEAK/Fn14 interaction stimulates human bronchial epithelial cells to produce IL-8 and GM-CSF. Biochem. Biophys. Res. Commun 2004;318:422–427. [PubMed: 15120617]
76. Kim SH, et al. TWEAK can induce pro-inflammatory cytokines and matrix metalloproteinase-9 in macrophages. Circ. J 2004;68:396–399. [PubMed: 15056843]
77. Michaelson JS, et al. Tweak induces mammary epithelial branching morphogenesis. Oncogene 2005;24:2613–2624. [PubMed: 15735761]
78. Nathan C. Points of control in inflammation. Nature 2002;420:846–852. [PubMed: 12490957]
79. Luster AD, Alon R, von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. Nature Immunol 2005;6:1182–1190. [PubMed: 16369557]
80. Saas P, et al. TWEAK stimulation of astrocytes and the proinflammatory consequences. Glia 2000;32:102–107. [PubMed: 10975915]
81. Chicheportiche Y, et al. Proinflammatory activity of TWEAK on human dermal fibroblasts and synoviocytes: blocking and enhancing effects of anti-TWEAK monoclonal antibodies. Arthritis Res 2002;4:126–133. [PubMed: 11879548]
82. Hosokawa Y, Hosokawa I, Ozaki K, Nakae H, Matsuo T. Proinflammatory effects of tumour necrosis factor-like weak inducer of apoptosis (TWEAK) on human gingival fibroblasts. Clin. Exp. Immunol 2006;146:540–549. [PubMed: 17100776]
83. Kamata K, et al. Involvement of TNF-like weak inducer of apoptosis in the pathogenesis of collagen-induced arthritis. J. Immunol 2006;177:6433–6439. [PubMed: 17056575]
84. Mueller AM, et al. Targeting fibroblast growth factor-inducible-14 signaling protects from chronic relapsing experimental autoimmune encephalomyelitis. J. Neuroimmunol 2005;159:55–65. [PubMed: 15652403]
85. Desplat-Jego S, et al. Anti-TWEAK monoclonal antibodies reduce immune cell infiltration in the central nervous system and severity of experimental autoimmune encephalomyelitis. Clin. Immunol 2005;117:15–23. [PubMed: 16027043]
86. Winkles JA, Tran NL, Berens ME. TWEAK and Fn14: new molecular targets for cancer therapy? Cancer Lett 2005;235:11–17. [PubMed: 15885893]
87. Watts GS, et al. Identification of Fn14/TWEAK receptor as a potential therapeutic target in esophageal adenocarcinoma. Int. J. Cancer 2007;121:2132–2139. [PubMed: 17594693]
88. Xu LG, Shu HB. TNFR-associated factor-3 is associated with BAFF-R and negatively regulates BAFF-R-mediated NF-κB activation and IL-10 production. J. Immunol 2002;169:6883–6889. [PubMed: 12471121]
89. Fotin-Mleczek M, et al. Tumor necrosis factor receptor-associated factor (TRAF) 1 regulates CD40-induced TRAF2-mediated NF-κB activation. J. Biol. Chem 2004;279:677–685. [PubMed: 14557256]
90. Hauer J, et al. TNF receptor (TNFR)-associated factor (TRAF) 3 serves as an inhibitor of TRAF2/5-mediated activation of the noncanonical NF-κB pathway by TRAF-binding TNFRs. Proc. Natl Acad. Sci. USA 2005;102:2874–2879. [PubMed: 15708970]
91. Trendelenburg G, et al. Serial analysis of gene expression identifies metallothionein-II as major neuroprotective gene in mouse focal cerebral ischemia. J. Neurosci 2002;22:5879–5888. [PubMed: 12122050]

92. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. J. Invest. Dermatol 2007;127:514–525. [PubMed: 17299434]

93. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol. Rev 2003;83:835–870. [PubMed: 12843410]

94. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70. [PubMed: 10647931]

95. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nature Rev. Cancer 2006;6:24–37. [PubMed: 16397525]

96. Winkles JA, et al. Role of TWEAK and Fn14 in tumor biology. Front. Biosci 2007;12:2761–2771. [PubMed: 17127278]

97. Kaduka Y, et al. TWEAK mediates anti-tumor effect of tumor-infiltrating macrophage. Biochem. Biophys. Res. Commun 2005;331:384–390. [PubMed: 15850771]

98. Witz IP. Yin-yang activities and vicious cycles in the tumor microenvironment. Cancer Res 2008;68:9–13. [PubMed: 18172289]

99. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nature Rev. Cancer 2003;3:401–410. [PubMed: 12778130]

100. Tonini T, Rossi F, Claudio PP. Molecular basis of angiogenesis and cancer. Oncogene 2003;22:6549–6556. [PubMed: 14528279]

101. Lu H, Ouyang W, Huang C. Inflammation, a key event in cancer development. Mol. Cancer Res 2006;4:221–233. [PubMed: 16603636]

102. Albini A, Tosetti F, Benelli R, Noonan DM. Tumor inflammatory angiogenesis and its chemoprevention. Cancer Res 2005;65:10637–10641. [PubMed: 16322203]

103. Jakubowski A, et al. Dual role for TWEAK in angiogenic regulation. J. Cell Sci 2002;115:267–274. [PubMed: 11839778]

104. Eccles SA, Welch DR. Metastasis: recent discoveries and novel treatment strategies. Lancet 2007;369:1742–1757. [PubMed: 17512859]

105. Gupta GP, Massague J. Cancer metastasis: building a framework. Cell 2006;127:679–695. [PubMed: 17110329]

106. Steeg PS. Tumor metastasis: mechanistic insights and clinical challenges. Nature Med 2006;12:895–904. [PubMed: 16892035]

107. Hoelzinger DB, Demuth T, Berens ME. Autocrine factors that sustain glioma invasion and paracrine biology in the brain microenvironment. J. Natl Cancer Inst 2007;99:1583–1593. [PubMed: 17971532]

108. Paleolog EM. Angiogenesis in rheumatoid arthritis. Arthritis Res 2002;4 Suppl. 3:S81–S90. [PubMed: 12110126]

109. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nature Rev. Immunol 2007;7:429–442. [PubMed: 17525752]

110. Rovin BH. The chemokine network in systemic lupus erythematous nephritis. Front. Biosci 2008;13:904–922. [PubMed: 17981599]

111. Campbell S, Michaelson J, Burkly L, Putterman C. The role of TWEAK/Fn14 in the pathogenesis of inflammation and systemic autoimmunity. Front. Biosci 2004;9:2273–2284. [PubMed: 15353286]

112. Schwartz N, et al. Urinary TWEAK and the activity of lupus nephritis. J. Autoimmun 2006;27:242–250. [PubMed: 17257812]

113. Hafler DA, et al. Multiple sclerosis. Immunol. Rev 2005;204:208–231. [PubMed: 15790361]

114. Baxter AG. The origin and application of experimental autoimmune encephalomyelitis. Nature Rev. Immunol 2007;7:904–912. [PubMed: 17917672]

115. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 1999;22:391–397. [PubMed: 10441299]

116. Huang J, Upadhyay UM, Tamargo RJ. Inflammation in stroke and focal cerebral ischemia. Surg. Neurol 2006;66:232–245. [PubMed: 16935624]

117. Simard JM, Kent TA, Chen M, Tarasov KV, Gerzanich V. Brain oedema in focal ischaemia: molecular pathophysiology and theoretical implications. Lancet Neurol 2007;6:258–268. [PubMed: 17303532]

118. Yepes M, Winkles JA. Inhibition of TWEAK activity as a new treatment for inflammatory and degenerative diseases. Drug News Perspect 2006;19:589–595. [PubMed: 17299600]
119. Yepes M. TWEAK and the central nervous system. Mol. Neurobiol 2007;35:255–265. [PubMed: 17917114]
120. Yepes M. Tweak and Fn14 in central nervous system health and disease. Front. Biosci 2007;12:2772–2781. [PubMed: 17485258]
121. Nash PT, Florin THJ. Tumour necrosis factor inhibitors. Med. J. Austr 2005;183:205–208.
122. Pastan I, Hassan R, Fitzgerald DJ, Kreitman RJ. Immunotoxin therapy of cancer. Nature Rev. Cancer 2006;6:559–565. [PubMed: 16794638]
123. Sun M, Fink PJ. A new class of reverse signaling costimulators belongs to the TNF family. J. Immunol 2007;179:4307–4312. [PubMed: 17878324]
124. Hansson GK, Robertson AK, Soderberg-Naucler C. Inflammation and atherosclerosis. Annu. Rev. Pathol 2006;1:297–329. [PubMed: 18039117]
125. Munoz-Garcia B, et al. Fn14 is upregulated in cytokine-stimulated vascular smooth muscle cells and is expressed in human carotid atherosclerotic plaques: modulation by atorvastatin. Stroke 2006;37:2044–2053. [PubMed: 16809572]
126. Sack M. Tumor necrosis factor-α in cardiovascular biology and the potential role for anti-tumor necrosis factor-α therapy in heart disease. Pharmacol. Ther 2002;94:123–135. [PubMed: 12191598]
127. Gius D, et al. Profiling microdissected epithelium and stroma to model genomic signatures for cervical carcinogenesis accommodating for covariates. Cancer Res 2007;67:7113–7123. [PubMed: 17671178]
128. Almstrup K, et al. Embryonic stem cell-like features of testicular carcinoma *in situ* revealed by genome-wide gene expression profiling. Cancer Res 2004;64:4736–4743. [PubMed: 15256440]
129. Hamill CA, Michaelson JS, Hahm K, Burkly LC, Kessler JA. Age-dependent effects of TWEAK/Fn14 receptor activation on neural progenitor cells. J. Neurosci. Res 2007;85:3535–3544. [PubMed: 17803219]
130. Felli N, et al. Multiple members of the TNF superfamily contribute to IFN-γ-mediated inhibition of erythropoiesis. J. Immunol 2005;175:1464–1472. [PubMed: 16034083]
131. Kaplan MJ, et al. The apoptotic ligands TRAIL, TWEAK, and Fas ligand mediate monocyte death induced by autologous Lupus T cells. J. Immunol 2002;169:6020–6029. [PubMed: 12421989]
132. Ren R, et al. Gene expression profiles identify a role for cyclooxygenase 2-dependent prostanoid generation in BMP6-induced angiogenic responses. Blood 2007;109:2847–2853. [PubMed: 17119124]

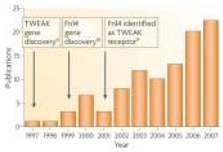

Figure 1. TWEAK–Fn14 axis publication summary

The number of TWEAK and/or Fn14 manuscripts published annually is shown. Publication numbers include both review articles and primary papers containing the terms TWEAK or Fn14 in the title or abstract with the exception that TWEAK was referred to as APO3L in the 1998 publication. The value shown for 2007 includes articles published online ahead of print.

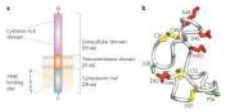

Figure 2. The Fn14 receptor

a | A schematic representation of the human Fn14 receptor is shown. Mature human Fn14 is only 102 amino acids (aa) in length, with a predicted molecular mass of 10,925 daltons and a theoretical isoelectric point of 8.24. **b** | High-resolution crystal structures for the TWEAK receptor-binding domain, the Fn14 extracellular domain (or cysteine-rich domain) or TWEAK–Fn14 complexes are presently unavailable. A homology model of the Fn14 cysteine-rich domain based on the X-ray crystal structure of the APRIL (A proliferation-inducing ligand)–BCMA (B-cell maturation antigen) complex is shown here. The cysteine residues (yellow) and the non-cysteine residues that have been shown to be important (red) or dispensable (green) for TWEAK binding to Fn14 are labelled and rendered as sticks. This figure is reproduced with permission from REF. [38] © (2006) The Biochemical Society.

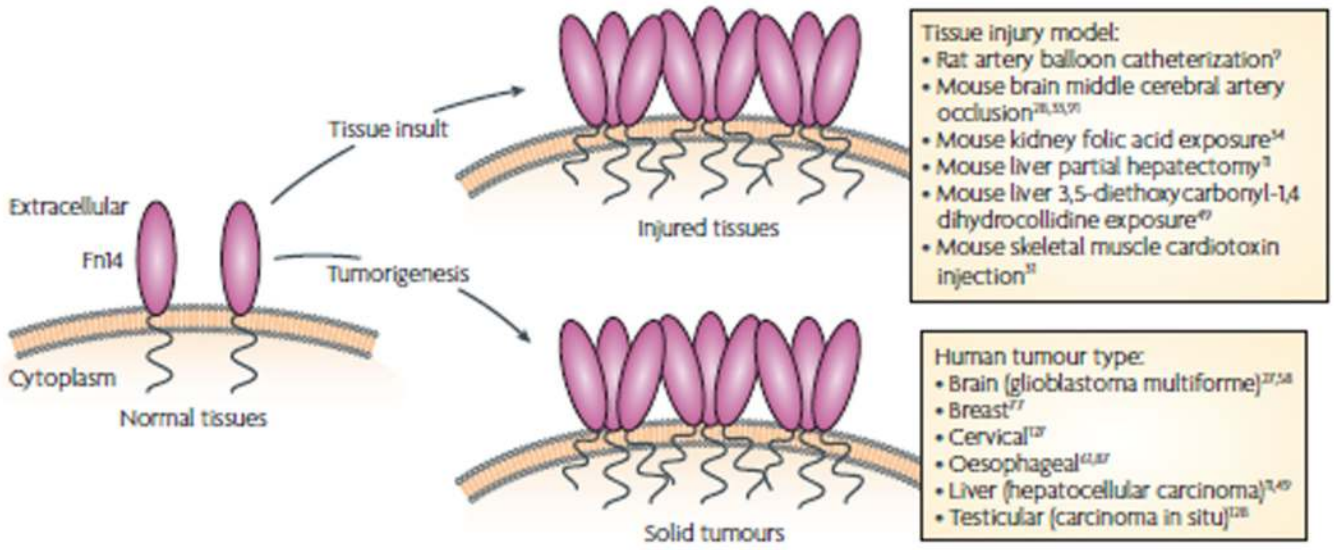

Figure 3. The *Fn14* gene is highly regulated *in vivo*

In most normal, healthy tissues *Fn14* expression is relatively low, but *Fn14* gene induction has been reported in various rodent models of tissue injury. Additionally, high levels of *Fn14* expression have been detected in several human tumour types. As illustrated in the figure, it is assumed that *Fn14* overexpression in injured tissues and solid tumours will promote Fn14 trimerization and multimerization, but this has not been confirmed experimentally.

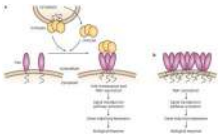

Figure 4. TWEAK-dependent and -independent Fn14 signalling

a | TWEAK is initially synthesized as a type II transmembrane protein (denoted here as membrane (m) TWEAK) but this protein can undergo intracellular proteolysis to generate a smaller, biologically active form that is released into the extracellular mileau (denoted here as soluble (s) TWEAK). As mTWEAK proteolytic processing does not occur with 100% efficiency, TWEAK can be detected on the surface of most cell types by fluorescence-activated cell-sorter analysis. It is established that sTWEAK can bind the Fn14 receptor and induce cellular responses, but it has not yet been reported that mTWEAK can act in a cell contact-dependent, juxtacrine manner to activate Fn14-positive cells. TWEAK is a trimeric cytokine, and it is likely that TWEAK binding promotes Fn14 trimerization, TNFR-associated factor (TRAF) association, signal pathway activation, changes in gene expression and thus, cellular responses. **b |** Ectopic *Fn14* overexpression studies conducted *in vitro* have indicated that when cellular Fn14 levels are elevated to a certain threshold value, TWEAK-independent Fn14 signalling can occur. It is likely that Fn14 overexpression on the cell surface induces monomer trimerization and trimer multimerization, which then triggers TRAF association and the subsequent molecular and cellular events. It is not presently known if TWEAK-independent Fn14 signalling occurs in cells that naturally express high levels of Fn14 *in vivo* (for example, certain tumour cells).

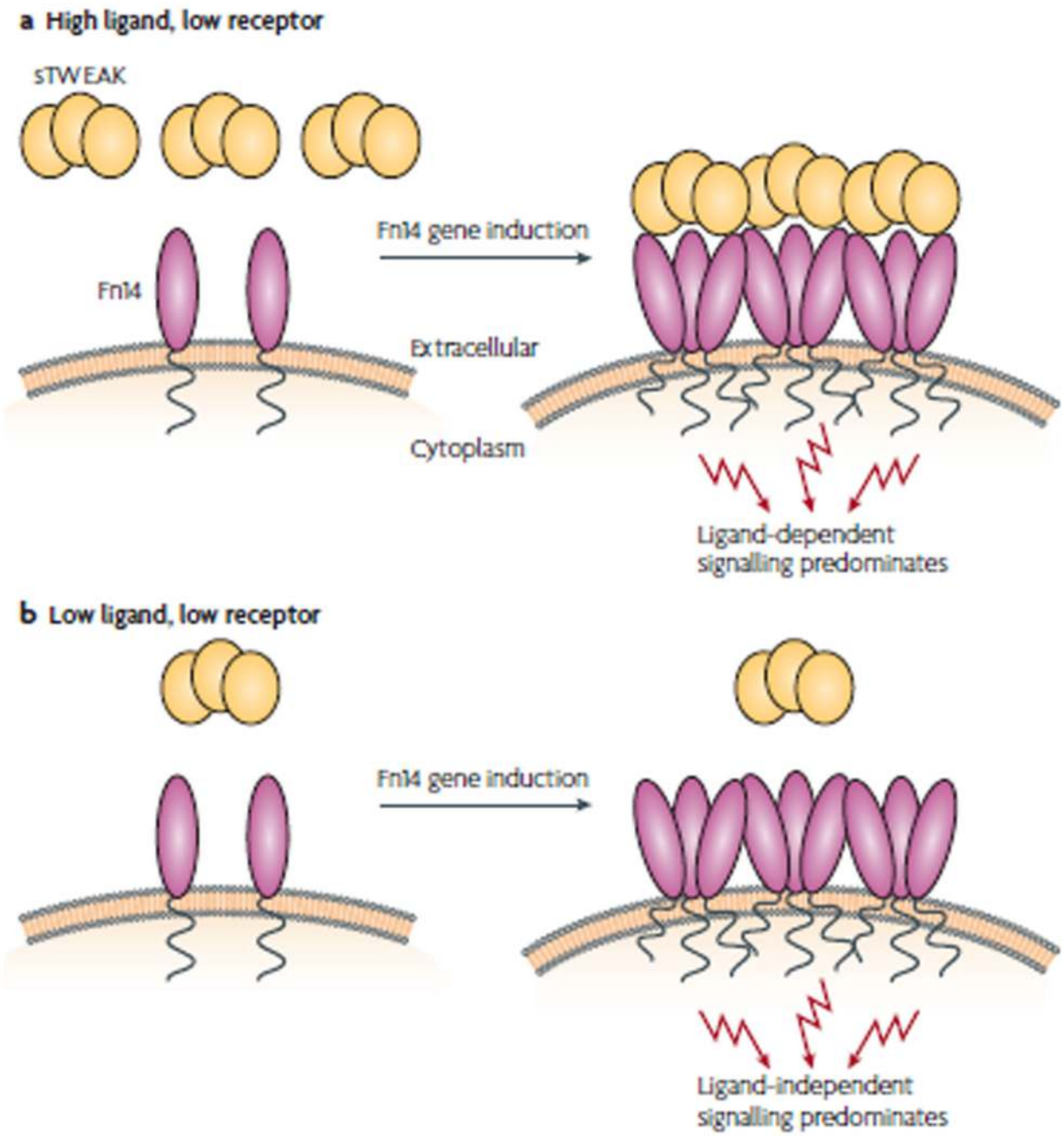

Figure 5. Hypothesis: TWEAK expression levels may dictate the predominant signalling mechanism in injured tissues and solid tumours

a | Tissues in which basal TWEAK expression levels are high and basal Fn14 expression levels are low, minimal Fn14 receptor activation is expected. However, an increase in Fn14 expression; for example, following tissue injury, would trigger TWEAK-dependent Fn14 signalling. **b** | Tissues in which both basal TWEAK and Fn14 expression levels are low, minimal Fn14 receptor activation is expected. In this situation, an increase in *Fn14* expression; for example, in response to tumour cell derived-growth factors, would trigger TWEAK-independent Fn14 signalling.

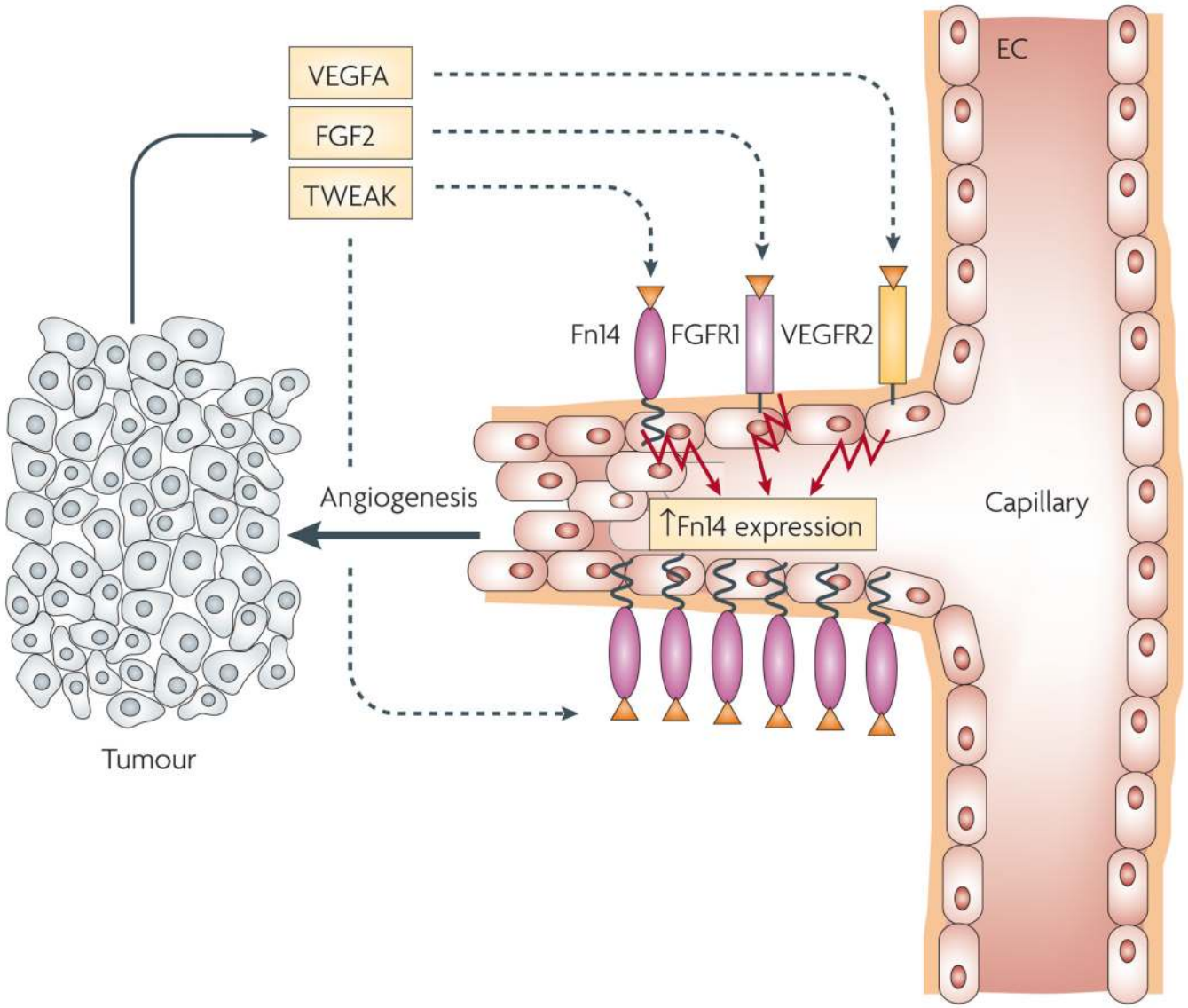

Figure 6. Hypothesis: some tumour angiogenic factors may act, at least in part, by increasing TWEAK-dependent Fn14 signalling in sprouting vessels

Tumours produce multiple angiogenic factors, including vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF2) and TWEAK. These three factors bind and activate the VEGFR2, FGFR1 and Fn14 receptors, respectively, on capillary endothelial cells. Each receptor is shown here as a monomer, but when bound to ligands, they either dimerize (FGFR1, VEGFR2) or trimerize (Fn14). Ligand–receptor engagement would activate intracellular signalling cascades and induce the expression of numerous genes, including *Fn14* (it is indicated here that all three angiogenic factors can induce *Fn14* expression in endothelial cells, but to date, TWEAK-regulated *Fn14* expression has only been reported in glioma cells). TWEAK levels in the tumour microenvironment are probably relatively high, so an increase in *Fn14* gene expression is likely to trigger additional (and perhaps persistent) cycles of TWEAK-dependent Fn14 signalling in the capillary endothelial cells. In this way, TWEAK, acting in concert with VEGFA and FGF2, would play a significant role in pathological sprouting angiogenesis.

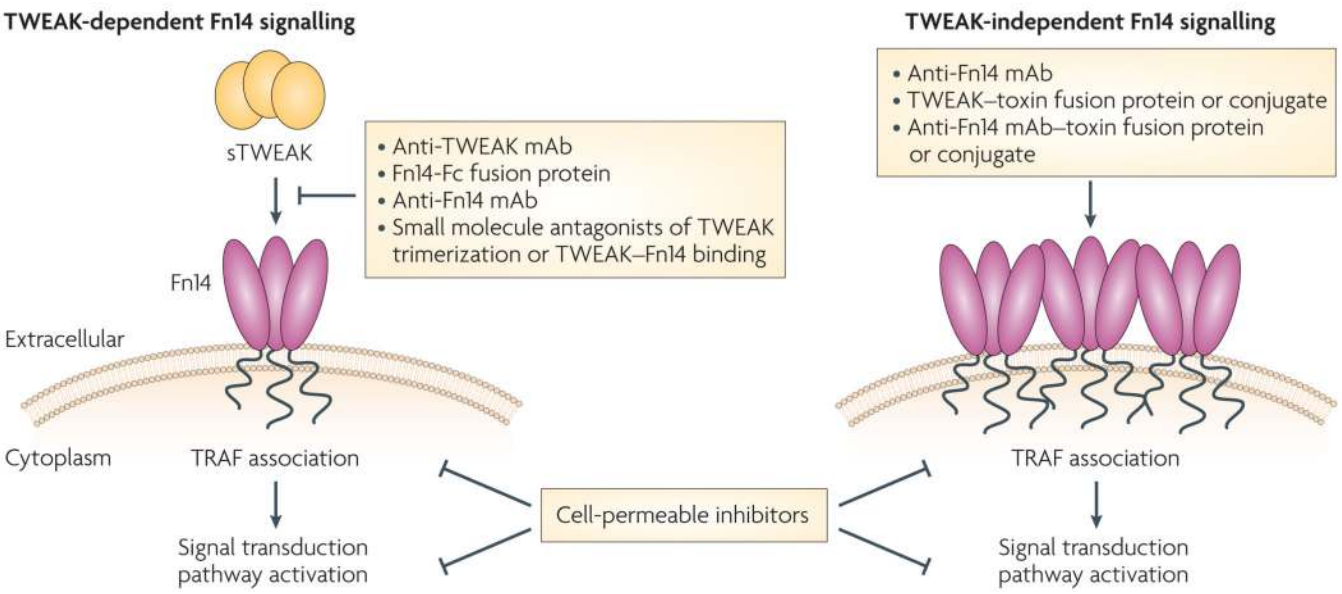

Figure 7. Potential therapeutic strategies for inhibiting Fn14-triggered pathological conditions

TWEAK-dependent Fn14 signalling is likely to be the predominant mechanism inducing pathological effects in humans. This pathway could be blocked using various protein- or small molecule-based TWEAK- or Fn14-targeted drugs that prevent TWEAK binding to Fn14. Indeed, the first two therapeutic strategies listed under TWEAK-dependent Fn14 signalling have shown efficacy in several rodent models of human disease. Alternatively, one could use membrane-permeable drugs that prevent Fn14–TRAF (TNFR-associated factor) association or inhibit downstream signalling events, but in this case it may prove more difficult to achieve TWEAK–Fn14 axis specificity. It is also possible that in tissues where the Fn14 receptor is overexpressed and TWEAK levels are low, TWEAK-independent Fn14 signalling could contribute to certain disease pathologies. This pathway could be inhibited using an anti-Fn14 monoclonal antibody (mAb) that blocks receptor multimerization or disassociates preformed multimers. Alternatively, Fn14-targeted toxins delivered as fusion proteins or chemical conjugates could be used to kill Fn14-overexpressing cells. One could also target Fn14–TRAF association or downstream signalling events as mentioned in the main text.

Table 1

TWEAK is a multifunctional cytokine

Cellular response (stimulate (+) or inhibit (−))	Cell type(s)	References
Proliferation (+)	Human endothelial cells	8,38,42–44
	Human smooth muscle cells	8
	Human liver tumour cell lines	26
	Murine EpH4 mammary epithelial cells	77
	Murine C2C12 myoblasts	31,45
	Murine primary myoblasts	31
	Murine primary astrocytes	29
	Murine synovial cells	83
	Rat liver NRC-1 cells	49
Proliferation (−)	Murine postnatal neural progenitor cells	129
Migration (+)	Human endothelial cells	43,44
	Human glioma cell lines	27
	Rat aortic smooth muscle cells	56
Survival (+)	Human endothelial cells	103
	Human glioma cell lines	71
Differentiation (+)	Murine RAW264.7 monocytes → osteoclasts	35
Differentiation (−)	Human immature erythroblasts → mature erythroblasts	130
	Human mesenchymal stem cells → chondrocytes	47
	Human osteoblast precursors → osteoblasts	47
	Murine MC3T3-E1 cells → osteoblasts	73
	Murine C2C12 myoblasts → myotubes*	31,45,46
Death (+)	Human HT-29 tumour cell line‡	4,20,32,36,39,41,42,80
	Human KATO-III tumour cell line‡	32,39
	Human MCF7 tumour cell line§	5
	Human HeLa tumour cell line§	5
	Human Kym-1 tumour cell line‖	6,39
	Human HSC3 tumour cell line	32,39
	Human SW480 tumour cell line	20
	Human peripheral blood mononuclear cells	131
	Murine mesangial cells‡	72
	Murine renal MCT cells¶	34

Cellular response (stimulate (+) or inhibit (−))	Cell type(s)	References
	Murine primary cortical neurons	33

*TWEAK treatment of differentiated myotubes promotes atrophy[54].

‡Requires interferon-γ (IFN-γ) co-treatment.

§Requires cycloheximide pre-treatment.

//Indirect effect mediated by TNF-α.

¶Requires TNF-α and IFN-γ co-treatment.

Table 2

Phenotypes of TWEAK–Fn14 axis genetically engineered mice

Model	Phenotype	Other information
TWEAK transgenic 1	• Enhanced experimental autoimmune encephalitis disease severity[29]	• Promoter, α1-antitrypsin; cDNA, mouse soluble TWEAK; tissue expression, liver (TWEAK levels in circulation of 300–600 ng ml^{-1})
TWEAK transgenic 2	• Progenitor (oval) cell hyperplasia[49]	• Promoter, α1-antitrypsin; cDNA, mouse full-length TWEAK; tissue expression, liver (TWEAK levels in circulation of 8–25 ng ml^{-1})
TWEAK transgenic 3	• Decreased muscle and body mass[54]	• Promoter, muscle creatine kinase; cDNA, mouse full-length TWEAK; tissue expression, skeletal muscle (TWEAK levels in circulation elevated approximately threefold, but concentration range not provided)
TWEAK knockout	• Enlarged spleen, excess natural killer cells, hypersensitive to lipopolysaccharide-induced death, stronger innate and adaptive responses to tumour challenge[30]	N/A
Fn14 knockout	• Reduced oval cell proliferation after 3,5-diethoxycarbonyl-1,4-dihydrocollidine-mediated liver injury[49]. • Reduced inflammatory and regenerative response after cardiotoxin-triggered skeletal muscle injury[31]. • Reduced brain tissue damage and oedema in esponse to focal ischaemic insult[52]. • Reduced kidney damage in chronic graft-versus-host model of systemic lupus erythematosus[50]	N/A

Table 3

Fn14 gene induction *in vitro*

Agent	Cell type	References
Angiotensin II	Rat aortic smooth muscle cells	9
Bone morphogenetic protein 6	Murine intra-embryonic endothelial cells	132
Epidermal growth factor	Murine NIH 3T3 fibroblasts	10
	Rat aortic smooth muscle cells	9
Fetal bovine serum	Human M426 fibroblasts	11
	Human aortic smooth muscle cells*	125
	Murine NIH 3T3 fibroblasts	10
	Rat aortic smooth muscle cells	9
Fibroblast growth factor 1	Human M426 fibroblasts	11
	Murine NIH 3T3 fibroblasts	10
Fibroblast growth factor 2	Human umbilical vein endothelial cells	43
	Murine NIH 3T3 fibroblasts	10
	Rat aortic smooth muscle cells	9
Interferon-γ	Human aortic smooth muscle cells*	125
	Human immature erythroblasts	130
	Human $CD14^+$ monocytes	30
	Human natural killer cells	30
	Human dendritic cells	30
	Murine renal MCT cells	34
Interleukin-1β	Human aortic smooth muscle cells*	125
	Human gingival fibroblasts	82
Bacterial lipopolysaccharide	Human THP-1 monocytic cells	25
Nerve growth factor	Rat PC12 cells	57
Platelet-derived growth factor-BB	Murine NIH 3T3 fibroblasts	10
	Rat aortic smooth muscle cells	9
Phorbol 12-myrisate 13-acetate	Human M426 fibroblasts	11
	Human $CD14^+$ monocytes	30
	Human natural killer cells	30
	Human dendritic cells	30
	Murine NIH 3T3 fibroblasts	10
	Rat aortic smooth muscle cells	9

Agent	Cell type	References
Transforming growth factor-β1	Human gingival fibroblasts	82
	Murine NIH 3T3 fibroblasts	10
Thrombin	Rat aortic smooth muscle cells	9
Tumour necrosis factor-α	Murine renal MCT cells	34
TNF-like weak inducer of apoptosis	Human glioma cells	58
Vascular endothelial growth factor A	Human umbilical vein endothelial cells	43

*This effect is inhibited by co-treatment with atorvastatin, a 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase inhibitor.