[Frontiers in Bioscience 12, 2761-2771, January 1, 2007]

Role of TWEAK and Fn14 in tumor biology

Jeffrey A. Winkles[1], Nhan L. Tran[2], Sharron A. N. Brown[1], Nichole Stains[1], Heather E. Cunliffe[2], Michael E. Berens[2]

[1]Departments of Surgery and Physiology, Center for Vascular and Inflammatory Diseases, and the Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland, USA, [2]Cancer and Cell Biology Division, Translational Genomics Research Institute, Phoenix, Arizona, USA

TABLE OF CONTENTS

1. ABSTRACT

The tumor necrosis factor (TNF) superfamily member TNF-like weak inducer of apoptosis (TWEAK) was initially described in a 1997 publication co-authored by investigators from the biotechnology company Biogen (now Biogen-Idec) and the University of Geneva. Four years later, researchers at the biotechnology company Immunex (now part of Amgen) reported the cloning and characterization of the human TWEAK receptor. A sequence database search revealed that the predicted TWEAK receptor amino acid sequence was identical to that of fibroblast growth factor-inducible 14 (Fn14), a small transmembrane protein described one year earlier in a publication from investigators at the American Red Cross Holland Laboratory. Recent studies have revealed that the TWEAK-Fn14 ligand-receptor pair likely plays an important role in a variety of cellular processes and in the pathogenesis of several human diseases, including atherosclerosis, stroke, rheumatoid arthritis and cancer. In this paper, we first summarize the general properties of these two proteins and then review the available data implicating TWEAK and Fn14 in multiple aspects of tumor biology.

2. INTRODUCTION

2.1. TWEAK and Fn14 structure

TWEAK (TNFSF12) was identified in 1997 as a TNF superfamily member with pro-apoptotic activity on interferon (IFN)-γ-treated HT-29 colon carcinoma cells (1). TWEAK is initially synthesized as a 249-amino acid (aa) type II transmembrane glycoprotein containing a C-terminal extracellular domain (206-aa), a transmembrane domain (25-aa), and an N-terminal intracellular domain (18-aa). In most cells, full-length TWEAK is proteolytically processed in the stalk region, and this 156-aa proteolytic product, which is biologically active, can function as a soluble cytokine (reviewed in 2, 3). It has recently been reported that full-length TWEAK, but not soluble TWEAK, may enter the cell nucleus, but the functional significance of this observation is currently unknown (4).

TWEAK activity is mediated via binding to a type I transmembrane protein named Fn14 (TNFRSF12A) (5-7). The Fn14 cell surface receptor is only 102-aa in length following signal peptide cleavage, making it the smallest TNF receptor (TNFR) superfamily member

identified to date (reviewed in 2). Indeed, the Fn14 extracellular domain containing the TWEAK-binding site is only 53-aa in length (8), and the Fn14 cytoplasmic tail, essential for signal transduction (9-12), is only 28-aa in length. TWEAK is the only TNF superfamily member that binds Fn14, and TWEAK does not bind to any other TNFR superfamily members (13).

2.2. The TWEAK-Fn14 signaling pathway

TWEAK binding to the Fn14 receptor promotes several cellular responses, depending on the cell type analyzed (see below), but the TWEAK signal transduction pathway is not well defined. Fn14, like other TNFR superfamily members, has no intrinsic protein kinase activity but instead associates with adaptor molecules that link cell surface receptor activation to intracellular signaling pathways. The TNFR-associated factors (TRAFs) are one group of adaptor molecules involved in TNFR superfamily signaling (reviewed in 14) and it has been shown that four TRAFs (TRAF1, 2, 3 and 5) bind to the Fn14 cytoplasmic tail (7, 9, 15). The Fn14 TRAF-binding site has been identified (9, 15) and mutated receptors missing this site are unable to signal (9-12). TWEAK binding to the Fn14 receptor is predicted to promote TRAF association, but this has not yet been confirmed experimentally.

TWEAK treatment of various Fn14-positive cell types has been shown to activate the nuclear factor (NF)-κB signaling pathway (9, 10, 12, 15-22). TWEAK-induced NF-κB activation is likely mediated, at least in part, by TRAF2 and TRAF5 (12, 15). TWEAK may also activate other signaling cascades; for example, Donohue et al. (16) detected TWEAK-stimulated extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) phosphorylation in human endothelial cells (ECs). It should be mentioned here that these signaling studies, as well as all of the studies described in Section 2.3 below, were conducted using recombinant soluble TWEAK protein. It is presently unknown whether full-length, membrane-anchored TWEAK is biologically active.

2.3. TWEAK biological activity *in vitro* and *in vivo*

TWEAK has been reported to stimulate cell proliferation (8, 16, 23-27), survival (10, 28), migration (16, 24, 29) and apoptosis (1, 22, 30-33). TWEAK can also promote (34) or inhibit (21, 35, 36) cellular differentiation. Finally, TWEAK treatment of numerous cell types has been shown to induce the expression of various pro-inflammatory molecules (1, 17-20, 24, 37-39). This is consistent with the ability of TWEAK to activate the NF-κB transcription factor (see above), an important mediator of the innate immune response (see Section 3.4).

TWEAK also has activity when administered *in vivo*. TWEAK can stimulate blood vessel formation (angiogenesis) (23, 40), and this property will be discussed in Section 3.5. TWEAK also appears to play a role in the regulation of neurovascular unit (NVU) permeability. The brain NVU consists of EC, pericytes, astrocytes and neurons. Polavarapu et al. (41) found that intracerebral TWEAK injection in mice induces NF-κB pathway activation, matrix metalloprotease (MMP)-9 expression, a disruption in NVU structure and an increase in blood-brain barrier permeability. More recently, it was reported that intravenous TWEAK administration up-regulates monocyte chemotactic protein (MCP)-1 and IFN-γ-induced protein (IP)-10 gene expression in mouse kidney, demonstrating for the first time that TWEAK has pro-inflammatory activity *in vivo* (20).

2.4. Phenotypic analysis of TWEAK-null and Fn14-null mice

Both TWEAK-null (knockout) and Fn14-null mice are viable, healthy and fertile. However, TWEAK-null mice have more natural killer (NK) cells in secondary hematopoietic tissues and are hypersensitive to bacterial endotoxin-induced death (due to an enhanced innate inflammatory response) (42). These mice also develop oversized spleens with expanded memory and T helper 1 (TH1) subtype cells upon aging. Finally, TWEAK-null mice have stronger innate and adaptive TH1-based responses against tumor challenge (discussed below in Section 3.3). Taken together, these results suggest that TWEAK normally functions *in vivo* to attenuate the innate immune response and the transition to adaptive TH1 immunity. It has not yet been reported whether Fn14-null mice also have immune system abnormalities. However, two groups have reported that Fn14-null mice have an abnormal response to tissue injury. Specifically, these mice show reduced progenitor cell expansion after liver injury (27) and they have less brain tissue damage and edema in response to a focal ischemic insult (43).

3. TWEAK AS A REGULATORY CYTOKINE IN CANCER

3.1. TWEAK gene expression in the tumor microenvironment

TWEAK gene expression has been detected in human tumor tissue specimens representing 13 different tumor types, including kidney (40), breast (40, 44), colon (40, 45), liver (39) and brain (29) tumors. In some tumor types, TWEAK mRNA levels are higher in tumor tissue compared to normal tissue, but this is not always the case (29, 40). Solid tumors contain many cell types, including cancer cells, blood and lymphatic vessel cells (EC, pericytes), stromal fibroblasts, and innate and adaptive immune system cells such as macrophages, dendritic cells, NK cells and T cells (reviewed in 46, 47). Most of these cell types are potential sources for the TWEAK found in tumor tissue specimens. Indeed, TWEAK mRNA and/or protein expression has been detected in cancer cells (11, 39, 40, 45), vascular EC (40), monocytes/macrophages (1, 19, 42, 48-51), NK cells (42) and dendritic cells (42). However, T and B cells do not express significant levels of TWEAK (or Fn14) (42).

3.2. TWEAK effects on tumor cell lines cultured *in vitro*

Studies to date have shown that many human tumor cell lines express the Fn14 receptor, and that TWEAK treatment of these cells can result in vastly different outcomes (reviewed in 11). For example, TWEAK stimulates Huh7 hepatocellular carcinoma cell

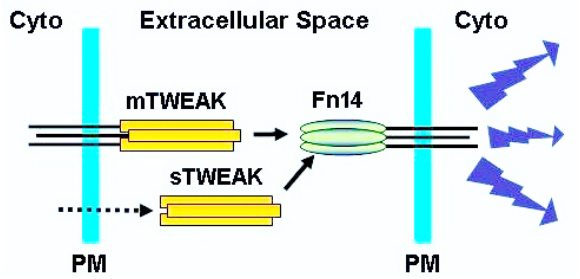

Figure 1. The TWEAK-Fn14 axis. Most cells express two TWEAK isoforms, a membrane-bound form (mTWEAK) and a soluble form (sTWEAK). TWEAK, like other TNF superfamily members, contains an extracellular TNF homology domain (yellow rectangle) and associates into non-covalently linked homotrimers. Trimeric TWEAK binds to Fn14 monomers containing a single cysteine-rich domain (green oval), which promotes receptor trimerization and signal transduction (purple arrows). In this diagram, TWEAK produced by one cell is acting on a neighboring cell via both a paracrine pathway (sTWEAK) and a juxtacrine pathway (mTWEAK), but to date TWEAK juxtacrine activity has not been confirmed. TWEAK can likely also function as an autocrine factor (i.e., the TWEAK produced by a cell binds the Fn14 receptors on that same cell), but this third mechanism of action is not illustrated here. Abbreviations: Cyto, cytoplasmic compartment; PM, plasma membrane.

proliferation (39) and U118 glioma cell migration (29), but it promotes HT-29 colon adenocarcinoma, HSC3 oral squamous cell carcinoma and Kym-1 rhabdomyosarcoma cell death (1, 30-33, 45). TWEAK-induced HT-29 cell death requires pretreatment with sensitizing agents or cytokines (1, 31, 33, 45), while TWEAK-induced Kym-1 cell death is mediated by TNF-α release (30, 33). In dramatic contrast to these reports indicating that TWEAK is a death-inducing factor for some tumor cell lines, when TWEAK is added to T98G or SF767 glioma cells, it does not cause cell death; instead, it actually suppresses the apoptotic activity of other cytokines and chemotherapeutic compounds (i.e., it is a survival factor) (10). This TWEAK effect is due, at least in part, to activation of the NF-κB signaling pathway and increased expression of the anti-apoptotic proteins BCL-X_L and BCL-W (10). This is an important finding, since it implies that the TWEAK produced in the tumor microenvironment may contribute to cancer therapy resistance.

It is presently unknown how TWEAK binding to Fn14 can promote tumor cell line-specific responses *in vitro* (proliferation, migration, survival, apoptosis). TWEAK activates the NF-κB signaling pathway in most if not all Fn14-positive cell types, and NF-κB activation can induce cell type- and stimulus-dependent cellular responses (reviewed in 52), so this is one possible mechanism. Alternatively, different Fn14-associated signaling complexes may form in the different tumor cell lines, and these complexes may couple to different signaling pathways that trigger particular cellular responses.

3.3. TWEAK as a potential regulator of tumor cell growth *in vivo*

TWEAK present in the tumor microenvironment may influence cancer cell growth via autocrine, paracrine and/or juxtacrine pathways (reviewed in 11) (Figure 1). Two groups have investigated whether TWEAK overexpression in tumor cell lines has an effect on subcutaneous tumor outgrowth in mice. Ho et al. (40) generated several stably-transfected human HEK293 cell lines that secreted similar levels of soluble human TWEAK and implanted them into immunodeficient Nu/Nu mice. These lines showed a significantly increased tumor growth rate compared to vector-transfected cells. More recently, Kaduka et al. (53) generated stably-transfected murine P815 and L5178Y cell lines that overexpressed full-length (membrane-bound) murine TWEAK and implanted them into syngeneic, immunocompetent DBA/2 mice. There was no difference in tumor growth rate between the TWEAK-overexpressing cells and the control cells. As mentioned earlier in Section 2.2, it is not known whether full-length TWEAK is biologically active. Although this group proposed in an earlier publication that the L5178Y transfectants released soluble TWEAK into cell culture medium (50), this conclusion was based on an indirect assay. Therefore, since it is unclear whether the TWEAK-overexpressing cells used in the Kakuda et al. (53) study release soluble TWEAK, their results cannot be directly compared to those of Ho et al. (40). In any case, more research is required to confirm and extend these initial reports.

As mentioned above, tumors contain cells involved in both the innate and adaptive immune response, including macrophages, NK cells and T cells. These cells produce an array of molecules that can exert either pro- or anti-tumorigenic effects (reviewed in 47, 54, 55). Two recent reports indicate that immune cell-derived TWEAK can have either a positive (42) or negative (53) effect on tumor 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 tumor growth was monitored (42). TWEAK deficiency completely inhibited B16.F10 tumor establishment and growth, and significantly attenuated B16.BL6 tumor growth. Additional research led the authors to conclude that TWEAK produced by cells of the innate immune system may normally act to suppress both innate and adaptive anti-tumor immunity. In the second study, Fn14-positive murine tumor cell lines were implanted subcutaneously into immunodeficient SCID mice or syngeneic, immunocompetent DBA/2 mice and then either an anti-mouse TWEAK monoclonal antibody or control IgG was administered intraperitoneally (53). TWEAK neutralization promoted tumor growth, and this effect could be suppressed if macrophage infiltration into the tumor site was inhibited using anti-CD11b antibodies. These authors concluded that TWEAK contributes to the anti-tumor effect of tumor-infiltrating macrophages.

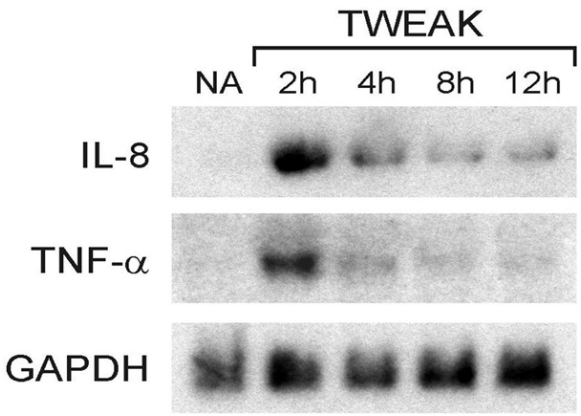

Figure 2. TWEAK regulation of IL-8 and TNF-α gene expression. Human EC were cultured in growth factor-deprived, reduced serum medium and then either left untreated (NA, no addition) or treated with recombinant TWEAK (100 ng/ml) for the indicated lengths of time. RNA was isolated and Northern blot hybridization analysis was conducted using the cDNA probes indicated on the left side.

3.4. TWEAK and tumor inflammation

Inflammation is a complex, dynamic process that occurs in tissues following traumatic, infectious, toxic or autoimmune injury (reviewed in 56). Vascular ECs are intricately involved in the inflammatory reaction; specifically, when they are physically damaged or exposed to bacterial endotoxin, oxidized lipoproteins or pro-inflammatory agents they become activated and express numerous cytokines (e.g., TNF-α), chemokines (e.g., IL-8, MCP-1) and adhesion molecules (e.g., intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1, E-selectin) that together orchestrate leukocyte transendothelial migration, a key feature of the inflammatory response (reviewed in 57-59). Inflammation is normally very tightly controlled but when this process is excessive or prolonged it contributes to the pathogenesis of numerous human diseases, including cancer (reviewed in 47, 60). Indeed, chronic, localized inflammatory conditions likely play an important role in tumor initiation and promotion (reviewed in 46, 54, 60). In addition, innate immune cells recruited to the tumor microenvironment, in particular tumor-associated macrophages (TAMs), contribute to all aspects of tumor progression, including growth, invasion, angiogenesis and metastasis (reviewed in 47, 55, 61, 62).

TWEAK treatment of many cell types activates the NF-κB signaling pathway (Section 2.2), a critical pathway linking inflammation and cancer (63, 64), and induces pro-inflammatory molecule expression (Section 2.3); therefore, it is reasonable to conclude that this cytokine may play a role in tumor inflammation. The possibility that TWEAK produced by tumor cells may act on neighboring EC and induce leukocyte infiltration is supported by studies demonstrating that TWEAK-stimulated EC express elevated levels of TNF-α (a key soluble mediator of inflammatory reactions), IL-8, MCP-1 (the major chemokine driving TAM recruitment), ICAM-1 and E-selectin (two important leukocyte adhesion molecules) (23, 24) (Figure 2). Furthermore, once innate immune system cells are established in the tumor, they are potential sources for TWEAK (Section 3.1), which could act on both vascular EC (and thus contribute to "inflammatory angiogenesis" (61)) and cancer cells. Finally, TWEAK present in the tumor microenvironment may act on the tumor-associated innate immune cells themselves, and this could stimulate additional growth factor, cytokine, chemokine and angiogenic factor production in the developing tumor.

3.5. TWEAK and tumor angiogenesis

Angiogenesis is the formation of new capillaries from the pre-existing vasculature. This is a highly regulated process that occurs during wound healing, in response to tissue ischemia, and during the menstrual cycle (reviewed in 65). Angiogenesis is driven by extracellular polypeptide factors that act on quiescent EC and induce protease production, proliferation, migration, and ultimately, vessel assembly and stabilization (reviewed in 65-68). It is now well established that angiogenesis is crucial for primary tumor growth and metastasis (reviewed in 66-68). Since TWEAK is produced by many of the cell types residing in the tumor microenvironment (Section 3.1) and it has pro-angiogenic activity *in vivo* (Section 2.3), it may function as a paracrine regulator of tumor angiogenesis. This possibility is supported by a study demonstrating that TWEAK-overexpressing HEK293 cells form large, highly-vascularized tumors when injected subcutaneously in mice (40). Additional studies; for example, comparing murine tumor cell growth and angiogenesis in syngeneic Fn14-null and wild-type mice, should clarify if TWEAK is an important tumor angiogenesis factor.

4. FN14 AS A TUMOR-SPECIFIC CELL SURFACE MARKER

4.1. Esophageal cancer

Cancer of the esophagus is one of the fastest growing cancers in the Western world, and this is a highly aggressive tumor, with a 5-year overall survival rate of 14% (reviewed in 69, 70). It is estimated that 13,770 Americans will die from esophageal cancer in 2006 (71). A recent transcriptional profiling study using RNA isolated from either normal esophageal mucosa (NE), Barrett's esophagus (BE), or esophageal adenocarcinoma (EAC) specimens and a cDNA microarray representing 8064 unique genes identified Fn14 as one of the 12 best candidate progression biomarkers in esophageal neoplasia (72). Indeed, microarray data analyses indicated that Fn14 mRNA expression was lower in (i) NE versus EAC, (ii) BE without concomitant EAC versus BE with concomitant EAC, and (iii) BE versus EAC tissue specimens. Quantitative real-time RT-PCR experiments confirmed that the Fn14 gene was overexpressed in cancerous esophageal specimens relative to non-cancerous esophageal specimens.

4.2. Liver cancer

Hepatocellular carcinoma **(**HCC), the most common form of liver cancer, is the fifth most common cancer worldwide (reviewed in 73). It is estimated that

liver/bile duct cancer will account for 16,200 deaths in the USA in 2006 (71). The first report indicating that the Fn14 gene was overexpressed in human HCC was published in 2000 (6). In this study, Fn14 mRNA levels were compared in HCC tissue versus adjacent non-cancerous liver tissue using Northern blot hybridization analysis. An elevated level of Fn14 gene expression was detected in three of the four tumor specimens examined. More recently, Jakubowski et al. (27) compared Fn14 expression in one normal liver sample versus one HCC sample by immunohistochemistry, and found increased anti-Fn14 antibody staining in the tumor sample.

4.3. Breast cancer

Breast cancer accounts for 25% of all female cancers, making it by far the most common malignant disease in Western women (reviewed in 74). It is estimated that 40,970 women will die from breast cancer in the USA in 2006 (71). Invasive ductal carcinoma is the most common type of invasive breast carcinoma, with a 10-year survival rate of 35-50%. Primary breast cancer cells frequently travel to distant organs, and the most common sites for metastatic spread are bone, liver and lung. It has been reported that Fn14 gene expression is elevated in human breast tumors (26). In this study, Fn14 mRNA expression was detected in 0/10 normal tissue specimens and 35/60 breast tumor specimens using an *in situ* hybridization approach. In a different set of samples, Fn14 protein expression was detected in 1/10 normal tissue specimens and 10/19 breast tumor specimens by immunohistochemistry. This group did not indicate whether elevated Fn14 levels were correlated with specific breast tumor characteristics (i.e, histologic grade, ERBB2 status, steroid receptor status), so it will be important to perform additional studies examining Fn14 expression levels in this tumor type.

4.4. Brain cancer

It is estimated that 18,820 Americans will be diagnosed with brain cancer in 2006, and this cancer will account for 12,820 deaths (71). Gliomas, primary brain tumors that derive from glial support cells, are the most common primary tumor of the adult central nervous system (reviewed in 75, 76). Adult gliomas of astrocytic origin (astrocytomas) comprise a spectrum of neoplasms that are generally classified into low-grade benign tumors (i.e., juvenile pilocytic astrocytoma, diffuse astrocytoma) and high-grade malignant tumors (i.e., anaplastic astrocytoma, glioblastoma multiforme (GBM)). Malignant glioma cells are highly invasive and their efficient infiltration into adjacent normal brain tissue prevents complete surgical removal or effective destruction by lethal radiation exposure (reviewed in 77, 78). Indeed, patients diagnosed with grade IV GBM, the most aggressive malignant glioma, have a median survival of 9-12 months after the onset of clinical symptoms (reviewed in 75, 76).

The first data indicating that Fn14 expression might be up-regulated in brain tumor cells was reported by Mariani et al. (79) in 2001. In this paper, the authors reported the results of cDNA microarray experiments in which they searched for genes regulated during glioma cell migration *in vitro*. They found that Fn14 was one of the ten most highly differentially expressed genes (3- to 4-fold induction after plating cells on a motility-enhancing substrate). Subsequent Northern blot and Western blot experiments confirmed this initial microarray data (29). Since an earlier study by Feng et al. (6) indicated that Fn14 mRNA expression was quite low in normal human brain tissue, this group next compared Fn14 gene expression levels in normal brain, pilocytic astrocytoma, low-grade astrocytoma, oligodendroglioma, anaplastic astrocytoma and GBM tissue specimens by quantitative real-time RT-PCR (29). Fn14 mRNA levels were very low in normal brain, as expected, and significantly higher in 13/16 GBM specimens analyzed. Interestingly, Fn14 mRNA expression is higher in GBM invasive rim cells relative to tumor core cells (80).

Recently, Fn14 mRNA expression levels were examined in a large number of clinically-annotated brain tumor samples by mining an Affymetrix gene expression database (80). In agreement with the earlier RT-PCR study mentioned above (29), Fn14 expression values were relatively low in normal brain specimens (n=24), but significantly higher in GBM samples (n=82). Principal component analysis was used to investigate the relationship of Fn14 expression across tumor samples and patient outcome. Kaplan-Meier survival curves were developed for two clusters. The median survival time of cluster 1 was 952 days (long term) while cluster 2 had a median survival time of 401 days (short term). Analysis of the Affymetrix expression value for Fn14 in the GBM specimens for each cluster revealed that patients in the short term survival cluster had higher expression of Fn14 than patients in the long term survival cluster. This is the first direct data indicating that high Fn14 mRNA expression levels in tumors correlate with poor patient outcome.

The Fn14 protein is also overexpressed in advanced brain tumors. Tran et al. (80) examined Fn14 expression levels in a panel of normal and cancerous brain specimens by immunohistochemistry. They found that while all nine of the control, non-tumorous brain specimens showed either negative or weak anti-Fn14 antibody staining, 23/27 GBM specimens showed either moderate or strong antibody staining. In GMB tissue, Fn14 immunoreactivity was observed in both the stationary, tumor core cells and the invading, tumor rim cells.

4.5. Mechanistic basis for Fn14 gene activation in tumors?

It is presently unknown why the Fn14 gene is overexpressed in certain solid tumors relative to control non-tumorous tissue, but there are several possible mechanisms. First, Fn14 gene amplification may occur during the development and progression of these tumors, and this could result in elevated Fn14 mRNA and protein production. Second, since fibroblast growth factor (FGF)-2, platelet-derived growth factor (PDGF)-BB, or vascular endothelial growth factor (VEGF)-A treatment of cells in culture increases Fn14 gene expression (5-7, 16) and these same growth factors are overproduced by tumors *in vivo*

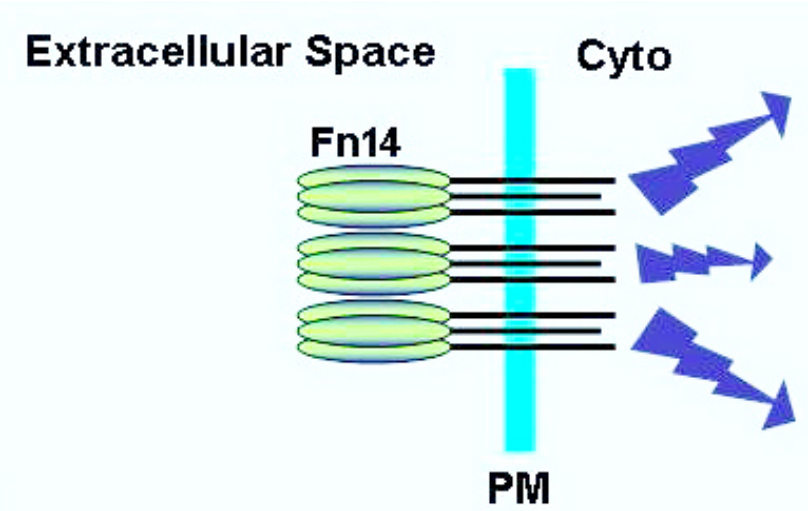

Figure 3. Ligand-independent Fn14 signaling. It has been reported that Fn14 overexpression in transfected cells can stimulate NF-κB activation and cellular responses. Therefore, it is possible that if tumor cells express the Fn14 receptor at a certain threshold level *in vivo*, the receptor could be activated even if TWEAK levels are low. This, in turn, could stimulate signaling cascades (purple arrows) and ultimately promote tumor cell proliferation, survival and/or invasive activity. Abbreviations: Cyto, cytoplasmic compartment; PM, plasma membrane.

(66-68), this could account for the elevated expression levels. Third, since the NF-κB transcription factor is constitutively activated in many tumors, including breast (81) and brain (82) tumors, and Fn14 is an NF-κB-inducible gene (80), this may be another potential mechanism for Fn14 overexpression in tumor tissue. Finally, Fn14 may be expressed at relatively high levels in human cancers as a result of intratumoral hypoxia (reviewed in 83). Hypoxia, a reduction in the normal level of tissue oxygen tension, increases hypoxia-inducible factor-1 (HIF-1) activity, and this transcription factor binds to hypoxia-responsive elements (HREs) within the promoters of genes encoding proteins involved in tumor growth, angiogenesis and metastasis (reviewed in 84). The human Fn14 promoter contains several putative HREs (unpublished data); in addition, it has been reported that Fn14 gene expression is upregulated in response to focal cerebral ischemia (22, 85). Therefore, HIF-1-mediated up-regulation of Fn14 gene expression may also contribute to Fn14 overexpression in tumors.

5. FN14 AS A TUMOR CELL REGULATORY MOLECULE

It is well established that TWEAK binding to Fn14 cell surface receptors activates intracellular signaling cascades and triggers cellular responses (see Sections 2.3 and 3.2). Several recent studies have indicated that a high level of Fn14 expression in cells is sufficient by itself to cause these biological effects. Indeed, when Fn14 is overexpressed in transiently-transfected NIH3T3 (9) or HEK293 (15) cells the NF-κB signaling pathway is activated. Furthermore, transient Fn14 overexpression in rat smooth muscle cells stimulates cell migration (15), while transient Fn14 overexpression in rat PC12 cells promotes growth cone lamelipodial formation and neurite outgrowth (86). It has also been reported that stably-transfected NIH3T3 cell lines expressing high levels of Fn14 have an altered morphology and decreased cell adhesion to extracellular matrix proteins (5).

Could elevated Fn14 expression in tumor cells *in vivo* contribute to the phenotypic properties of these cells? This possibility is supported by recent studies using human glioma cell lines (10, 11, 80). In these studies, cell lines that express relatively low levels of endogenous Fn14 were infected with adenoviruses encoding either the human Fn14 receptor or a control protein (LacZ) and several different *in vitro* assays were conducted. Forced Fn14 overexpression in glioma cells stimulated cell migration (11, 80), invasion (80) and survival (10). Additional studies revealed that the migration and survival effects do not occur if a truncated, signaling-deficient Fn14 mutant is overexpressed (10, 11, 80), and the invasion effect does not occur if Rac1 expression is down-regulated using siRNA duplexes (80). The likely explanation for ligand-independent Fn14 signaling is that when Fn14 receptors are overexpressed in cells they spontaneously oligomerize, and this receptor clustering promotes TRAF association and signaling cascade activation (Figure 3).

6. SUMMARY

In this review, we have summarized the presently available data indicating that TWEAK and Fn14 are (i) expressed in the tumor microenvironment and, (ii) likely to play a role in tumor biology. These studies indicate that TWEAK binding to Fn14 receptor-

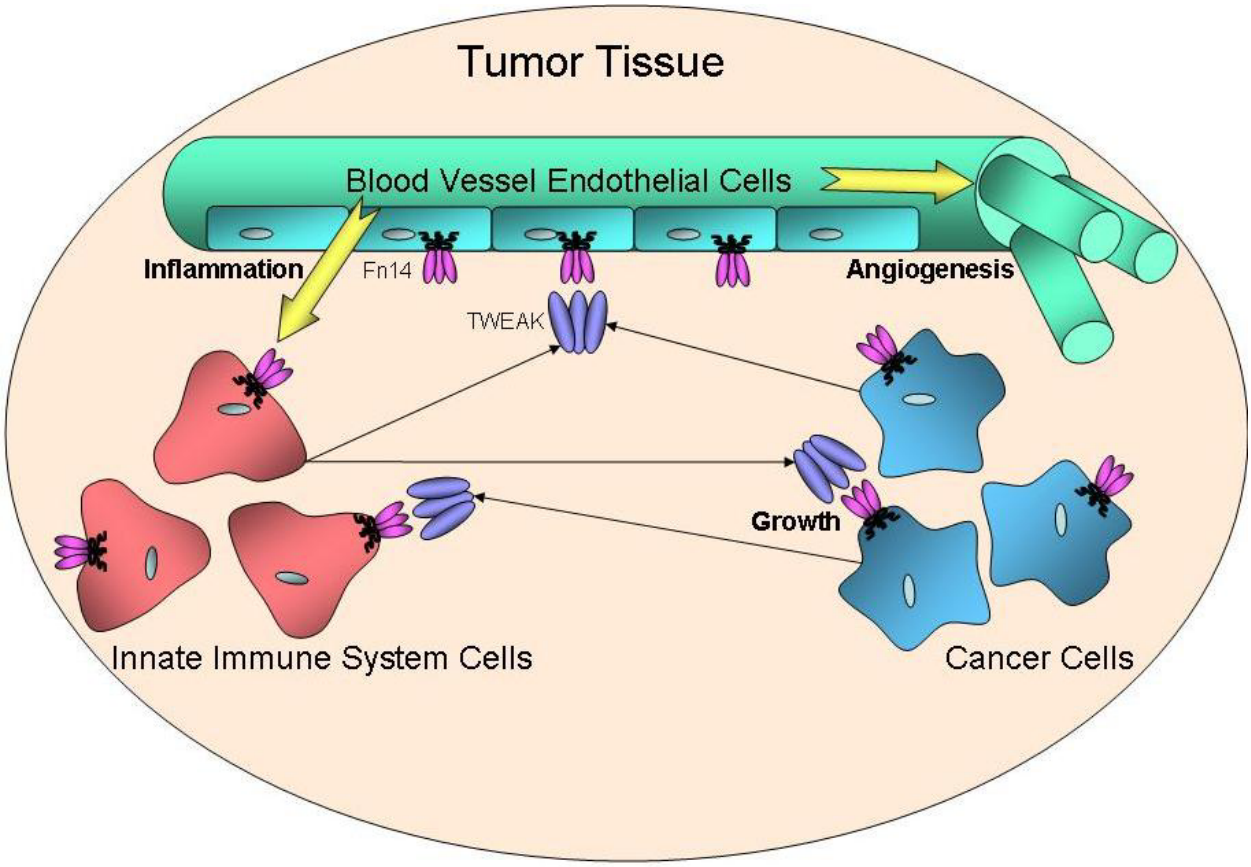

Figure 4. TWEAK may play a role in tumor growth, inflammation and angiogenesis. Solid tumors contain multiple cell types, including cancer cells, vascular EC, stromal fibroblasts and cells involved in innate (e.g., macrophages, granulocytes, NK cells) and adaptive (e.g., T cells) immunity. Only a subset of these cells are shown here. TWEAK and Fn14 are expressed by many of the cell types residing in tumors. In this diagram, TWEAK paracrine activity in tumor tissue, but not autocrine or juxtacrine activity, is illustrated. In the initial stages of tumor progression, cancer cell-derived TWEAK could act on EC and induce both tumor inflammation and angiogenesis. Subsequently, cancer cell-derived TWEAK could act on tumor-associated macrophages and other innate immune response cell types, inducing the expression of growth factors, proteolytic enzymes, cytokines, chemokines and angiogenic factors. Also, immune cell-derived TWEAK could act on EC, stimulating additional leukocyte recruitment and angiogenesis, and neighboring cancer cells, stimulating proliferation, invasion and metastasis.

positive cells may regulate several of the key events associated with tumor initiation and progression, including inflammation and angiogenesis (Figure 4). The availability of TWEAK-null and Fn14-null mice (Section 2.4), as well as anti-TWEAK monoclonal antibodies (27, 36, 87) and Fn14-Fc decoy receptors (43, 85) that neutralize TWEAK activity *in vivo*, should now allow investigators to directly test whether TWEAK-Fn14 interactions play a significant role in tumor growth, angiogenesis, invasion and/or metastasis. If these additional studies indicate that this is in fact the case, then TWEAK should be considered as a potential target for anti-cancer therapy in humans.

The observation that Fn14 gene expression is elevated in certain high-grade tumors could have clinical implications as well. First, Fn14 could be a diagnostic or prognostic marker for these cancers. Second, since Fn14 is on the cell surface, it could serve as a targeting molecule for antibody-based or ligand-based cytotoxin tumor therapy. Finally, the additional observation that Fn14 overexpression in glioma cells (Section 5) and breast cancer cells (unpublished data) increases their invasive capacity indicates that agents that inhibit Fn14 expression (e.g., siRNA duplexes) or block Fn14 signaling (e.g., NF-κB inhibitors) should be evaluated for their anti-invasive activity in animal models. In conclusion, although the TWEAK/Fn14 studies conducted to date have revealed the potential importance of these two proteins in tumor biology, much more pre-clinical research is required in order to determine whether TWEAK or Fn14 are new molecular targets for cancer drug development.

7. ACKNOWLEDGMENTS

Research in the author's laboratories is supported, in part, by NIH Grants R01 HL-39727 (JAW)

and R01 NS-42262 (MEB), Susan G. Komen Breast Cancer Foundation Grants BCTR0503968 (JAW) and BCTR0504515 (HEC), Ruth L. Kirschstein National Research Service Award F32 CA-112986 (NLT) and a Russell Becker-American Brain Tumor Association Grant (NLT).

8. REFERENCES

1. Chicheportiche, Y., P. R. Bourdon, H. Xu, Y. Hsu, H. Scott, C. Hession, I. Garci and J. L. Browning: TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. *J Biol Chem* 272, 32401-32410 (1997)

2. Wiley, S. R. and J. A. Winkles: TWEAK, a member of the TNF superfamily, is a multifunctional cytokine that binds the TweakR/Fn14 receptor. *Cytokine Growth Factor Rev* 14, 241-249 (2003)

3. Campbell, S., J. Michaelson, L. Burkly and C. Putterman: The role of TWEAK/Fn14 in the pathogenesis of inflammation and systemic autoimmunity. *Front Biosci* 9, 2273-2284 (2004)

4. Baxter, F. O., P. J. Came, K. Abell, B. Kedjouar, M. Huth, K. Rajewsky, M. Pasparakis and C. J. Watson: IKKβ/2 induces TWEAK and apoptosis in mammary epithelial cells. *Development* 133, 3385-3394 (2006)

5. Meighan-Mantha, R. L., D. K. W. Hsu, Y. Guo, S. A. N. Brown, S. Y. Feng, K. A. Peifley, G. F. Alberts, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, C. M. Richards and J. A. Winkles: The mitogen-inducible Fn14 gene encodes a type I transmembrane protein that modulates fibroblast adhesion and migration. *J Biol Chem* 274, 33166-33176 (1999)

6. Feng, S. Y., Y. Guo, V. M. Factor, S. S. Thorgeirsson, D. W. Bell, J. R. Testa, K. A. Peifley and J. A. Winkles: The Fn14 immediate-early response gene is induced during liver regeneration and highly expressed in both human and murine hepatocellular carcinomas. *Am J Pathol* 156, 1253-1261 (2000)

7. Wiley, S. R., L. Cassiano, T. Lofton, T. Davis-Smith, J. A. Winkles, V. Lindner, H. Liu, T. O. Daniel, C. A. Smith and W. C. Fanslow: A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis. *Immunity* 15, 837-846 (2001)

8. Brown, S. A. N., H. N. Hanscom, H. Vu, S. A. Brew and J. A. Winkles: TWEAK binding to the Fn14 cysteine-rich domain depends on charged residues located in both the A1 and D2 modules. *Biochem J* 397, 297-304 (2006)

9. Brown, S. A. N., C. M. Richards, H. N. Hanscom, S. L. Feng and J. A. Winkles: The Fn14 cytoplasmic tail binds tumour necrosis factor receptor-associated factors 1, 2, 3 and 5 and mediates nuclear factor-κB activation. *Biochem J* 371, 395-403 (2003)

10. Tran, N. L., W. S. McDonough, B. A. Savitch, T. F. Sawyer, J. A. Winkles and M. E. Berens: 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* 280, 3483-3492 (2005)

11. Winkles, J. A., N. L. Tran and M. E. Berens: TWEAK and Fn14: New molecular targets for cancer therapy? *Cancer Lett* 235, 11-17 (2005)

12. Saitoh, T., M. Nakayama, H. Nakano, H. Yagita, N. Yamamoto and S. Yamaoka: TWEAK induces NF-κB2 p100 processing and long lasting NF-κB activation. *J Biol Chem* 278, 36005-36012 (2003)

13. Bossen, C., K. Ingold, A. Tardivel, J. L. Bodmer, O. Gaide, S. Hertig, C. Ambrose, J. Tschopp and P. Schneider: Interactions of tumor necrosis factor (TNF) and TNF receptor family members in the mouse and human. *J Biol Chem* 281, 13964-13971 (2006)

14. Dempsey, P. W., S. E. Doyle, J. Q. He and G. Cheng: The signaling adaptors and pathways activated by TNF superfamily. *Cytokine Growth Factor Rev* 14, 193-209 (2003)

15. Han, S., K. Yoon, K. Lee, K. Kim, H. Jang, N. K. Lee, K. Hwang and L. S. Young: TNF-related weak inducer of apoptosis receptor, a TNF receptor superfamily member, activates NF-κB through TNF receptor-associated factors. *Biochem Biophys Res Commun* 305, 789-796 (2003)

16. Donohue, P. J., C. M. Richards, S. A. N. Brown, H. N. Hanscom, J. Buschman, S. Thangada, T. Hla, M. S. Williams and J. A. Winkles: TWEAK is an endothelial cell growth and chemotactic factor that also potentiates FGF-2 and VEGF-A mitogenic activity. *Arterioscler Thromb Vasc Biol* 23, 594-600 (2003)

17. Xu, H., A. Okamoto, J. Ichikawa, T. Ando, K. Tasaka, K. Masuyama, H. Ogawa, H. Yagita, K. Okumura and A. Nakao: TWEAK/Fn14 interaction stimulates human bronchial epithelial cells to produce IL-8 and GM-CSF. *Biochem Biophys Res Commun* 318, 422-427 (2004)

18. Jin, L., A. Nakao, M. Nakayama, N. Yamaguchi, Y. Kojima, N. Nakano, R. Tsuboi, K. Okumura, H. Yagita and H. Ogawa: Induction of RANTES by TWEAK/Fn14 interaction in human keratinocytes. *J Invest Dermatol* 122, 1175-1179 (2004)

19. Kim, S. H., Y. J. Kang, W. J. Kim, D. K. Woo, Y. Lee, D. I. Kim, Y. B. Park, B. S. Kwon, J. E. Park and W. H. Lee: TWEAK can induce pro-inflammatory cytokines and matrix metalloproteinase-9 in macrophages. *Circ J* 68, 396-399 (2004)

20. Campbell, S., L. C. Burkly, H. X. Gao, J. W. Berman, L. Su, B. Browning, T. Zheng, L. Schiffer, J. S. Michaelson and C. Putterman: Proinflammatory effects of TWEAK/Fn14 interactions in glomerular mesangial cells. *J Immunol* 176, 1889-1898 (2006)

21. Dogra, C., H. Changotra, S. Mohan and A. Kumar. 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* 281, 10327-10336 (2006)

22. Potrovita, I., W. Zhang, L. Burkly, K. Hahm, J. Lincecum, M. Z. Wang, M. H. Maurer, M. Rossner, A. Schneider and M. Schwaninger: Tumor necrosis factor-like weak inducer of apoptosis-induced neurodegeneration. *J Neurosci* 24, 8237-8244 (2004)

23. Lynch, C. N., Y. C. Wang, J. K. Lund, Y. Chen, J. A. Leal and S. R. Wiley: TWEAK induces angiogenesis and proliferation of endothelial cells. *J Biol Chem* 274, 8455-8459 (1999)

24. Harada, N., M. Nakayama, H. Nakano, Y. Fukuchi, H. Yagita and K. Okumura: Pro-inflammatory effect of TWEAK/Fn14 interaction on human umbilical vein endothelial cells. *Biochem Biophys Res Commun* 299, 488-493 (2002)

25. Desplat-Jego, S., S. Varriale, R. Creidy, R. Terra, D. Bernard, M. Khrestchatisky, S. Izui, Y. Chicheportiche and J. Boucraut: TWEAK is expressed by glial cells, induces astrocyte proliferation and increases EAE severity. *J Neuroimmunol* 133, 116-123 (2002)
26. Michaelson, J. S., S. Cho, B. Browning, T. S. Zheng, J. M. Lincecum, M. Z. Wang, Y. M. Hsu and L. C. Burkly: Tweak induces mammary epithelial branching morphogenesis. *Oncogene* 24, 2613-2624 (2005)
27. Jakubowski, A., C. Ambrose, M. Parr, J. M. Lincecum, M. Z. Wang, T. S. Zheng, B. Browning, J. S. Michaelson, M. Baetscher, B. Wang, D. M. Bissell and L. C. Burkly: TWEAK induces liver progenitor cell proliferation. *J Clin Invest* 115, 2330-2340 (2005)
28. Jakubowski, A., B. Browning, M. Lukashev, I. Sizing, J. S. Thompson, C. D. Benjamin, Y. Hsu, C. Ambrose, T. S. Zheng and L. C. Burkly: Dual role for TWEAK in angiogenic regulation. *J Cell Sci* 115, 267-274 (2002)
29. Tran, N. L., W. S. McDonough, P. J. Donohue, J. A. Winkles, T. J. Berens, K. R. Ross, D. B. Hoelzinger, C. Beaudry, S. W. Coons and M. E. Berens: The human Fn14 receptor gene is up-regulated in migrating glioma cells in vitro and overexpressed in advanced glial tumors. *Am J Pathol* 162, 1313-1321 (2003)
30. Schneider, P., R. Schwenzer, E. Haas, F. Muhlenbeck, G. Schubert, P. Scheurich, J. Tschopp and H. Wajant: TWEAK can induce cell death via endogenous TNF and TNF receptor 1. *Eur J Immunol* 29, 1785-1792 (1999)
31. Wilson, C. A. and J. L. Browning: 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* 9, 1321-1333 (2002)
32. Nakayama, M., K. Ishidoh, N. Kayagaki, Y. Kojima, N. Yamaguchi, H. Nakano, E. Kominami, K. Okumura and H. Yagita: Multiple pathways of TWEAK-induced cell death. *J Immunol* 168, 734-743 (2002)
33. Nakayama, M., K. Ishidoh, Y. Kojima, N. Harada, E. Kominami, K. Okumura and H. Yagita: Fibroblast growth factor-inducible 14 mediates multiple pathways of TWEAK-induced cell death. *J Immunol* 170, 341-348 (2003)
34. Polek, T. C., M. Talpaz, B. G. Darnay and T. Spivak-Kroizman: 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* 278, 32317-32323 (2003)
35. Felli, N., F. Pedini, A. Zeuner, E. Petrucci, U. Testa, C. Conticello, M. Biffoni, C. A. Di Cataldo, J. A. Winkles, C. Peschle and R. De Maria: Multiple members of the TNF superfamily contribute to IFN-γ-mediated inhibition of erythropoiesis. *J Immunol* 175, 1464-1472 (2005)
36. Perper, S. J., B. Browning, L. C. Burkly, S. Weng, C. Gao, K. Giza, L. Su, L. Tarilonte, T. Crowell, L. Rajman, L. Runkel, M. Scott, G. J. Atkins, D. M. Findlay, T. S. Zheng and H. Hess: TWEAK is a novel arthritogenic mediator. *J Immunol* 177, 2610-2620 (2006)
37. Saas, P., J. Boucraut, P. R. Walker, A. Quiquerez, M. Billot, S. Desplat-Jego, Y. Chicheportiche and P. Dietrich: TWEAK stimulation of astrocytes and the proinflammatory consequences. *Glia* 32, 102-107 (2000)
38. Chicheportiche, Y., R. Chicheportiche, I. Sizing, J. Thompson, C. B. Benjamin, C. Ambrose and J. Dayer: Proinflammatory activity of TWEAK on human dermal fibroblasts and synoviocytes: blocking and enhancing effects of anti-TWEAK monoclonal antibodies. *Arthritis Res* 4, 126-133 (2002)
39. Kawakita, T., K. Shiraki, Y. Yamanaka, Y. Yamaguchi, Y. Saitou, N. Enokimura, N. Yamamoto, H. Okano, K. Sugimoto, K. Murata and T. Nakano: Functional expression of TWEAK in human hepatocellular carcinoma: possible implication in cell proliferation and tumor angiogenesis. *Biochem Biophys Res Commun* 318, 726-733 (2004)
40. Ho, D. H., H. Vu, S. A. N. Brown, P. J. Donohue, H. N. Hanscom and J. A. Winkles: Soluble tumor necrosis factor-like weak inducer of apoptosis overexpression in HEK293 cells promotes tumor growth and angiogenesis in athymic nude mice. *Cancer Res* 64, 8968-8972 (2004)
41. Polavarapu, R., M. C. Gongora, J. A. Winkles and M. Yepes: Tumor necrosis factor-like weak inducer of apoptosis increases the permeability of the neurovascular unit through nuclear factor-κB pathway activation. *J Neurosci* 25, 10094-10100 (2005)
42. Maecker, H., E. Varfolomeev, F. Kischkel, D. Lawrence, H. LeBlanc, W. Lee, S. Hurst, D. Danilenko, J. Li, E. Filvaroff, B. Yang, D. Daniel and A. Ashkenazi: TWEAK attenuates the transition from innate to adaptive immunity. *Cell* 123, 931-944 (2005)
43. Zhang, X., J. A. Winkles, M. C. Gongora, R. Polavarapu, J. S. Michaelson, K. Hahm, L. Burkly, M. Friedman, X. J. Li and M. Yepes: TWEAK-Fn14 pathway inhibition protects the integrity of the neurovascular unit during cerebral ischemia. *J Cereb Blood Flow Metab* advance online publication 12 july (2006).
44. Zhao, H., A. Langerod, Y. Ji, K. W. Nowels, J. M. Nesland, R. Tibshirani, I. K. Bukholm, R. Karesen, D. Botstein, A. L. Borresen-Dale and S. S. Jeffrey: Different gene expression patterns in invasive lobular and ductal carcinomas of the breast. *Mol Biol Cell* 15, 2523-2536 (2004)
45. Kawakita, T., K. Shiraki, Y. Yamanaka, Y. Yamaguchi, Y. Saitou, N. Enokimura, N. Yamamoto, H. Okano, K. Sugimoto, K. Murata and T. Nakano: Functional expression of TWEAK in human colonic adenocarcinoma cells. *Int J Oncol* 26, 87-93 (2005)
46. Vakkila, J. and M. T. Lotze: Inflammation and necrosis promote tumour growth. *Nat Rev Immunol* 4, 641-648 (2004)
47. de Visser, K. E., A. Eichten and L. M. Coussens: Paradoxical roles of the immune system during cancer development. *Nat Rev Cancer* 6, 24-37 (2006)
48. Chicheportiche, Y., L. Fossati-Jimack, S. Moll, N. Ibnou-Zekri and S. Izui: Down-regulated expression of TWEAK mRNA in acute and chronic inflammatory pathologies. *Biochem Biophys Res Commun* 279, 162-165 (2000)
49. Nakayama, M., N. Kayagaki, N. Yamaguchi, K. Okumura and H. Yagita: Involvement of TWEAK in interferon gamma-stimulated monocyte cytotoxicity. *J Exp Med* 192, 1373-1380 (2000)
50. Nakayama, M., N. Harada, K. Okumura and H. Yagita: Characterization of murine TWEAK and its receptor

(Fn14) by monoclonal antibodies. *Biochem Biophys Res Commun* 306, 819-825 (2003)
51. Munoz-Garcia, B., J. L. Martin-Ventura, E. Martinez, S. Sanchez, G. Hernandez, L. Ortega, A. Ortiz, J. Egido and L. M. Blanco-Colio: Fn14 is upregulated in cytokine-stimulated vascular smooth muscle cells and is expressed in human carotid atherosclerotic plaques: modulation by atorvastatin. *Stroke* 37, 2044-2053 (2006)
52. Luo, J. L., H. Kamata and M. Karin: IKK/NF-κB signaling: balancing life and death--a new approach to cancer therapy. *J Clin Invest* 115, 2625-2632 (2005)
53. Kaduka, Y., K. Takeda, M. Nakayama, K. Kinoshita, H. Yagita and K. Okumura: TWEAK mediates anti-tumor effect of tumor-infiltrating macrophage. *Biochem Biophys Res Commun* 331, 384-390 (2005)
54. Coussens, L. M. and Z. Werb: Inflammation and cancer. *Nature* 420, 860-867 (2002)
55. Lu, H., W. Ouyang and C. Huang: Inflammation, a key event in cancer development. *Mol Cancer Res* 4, 221-233 (2006)
56. Nathan, C: Points of control in inflammation. *Nature* 420, 846-852 (2002)
57. Krishnaswamy, G., J. Kelley, L. Yerra, J. K. Smith and D. S. Chi: Human endothelium as a source of multifunctional cytokines: molecular regulation and possible role in human disease. *J Interferon Cytokine Res* 19, 91-104 (1999)
58. Muller, W. A: Leukocyte-endothelial cell interactions in the inflammatory response. *Lab Invest* 82, 521-533 (2002)
59. Ulbrich, H., E. E. Eriksson and L. Lindbom: Leukocyte and endothelial cell adhesion molecules as targets for therapeutic interventions in inflammatory disease. *Trends Pharmacol Sci* 24, 640-647 (2003)
60. Balkwill, F., K. A. Charles and A. Mantovani: Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* 7, 211-217 (2005)
61. Albini, A., F. Tosetti, R. Benelli and D. M. Noonan: Tumor inflammatory angiogenesis and its chemoprevention. *Cancer Res* 65, 10637-10641 (2005)
62. Lewis, C. E. and J. W. Pollard: Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 66, 605-612 (2006)
63. Bonizzi, G. and M. Karin: The two NF-κB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 25, 280-288 (2004)
64. Karin, M. and F. R. Greten: NF-κB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* 5, 749-759 (2005)
65. Carmeliet, P: Angiogenesis in health and disease. *Nat Med* 9, 653-660 (2003)
66. Bergers, G. and L. E. Benjamin: Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 3, 401-410 (2003)
67. Tonini, T., F. Rossi and P. P. Claudio: Molecular basis of angiogenesis and cancer. *Oncogene* 22, 6549-6556 (2003)
68. Cao, Y: Antiangiogenic cancer therapy. *Semin Cancer Biol* 14, 139-145 (2004)
69. Tew, W. P., D. P. Kelsen and D. H. Ilson: Targeted therapies for esophageal cancer. *Oncologist* 10, 590-601 (2005)
70. Layke, J. C. and P. P. Lopez: Esophageal cancer: a review and update. *Am Fam Physician* 73, 2187-2194 (2006)
71. Jemal, A., R. Siegel, E. Ward, T. Murray, J. Xu, C. Smigal and M. J. Thun: Cancer statistics, 2006. *CA Cancer J Clin* 56, 106-130 (2006)
72. Wang, S., M. Zhan, J. Yin, J. M. Abraham, Y. Mori, F. Sato, Y. Xu, A. Olaru, A. T. Berki, H. Li, K. Schulmann, T. Kan, J. P. Hamilton, B. Paun, M. M. Yu, Z. Jin, Y. Cheng, T. Ito, C. Mantzur, B. D. Greenwald and S. J. Meltzer: Transcriptional profiling suggests that Barrett's metaplasia is an early intermediate stage in esophageal adenocarcinogenesis. *Oncogene* 25, 3346-3356 (2006)
73. Avila, M. A., C. Berasain, B. Sangro and J. Prieto: New therapies for hepatocellular carcinoma. *Oncogene* 25, 3866-3884 (2006)
74. Weigelt, B., J. L. Peterse and L. J. van't Veer: Breast cancer metastasis: markers and models. *Nat Rev Cancer* 5, 591-602 (2005)
75. Rich, J. N. and D. D. Bigner: Development of novel targeted therapies in the treatment of malignant glioma. *Nat Rev Drug Discov* 3, 430-446 (2004)
76. Louis, D. N: Molecular pathology of malignant gliomas. *Ann Rev Pathol Mech Dis* 1, 97-117 (2006)
77. Giese, A., R. Bjerkvig, M. E. Berens and M. Westphal: Cost of migration: invasion of malignant gliomas and implications for treatment. *J Clin Oncol* 21, 1624-1636 (2003)
78. Salhia, B., N. L. Tran, M. Symons, J. A. Winkles, J. T. Rutka and M. E. Berens: Molecular pathways triggering glioma cell invasion. *Expert Rev Mol Diagn* 6, 613-626 (2006)
79. Mariani, L., C. Beaudry, W. S. McDonough, D. B. Hoelzinger, T. Demuth, K. R. Ross, T. Berens, S. W. Coons, G. Watts, J. M. Trent, J. S. Wei, A. Giese and M. E. Berens: Glioma cell motility is associated with reduced transcription of proapoptotic and proliferation genes: a cDNA microarray analysis. *J Neurooncol* 53, 161-176 (2001)
80. Tran, N. L., W. S. McDonough, B. A. Savitch, S. P. Fortin, J. A. Winkles, M. Symons, M. Nakada, H. E. Cunliffe, G. Hostetter, D. B. Hoelzinger, J. L. Rennert, J. S. Michaelson, L. C. Burkly, C. A. Lipinski, J. C. Loftus, L. Mariani and M. E. Berens: Increased fibroblast growth factor-inducible 14 expression levels promote glioma cell invasion via Rac1 and NF-κB and correlate with poor patient outcome. *Cancer Res* 66, 9535-9542 (2006)
81. Biswas, D. K., Q. Shi, S. Baily, I. Strickland, S. Ghosh, A. B. Pardee and J. D. Iglehart: NF-κB activation in human breast cancer specimens and its role in cell proliferation and apoptosis. *Proc Natl Acad Sci USA* 101, 10137-10142 (2004)
82. Nagai, S., K. Washiyama, M. Kurimoto, A. Takaku, S. Endo and T. Kumanishi: Aberrant nuclear factor-κB activity and its participation in the growth of human malignant astrocytoma. *J Neurosurg* 96, 909-917 (2002)
83. Harris, A. L: Hypoxia--a key regulatory factor in tumour growth. *Nat Rev Cancer* 2, 38-47 (2002)
84. Semenza, G. L: Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* 3, 721-732 (2003)
85. Yepes, M., S. A. N. Brown, E. G. Moore, E. P. Smith, D. A. Lawrence and J. A. Winkles: A soluble Fn14-Fc

decoy receptor reduces infarct volume in a murine model of cerebral ischemia. *Am J Pathol* 166, 511-520 (2005)
86. Tanabe, K., I. Bonilla, J. A. Winkles and S. M. Strittmatter: Fibroblast growth factor-inducible-14 is induced in axotomized neurons and promotes neurite outgrowth. *J Neurosci* 23, 9675-9686 (2003)
87. Desplat-Jego, S., R. Creidy, S. Varriale, N. Allaire, Y. Luo, D. Bernard, K. Hahm, L. Burkly and J. Boucraut: Anti-TWEAK monoclonal antibodies reduce immune cell infiltration in the central nervous system and severity of experimental autoimmune encephalomyelitis. *Clin Immunol* 117, 15-23 (2005)

Abbreviations: aa: amino acid; BE: Barrett's esophagus; EAC: esophageal adenocarcinoma; EC: endothelial cell; ERK, extracellular signal-regulated kinase; FGF: fibroblast growth factor; Fn14: fibroblast growth factor-inducible 14; GBM: glioblastoma multiforme; HCC: hepatocellular carcinoma; HIF: hypoxia-inducible factor; HRE: hypoxia-responsive element; ICAM: intercellular adhesion molecule; IFN: interferon; IP: IFN-γ-induced protein; JNK: c-Jun N-terminal kinase; MCP: monocyte chemotactic protein; MMP: matrix metalloprotease; NE: normal esophageal; NF: nuclear factor; NK: natural killer; NVU: neurovascular unit; PDGF: platelet-derived growth factor; TAM: tumor-associated macrophage; TH: T helper; TNF: tumor necrosis factor; TNFR: TNF receptor; TRAF: TNFR-associated factor; TWEAK: TNF-like weak inducer of apoptosis; VEGF: vascular endothelial growth factor.

Key Words: Tumor necrosis factor, TNF, TWEAK, Fn14, Tumor, Cancer, Neoplasia, Inflammation, Angiogenesis

Correspondence to: Jeffrey A. Winkles, Ph.D., Center for Vascular and Inflammatory Diseases, University of Maryland School of Medicine, 800 West Baltimore Street, Room 320, Baltimore, MD 21201. Tel: 410-706-8172, Fax: 410-706-8234, E-mail: jwinkles@som.umaryland.edu

http://www.bioscience.org/current/vol12.htm