

The facilitator in acute kidney injury: TWEAK

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THE PLOT: ACUTE KIDNEY INJURY

Most of the mass of the kidneys is composed of tubular cells. Loss of renal tubular cells characterizes both acute and chronic failure of renal function¹. Tubular cells may be lost by shedding, death or differentiation into fibroblasts. In human studies tubular cell death was the best histopathological correlate of renal dysfunction during acute kidney injury (AKI).^{2,3} In addition sublethally injured tubular cells secrete mediators of inflammation that further aggravate AKI. However, the factors that modulate tubular cell injury and the interplay between different factors is incompletely understood. In fact, AKI is a paradigmatic situation in which an insufficient understanding of the mechanisms of injury precludes the implementation of effective physiopathology-based approaches for prevention and treatment. Numerous factors contributing to injury have been identified and many of them have been shown to be effective targets in animal models of injury.⁴ However the few attempts to translate this information to the clinical situation have failed. We now review the evidence involving a novel cytokine-ligand pair, TWEAK and Fn14, in AKI.⁵⁻⁷ We feel that they may become important targets in the treatment of AKI because they are «team players», they behave as «facilitators» that amplify injurious responses initiated by other stimuli. Thus

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their targeting has the potential to dampen injury induced by a wide range of stimuli.

THE STARS: TWEAK AND Fn14

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK, Apo3L, TNFSF12) is a member of the tumor necrosis factor superfamily (TNFSF) of structurally-related cytokines⁸ (fig. 1). The human TWEAK gene is located at chromosome 17 and encodes a 249-amino acid (aa) type II transmembrane glycoprotein (30 kDa). The intracellular domain contains a putative serine phosphorylation site. The extracellular domain contains the receptor-binding

site. TWEAK may be expressed as a membrane-bound protein (mTWEAK) and as a 156-aa, 18kDa soluble protein, (sTWEAK) that results from proteolysis of mTWEAK.^{9,10} mTWEAK, but not sTWEAK, may enter the cell nucleus, although the relevance of this observation is unknown.¹¹

The TWEAK receptor was initially and erroneously reported to be the tumor necrosis factor receptor superfamily (TNFRSF) member death receptor 3 (DR3).¹² In 2001 the TWEAK receptor was cloned and shown to be the previously described human fibroblast growth factor-inducible 14 (Fn14, TNFRSF12A)¹³⁻¹⁵ (fig. 1). The human Fn14 gene is located at chromosome 16 and encodes a

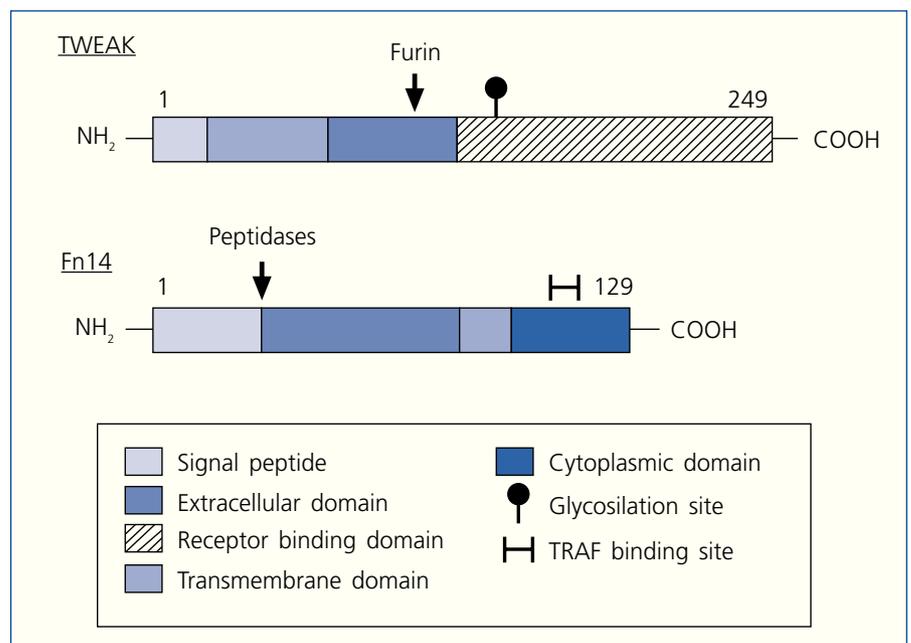


Figure 1. Structure of TWEAK and Fn14. Furin and poorly characterized peptidases may cleave TWEAK and Fn14, respectively, giving rise to soluble proteins.

129-aa type I transmembrane protein (14 kDa). Fn14 is processed into a 102-aa mature protein, making it the smallest TNFRSF member to date.^{9,16} The Fn14 extracellular domain (53-aa) contains the TWEAK-binding site.¹⁷ The intracellular domain (29-aa) contains a TNFR-associated factor (TRAF)-binding site with three potentially phosphorylated threonines. TRAF binding has been implicated in signal transduction.¹⁸ TWEAK trimerizes and binds to Fn14 monomers, promoting receptor trimerization and signal transduction.¹⁸⁻²¹

Although Fn14 was the only characterized TWEAK receptor, some TWEAK actions may be mediated independently of Fn14.²² Indeed CD163 was recently shown to either act as a TWEAK scavenger in pathological conditions or serve as an alternate receptor for TWEAK in cells lacking Fn14/TweakR.²³

Human and murine TWEAK are closely related, with a 93% homology in the receptor-binding domain. Furthermore, human and murine Fn14 have a 90% homology in their overall sequences, human TWEAK can bind to murine Fn14 and vice-versa.²⁴ In fact this pathway is remarkably conserved throughout evolution and putative TWEAK and Fn14 ESTs can be found in species as primitive as zebrafish.²⁵ The phylogenetical conservation suggests an important biological role.

TWEAK is widely expressed, and can be found at high levels in pancreas, intestine, heart, brain, lung, ovary and skeletal muscle, and at lower levels in liver and kidney.^{8,26} TWEAK mRNA levels were initially reported to be reduced in mice with both acute (induced by lipopolysaccharide) or chronic (autoimmune pathologies like lupus erythematosus or hemolytic anemia) inflammatory processes in numerous tissues and peritoneal macrophages.²⁷ In the same line of evidence, normal human arteries release sTWEAK but sTWEAK release from atherosclerotic arteries is reduced.²⁸ Low serum sTWEAK is a marker for subclinical atherosclerosis of potential clinical relevance.²⁸ However, elevated TWEAK expression was found in other inflammatory conditions such as experimental autoimmune encephalitis.²⁹

By contrast to TWEAK, resting tissue levels of Fn14 are usually low. As

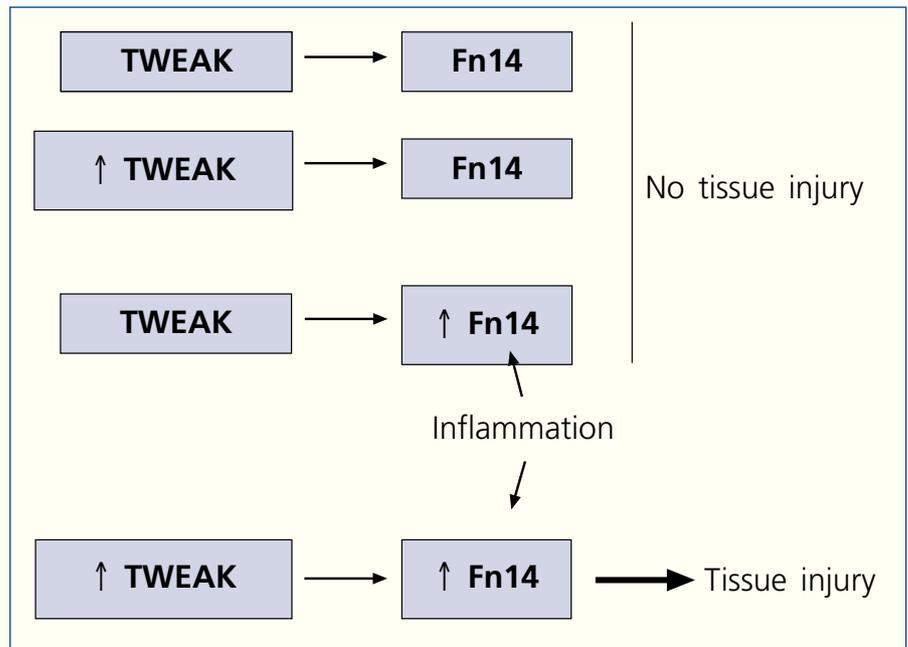


Figure 2. TWEAK actions may be regulated by the amount of receptor. Fn14 expression in normal tissues is usually low, but it is rapidly induced upon tissue injury.

an example, it is undetectable in normal arteries.²⁸ Fn14 initially was identified as an early response gene whose transcription was induced by FGF-1 (fibroblast growth factor-1) in fibroblasts.¹⁴ Numerous cell culture and in vivo studies have demonstrated rapid induction and increased expression of Fn14 upon activation of diverse cell types by multiple stimuli, as well as in vivo in the course of liver, artery, central nervous system or kidney injury or in tumor cells.^{19,16,13,30,31} It has been postulated that a reduced Fn14 expression limits the actions of TWEAK in healthy tissues and rapid and quantitatively important upregulation of this receptor sensitizes to TWEAK actions^{28,32} (fig. 2). This sensitization would promote TWEAK activity in the presence of only mildly increased or even reduced TWEAK expression.

In experimental models of AKI and autoimmune injury renal TWEAK and, more significantly, Fn14 expression is upregulated.^{31,33} The potential sources of TWEAK in the kidney include infiltrating monocytes and T lymphocytes.^{34,35} In particular T cells from lupus patients express TWEAK.³⁴ Other renal sources of TWEAK include tubular epithelial cells.³¹ In addition, mesangial cells also express TWEAK and Fn14.³⁶ Defining the role of a pleiotropic cytokine such

as TWEAK in AKI is a challenge that has been approached by functional studies in cell cultures and experimental models.³²

THE ROLES: HEROES AND VILLAINS

Evidence suggests that TWEAK and Fn14 play a role in the pathogenesis of human diseases, including atherosclerosis, stroke, rheumatoid arthritis, autoimmune kidney injury, AKI and cancer.^{5-7,37,38,25} TWEAK stimulates, in a cell-type- and microenvironment-dependent fashion, cell proliferation,³⁹⁻⁴³ survival,⁴⁴ migration,^{41,45,46} cell growth⁹ and apoptosis.^{8,47-51} TWEAK can also promote²² or inhibit⁵²⁻⁵⁴ cell differentiation. Finally, TWEAK induces the expression of pro-inflammatory molecules.^{8,27,36,41,45,55-58} *In vivo*, TWEAK can stimulate blood vessel formation (angiogenesis)^{40,59} and regulates neurovascular unit permeability.⁶⁰

A CROWDED PLACE: TWEAK AND INFLAMMATION

TWEAK promotes sustained NFκB activation in murine tubular epithelial cells. In tubular cells TWEAK increased NFκB DNA-binding and transcriptional activity, IκB-α phosphorylation

and RelA nuclear translocation via Fn14.³² As a consequence, TWEAK induced the mRNA expression and secretion of MCP-1, IL-6 and RANTES, which was prevented by the RelA inhibitor parthenolide. The effect of TWEAK on chemokines (previously known as intercrines),⁶¹ such as MCP-1 and RANTES is particularly interesting as it allows the recruitment of further inflammatory cells and amplification of inflammation.

TNF α , used as a control for NF κ B activation, yielded a different pattern of NF κ B DNA-binding complexes.³² This suggests that TWEAK induces other biological responses also dependent on NF κ B that differ from those elicited by TNF α . Thus, TNF α and TWEAK would not be redundant cytokines in renal injury. In this regard, the persistent NF κ B activation induced by TWEAK in tubular cells is consistent with the recently identified non-canonical NF κ B activation, which is not activated by TNF α .⁶²

Systemic administration of TWEAK activates kidney NF κ B and has a proinflammatory effect in the renal tubulointerstitium characterized by increased MCP-1, RANTES and IL-6 mRNA.³² Immunohistochemistry showed that RelA translocated to the nucleus of tubular cells and that these cells were the main site of expression of MCP-1 and RANTES. The expression of chemokines by tubular cells was followed by increased renal expression of the mRNA for the MCP-1 receptor, CCR2, which is expressed by macrophages, as well as by an increased number of interstitial macrophages. This demonstrates that TWEAK induces tubulointerstitial inflammation *in vivo*. However, TWEAK alone did not modify serum creatinine.

TWEAK had recently been found to increase MCP-1 expression in cultured glomerular mesangial cells and to upregulate whole kidney MCP-1 mRNA.³⁶ However, that report did not address whether there was a concomitant increase of chemokine protein, which were the cells responsible for mRNA expression and whether TWEAK elicited renal inflammation. In fact, the context of the report (cell culture studies in mesangial cells) suggested that the site of action of TWEAK would be the glo-

merulus. Our findings demonstrate that in normal kidneys TWEAK elicits tubulointerstitial, but not glomerular inflammation.³² TWEAK contributes to glomerular injury when the Fn14 receptor has been previously upregulated locally by injurious stimuli.³³

THE KILLING FIELDS

Apoptosis contributes to renal cell loss.⁶³ In addition to nephrotoxins and other forms of cell stress,^{64,65} several members of the TNF ligand superfamily induce apoptotic renal cell death, including TRAIL, FasL and TNF- α .⁶⁶⁻⁶⁸ The name TWEAK derives from its weak capacity to induce apoptosis. TWEAK frequently requires co-stimuli, such as IFN γ , to induce apoptosis.³⁵ However, TWEAK alone may induce apoptosis in primary neurons and mesangial cells and this effect is magnified in the presence of IFN γ .^{36,48} TWEAK also mediates activated CD4⁺ T cell-induced apoptosis of monocytes and macrophages (T cells presumably release concomitantly other mediators).⁶⁹ By contrast, non-stimulated tubular epithelial cells are resistant to TWEAK-induced apoptosis and the lethal effect only becomes apparent in the presence of several (not one) inflammatory mediators released during AKI, such as IFN γ and TNF- α .^{31,70,71} This requirement for both IFN γ and TNF α in order to sensitize to cell death in the presence of TWEAK is novel. TNF α /IFN γ increased Fn14 expression in tubular cells. Upregulation of Fn14 expression may underlie the sensitization to apoptosis. However, the level of Fn14 expression is not the only mechanism involved, since IFN γ or TNF α alone also increase Fn14 expression but do not sensitize to cell death. Functional inhibition studies suggested that autocrine activation of the FasL/Fas system mildly contributed to cell death.^{31,72}

The signaling pathways leading from Fn14 to cell death remains poorly understood and there is evidence that it may differ with cell type and the cell microenvironment. The lack of a death domain (DD) suggests that direct recruitment of DD-containing adaptor proteins is not the primary mode of inducing cell death. Evidence from caspase inhibitor studies also points to

several pathways for cell death. In tubular cells, the combination of TWEAK, TNF α and IFN γ resulted in activation of caspase-8, proteolysis of Bid, release of cytochrome c to the cytosol and activation of caspase-9 and caspase-3, suggesting activation of a canonical cell death receptor pathway with recruitment of the mitochondrial pathway. No evidence supporting the participation of endoplasmic reticulum stress was found. The apoptotic endoplasmic reticulum response is preserved in tubular cells exposed to paracetamol or tunicamycin.⁶⁴ Most extracellular inputs are not processed in isolation, rather, multiple inputs are perceived by cells in a proinflammatory milieu.⁷³ Thus, it is difficult to assign the final output to a single stimulus. It is conceivable that similar apoptotic pathways engaged by both TNF α and TWEAK in the presence of IFN γ cooperate in cell death induction. Indeed, TNF α may induce a delayed apoptosis in tubular cells, but the time-course differs from that induced by TWEAK/TNF α /IFN γ , as there is a lag period of 48 h, which was confirmed in cells treated with TNF α /IFN γ .³¹ In addition, the intracellular molecular mechanisms differ between death induced by TNF alone and by the cytokine combination. The pan-caspase inhibitor zVAD prevented the activation of caspases, and also prevented apoptosis induced by TWEAK/TNF α /IFN γ .³¹ However, zVAD transformed the mode of cell death to necrosis and even increased the rate of cell death. Although the induction of necrosis in cells exposed to members of the TNF superfamily when caspases are inhibited had been previously observed,⁷⁴ zVAD did not promote TNF α -induced necrosis in tubular epithelium.³¹ This response is cell type-specific and suggests that pan-caspase inhibition may not be an adequate therapeutic approach to renal injury in which inflammation is contributing the cell death. In tubular cells Fn14 and reactive oxygen species were required for the necrotic response.

THE OUTCOME: PROTECTION

As outlined above cell culture data suggest that TWEAK may be deleterious in inflamed kidney. However, potential proliferative actions of TWEAK on

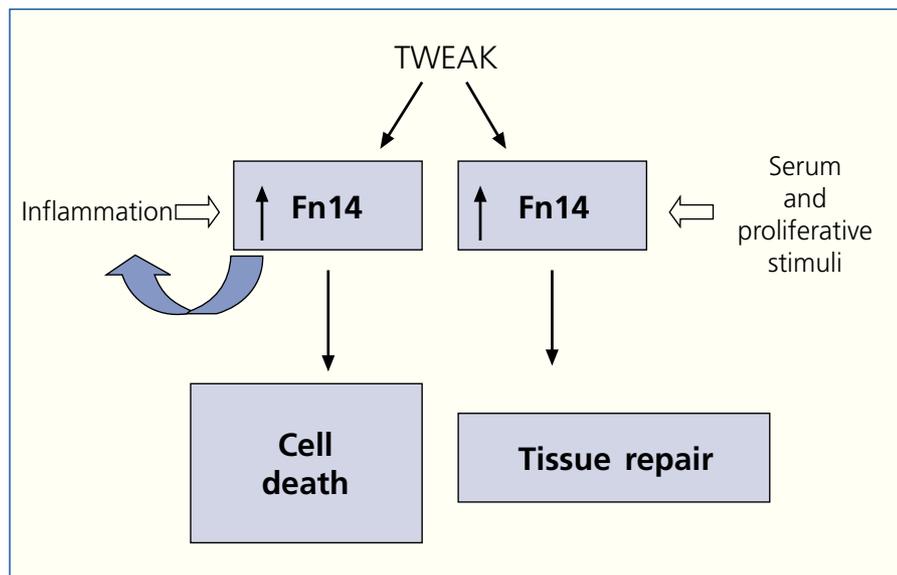


Figure 3. TWEAK the facilitator. Cells responses to TWEAK depend both on the cell type and the microenvironment. Different microenvironmental conditions may lead to increased Fn14 expression, but the consequences of the engagement of this increased amount of receptors are not uniform. In an inflammatory microenvironment Fn14 engagement leads to tubular cell death. By contrast, Fn14 engagement in an environment rich in proliferative and survival stimuli may lead to further cell proliferation and tissue repair. Inflammatory cytokines increase Fn14 expression which, in turn, allows the secretion of more inflammatory cytokines upon TWEAK binding, thus amplifying the inflammatory response.

tubular cells or progenitor cells or the recruitment of macrophages that participate in tissue repair might confer a protective role for TWEAK.²⁵ In this regard, an initial interpretation of the low serum levels of TWEAK in atherosclerosis was that they may be contributing to injury. Functional inhibition of TWEAK *in vivo* was required to understand the outcome of these different potential actions of TWEAK and the end-result of its interactions with different components of the microenvironment. AKI induced by a folic acid overdose is an animal model which has a human counterpart in the rare cases of accidental parental overdose of folic acid.⁷⁵ In addition, it shares with human AKI processes such as tubular cell death, inflammation, and tubular cell proliferation leading to spontaneous recovery and mild fibrosis.^{70,76} In this model, expression of TWEAK and Fn14 increased both at the mRNA and protein level.³¹ There was a 13-fold increase in Fn14 mRNA and a 2.5-fold increase in tubular cell Fn14 receptor. Fn14 was localized to injured, dilated proximal and distal tubules.³¹ While Fn14 was already very high at 24 h, TWEAK expression progressively increased, peaking at 72 h. In addition,

renal expression of IFN γ and TNF α is increased in AKI.^{70,32} A neutralizing anti-TWEAK antibody decreased peak serum creatinine and did not interfere with recovery which was achieved by day 7 in control and treated mice.³² Neutralization of TWEAK also resulted in significantly milder histological injury. TWEAK did not participate in the early (24 h) inflammatory response, which was TWEAK-independent. However, it amplified subsequent inflammation and when TWEAK was neutralized the tubular expression of certain inflammatory molecules (MCP-1, RANTES) and interstitial inflammation by macrophages was decreased at 72 h. The *in vivo* effect of TWEAK on inflammatory mediators appears to be selective. As an example, kidney IL-6 in the course of injury was TWEAK-independent. Thus, although TWEAK may modulate IL-6 expression in cultured tubular cells, other stimuli are more important regulators of IL-6 expression in AKI.

EPILOGUE

The Oxford English dictionary defines TWEAK as «improving by making fine adjustments». This meaning closely represents the role of TWEAK in kid-

ney injury. TWEAK regulates a wide range of cellular processes that are involved both in the generation and the recovery from renal injury. Interestingly the precise effect of TWEAK over kidney cells is modulated by the cell microenvironment, allowing it to fine-tune the renal response to injury (fig. 3). TWEAK interacts with the cell microenvironment to elicit cell-specific responses. In the context of kidney inflammation during AKI the overall effect of TWEAK is negative for the kidney, promoting inflammation and cell death. In this context, TWEAK antagonism improves renal function. TWEAK regulates a wide range of cellular processes that are involved both in the generation of and the recovery from renal injury. Given the pleiotropic actions of TWEAK, that include modulating the proliferation of progenitor cells,⁷⁷ agonism of TWEAK should also be explored in certain clinical contexts. In addition, the possible role of TWEAK as a biomarker should be studied as urinary TWEAK levels were found to be elevated in patients with active lupus nephritis⁷⁸ and low serum TWEAK levels are a marker for subclinical atherosclerosis.²⁸

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REFERENCES

- Ortiz A, Justo P, Sanz A, Lorz C, Egido J. Targeting apoptosis in acute tubular necrosis. *Biochem Pharmacol* 2003; 66: 1589-94.
- Solez K, Morel-Maroger L, Sraer JD. The morphology of «acute tubular necrosis» in man: analysis of 57 renal biopsies and a comparison with the glycerol model. *Medicine (Baltimore)* 1979; 58: 362-76.

3. Olsen TS, Olsen HS, Hansen HE. Tubular ultrastructure in acute renal failure in man: epithelial necrosis and regeneration. *Virchows Arch A Pathol Anat Histopathol* 1985; 406: 75-89.
4. Lorz C, Benito-Martín A, Justo P, Sanz AB, Sánchez Niño MD, Egido J, Ortiz A. Modulation of renal tubular cell survival: where is the evidence? *Current Medicinal Chemistry* 2006; 13: 763-771.
5. Winkles JA. The TWEAK-Fn14 cytokine-receptor axis: discovery, biology and therapeutic targeting. *Nat Rev Drug Discov* 2008; [Epub ahead of print].
6. 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. PMID: 17981048.
7. Sanz AB, Moreno JA, Sánchez-Nino MD, Ucero AC, Benito A, Santamaría B, Justo P, Izquierdo MC, Egido J, Blanco-Colio LM, Ortiz A. TWEAKing renal injury. *Front Biosci* 2008; 13: 580-589.
8. Chicheportiche Y, Bourdon PR, Xu H, Hsu Y, Scott H, Hession C, Garci I, Browning JL. TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. *J Biol Chem* 1997; 272: 32401-32410.
9. 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.
10. 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.
11. Baxter FO, Came PJ, Abell K, Kedjouar B, Huth M, Rajewsky K, Pasparakis M, Watson CJ. IKK beta/2 induces TWEAK and apoptosis in mammary epithelial cells. *Development* 2006; 133: 3385-3394.
12. Marsters SA, Sheridan JP, Pitti RM, Brush J, Goddard A, Ashekenazi A. Identification of a ligand for the death-domain containing receptor Apo3. *Curr Biol* 1998; 8: 525-8.
13. Wiley SR, Cassiano L, Lofton T, Davis-Smith T, Winkles JA, Lindner V, Liu H, Daniel TO, Smith CA, Fanslow WC. A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis. *Immunity* 2001; 15: 837-846.
14. Meighan-Mantha RL, Hsu DK, Guo Y, Brown SA, Feng SL, Peifley KA, Alberts GF, Copeland NG, Gilbert DJ, Jenkins NA, Richards CM, Winkles JA. The mitogen-inducible Fn14 gene encodes a type I transmembrane protein that modulates fibroblast adhesion and migration. *J Biol Chem* 1999; 274: 33166-33176.
15. 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.
16. Feng SL, Guo Y, Factor VM, Thorgeirsson SS, Bell DW, Testa JR, Peifley KA, Winkles JA. 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.
17. 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.
18. Brown SA, 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-kappa B activation. *Biochem J* 2003; 371: 395-403.
19. Tran NL, McDonough WS, Savitch BA, Sawyer TF, Winkles JA, Berens ME. The tumor necrosis factor-like weak inducer of apoptosis (TWEAK)-fibroblast growth factor-inducible 14 (Fn14) signaling system regulates glioma cell survival via NF-kappaB pathway activation and BCLXL/BCL-W expression. *J Biol Chem* 2005; 280: 3483-3492.
20. Winkles JA, Tran NL, Berens ME. TWEAK and Fn14: New molecular targets for cancer therapy? *Cancer Lett* 2005; 235: 11-17.
21. Saitoh T, Nakayama M, Nakano H, Yagita H, Yamamoto N, Yamaoka S. TWEAK induces NF-kappaB p100 processing and long lasting NF-kappaB activation. *J Biol Chem* 2003; 278: 36005-36012.
22. 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.
23. Bover LC, Cardó-Vila M, Kuniyasu A, Sun J, Rangel R, Takeya M, Aggarwal BB, Arap W, Pasqualini R. A previously unrecognized protein-protein interaction between TWEAK and CD163: potential biological implications. *J Immunol* 2007; 178: 8183-94. PMID: 17548657.
24. Bossen C, Ingold K, Tardivel A, Bodmer JL, Gaide O, Hertig S, Ambrose C, Tschopp J, Schneider P. Interactions of tumor necrosis factor (TNF) and TNF receptor family members in the mouse and human. *J Biol Chem* 2006; 281: 13946-13971.
25. Zheng TS, Burkly LC. No End In Site: TWEAK/Fn14 Activation and Autoimmunity Associated End-Organ Pathologies. *J Leukocyte Biology* 2008 (in press).
26. Marsters SA, Sheridan JP, Pitti RM, Brush J, Goddard A, Ashekenazi A. Identification of a ligand for the death-domain-containing receptor Apo3. *Curr Biol* 1998; 8: 525-528.
27. Chicheportiche Y, Chicheportiche R, Sizing I, Thompson J, Benjamin CB, Ambrose C, Daver JM. Proinflammatory activity of TWEAK on human dermal fibroblasts and synoviocytes: blocking and enhancing effects of anti-TWEAK monoclonal antibodies. *Arthritis Res* 2002; 4: 126-33.
28. Blanco-Colio LM, Martín-Ventura JL, Muñoz-García B, Orbe J, Páramo JA, Michel JB, Ortiz A, Meilhac O, Egido J. Identification of Soluble Tumor Necrosis Factor-Like Weak Inducer of Apoptosis (sTWEAK) as a Possible Biomarker of Sub-clinical Atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007; 27: 916-922.
29. Desplat-Jego S, Varriale S, Creidy R, Terra R, Bernard D, Khrestchatisky M, Izui S, Chicheportiche Y, Boucrat J. TWEAK is expressed by glial cells, induces astrocyte proliferation and increases EAE severity. *J Neuroimmunol* 2002; 133: 116-123.
30. 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.
31. Justo P, Sanz AB, Sánchez-Niño MD, Winkles JA, Lorz C, Egido J, Ortiz A. Cytokine cooperation in renal tubular cell injury: the role of TWEAK. *Kidney Int* 2006; 70: 1750-1758.
32. Sanz AB, Justo P, Sánchez-Niño MD, Blanco-Colio LM, Winkles JA, Krezdler M, Jakubowski A, Egido J, Ruiz-Ortega M, Ortiz A. The Cytokine TWEAK Modulates Renal Tubulointerstitial Inflammation. *J Am Soc Nephrol* 2008; 19: 695-703.
33. Zhao Z, Burkly LC, Campbell S, Schwartz N, Molano A, Choudhury A, Eisenberg RA, Michaelson JS, Putterman C. 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.
34. Kaplan JM, Lewis EE, Shelden EA, Somers E, Pavlic R, McCune WJ, Richardson BC. The apoptotic ligands TRAIL, TWEAK, and Fas ligand mediate monocyte death induced by autologous lupus T cells. *J Immunol* 2002; 169: 6020-6029.
35. Nakayama M, Kayagaki N, Yamaguchi N, Okumura K, Yagita H. Involvement of TWEAK in interferon gamma-stimulated monocyte cytotoxicity. *J Exp Med* 2000; 192: 1373-1380.
36. Campbell S, Burkly LC, Gao HX, Berman JW, Su L, Browning B, Zheng T, Schiffer L, Michaelson JS, Putterman C. Proinflammatory effects of TWEAK/Fn14 interactions in glomerular mesangial cells. *J Immunol* 2006; 176: 1889-1898.
37. Winkles JA, Tran NL, Brown SA, Stains N, Cunliffe HE, Berens ME. Role of TWEAK and Fn14 in tumor biology. *Front Biosci* 2007; 12: 2761-2771.
38. Blanco-Colio M, Martín-Ventura JL, Muñoz-García B, Moreno JA, Meilhac O, Ortiz A, Egido J. TWEAK and Fn14. New players in the pathogenesis of atherosclerosis. *Front Biosci* 2007; 12: 3648-3655.
39. Donohue PJ, Richards CM, Brown SAN, Hanscom HN, Buschman J, Thangada S, Hla T, Williams MS, Winkles JA. 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.
40. Lynch CN, Wang YC, Lund JK, Chen Y, Leal JA, Wiley SR. TWEAK induces angiogenesis and proliferation of endothelial cells. *J Biol Chem* 1999; 274: 8455-8459.
41. Harada N, Nakayama M, Nakano H, Fukuchi Y, Yagita H, Okumura K. Pro-inflammatory effect of TWEAK/Fn14 inte-

- reaction on human umbilical vein endothelial cells. *Biochem Biophys Res Commun* 2002; 299: 488-493.
42. Michaelson JS, Cho S, Browning B, Zheng TS, Lincecum JM, Wang MZ, Hsu YM, Burkly LC. TWEAK induces mammary epithelial branching morphogenesis. *Oncogene* 2005; 24: 2613-2624.
 43. Jakubowski A, Ambrose C, Parr M, Lincecum JM, Wang MZ, Zheng TS, Browning B, Michaelson JS, Baetscher M, Wang B, Bissell DM, Burkly LC. TWEAK induces liver progenitor cell proliferation. *J Clin Invest* 2005; 115: 2330-2340.
 44. Jakubowski A, Browning B, Lukashev M, Sizing I, Thompson JS, Benjamin CD, Hsu Y, Ambrose C, Zheng TS, Burkly LC. Dual role for TWEAK in angiogenic regulation. *J Cell Sci* 2002; 115: 267-274.
 45. Jin L, Nakao A, Nakayama M, Yamaguchi N, Kojima Y, Nakano N, Tsuboi R, Okumura K, Yagita H, Ogawa H. Induction of RANTES by TWEAK/Fn14 interaction in human keratinocytes. *J Invest Dermatol* 2004; 122: 1175-1179.
 46. Tran NL, McDonough WS, Donohue PJ, Winkles JA, Berens TJ, Ross KR, Hoelzinger DB, Beaudry C, Coons SW, Berens ME. 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.
 47. Nakayama M, Ishidoh K, Kayagaki N, Kojima Y, Yamaguchi N, Nakano H, Kominami E, Okumura K, Yagita H. Multiple pathways of TWEAK-induced cell death. *J Immunol* 2002; 168: 734-743.
 48. Potrovita I, Zhang W, Burkly L, Hahm K, Lincecum J, Wang MZ, Maurer MH, Rossner M, Schneider A, Schwaninger M. Tumor necrosis factor-like weak inducer of apoptosis-induced neurodegeneration. *J Neurosci* 2004; 24: 8237-8244.
 49. Schneider P, Schwenzler R, Haas E, Muhlenbeck F, Schubert G, Scheurich P, Tschopp J, Wajant H. TWEAK can induce cell death via endogenous TNF and TNF receptor 1. *Eur J Immunol* 1999; 29: 1785-1792.
 50. 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.
 51. Nakayama M, Ishidoh K, Kojima Y, Harada N, Kominami E, Okumura K, Yagita H. Fibroblast growth factor-inducible 14 mediates multiple pathways of TWEAK-induced cell death. *J Immunol* 2003; 170: 341-348.
 52. Felli N, Pedini F, Zeuner A, Petrucci E, Testa U, Conticello C, Biffoni M, Di Cataldo A, Winkles JA, Peschle C, De Maria R. Multiple members of the TNF superfamily contribute to IFN-gamma-mediated inhibition of erythropoiesis. *J Immunol* 2005; 1: 1464-1472.
 53. 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-kappaB and degradation of myoD protein. *J Biol Chem* 2006; 281: 10327-10336.
 54. Perper SJ, Browning B, Burkly LC, Weng S, Gao C, Giza K, Su L, Tarilonte L, Crowell T, Rajman L, Runkel L, Scott M, Atkins GJ, Findlay DM, Zheng TS, Hess H. TWEAK is a novel arthritogenic mediator. *J Immunol* 2006; 177: 2610-2620.
 55. Xu H, Okamoto A, Ichikawa J, Ando T, Tasaka K, Masuyama K, Ogawa H, Yagita H, Okumura K, Nakao A. TWEAK/Fn14 interaction stimulates human bronchial epithelial cells to produce IL-8 and GM-CSF. *Biochem Biophys Res Commun* 2004; 318: 422-427.
 56. Kim SH, Kang YJ, Kim WJ, Woo DK, Lee Y, Kim DI, Park YB, Kwon BS, Park JE, Lee WH. TWEAK can induce pro-inflammatory cytokines and matrix metalloproteinase-9 in macrophages. *Circ J* 2004; 68: 396-399.
 57. Saas P, Boucraut J, Walker PR, Quiquerez AL, Billot M, Desplat-Jego S, Chicheportiche Y, Dietrich PY. TWEAK stimulation of astrocytes and the proinflammatory consequences. *Glia* 2000; 32: 102-7.
 58. Kawakita T, Shiraki K, Yamanaka Y, Yamaguchi Y, Saitou Y, Enokimura N, Yamamoto N, Okano H, Sugimoto K, Murata K, Nakano T. Functional expression of TWEAK in human hepatocellular carcinoma: possible implication in cell proliferation and tumor angiogenesis. *Biochem Biophys Res Commun* 2004; 318: 726-733.
 59. Ho DH, Vu H, Brown SAN, Donohue PJ, Hanscom HN, Winkles JA. 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.
 60. 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-kappaB pathway activation. *J Neurosci* 2005; 25: 10094-10100.
 61. Gómez-Chiarri M, Ortiz A, Seron D, Glez E, Egido J. The intercrine superfamily and renal disease. *Kidney Int* 1993; Suppl. 39: S81-S85.
 62. Beinke S, Ley SC. Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. *Biochem J* 2004; 382 (Pt 2): 393-409.
 63. Sanz AB, Santamaría B, Ruiz Ortega M, Egido J, Ortiz A. Mechanisms of renal apoptosis in health and disease. *J Am Soc Nephrol* 2007; Accepted.
 64. Lorz C, Justo P, Sanz A, Subirá D, Egido J, Ortiz A. Paracetamol nephrotoxicity: a role for ER stress. *J Am Soc Nephrol* 2004; 15: 380-9.
 65. Justo P, Lorz C, Sanz A, Egido J, Ortiz A. Intracellular mechanisms of cyclosporine A-induced tubular cell apoptosis. *J Am Soc Nephrol* 2003; 14: 3072-3080.
 66. Ortiz A, Lorz C, Egido J. New kids in the block: the role of FasL and Fas in kidney damage. *J Nephrol* 1999; 12: 150-158.
 67. Ortiz A, Lorz C, Egido J. The Fas ligand/Fas system in renal injury. *Nephrol Dial Transplant* 1999; 14: 1831-1834.
 68. Ortiz A, González-Cuadrado S, Bustos C, Alonso J, Gómez-Guerrero C, López-Armada MJ, González E, Plaza JJ, Egido J. Tumor necrosis factor and glomerular damage. *J Nephrol* 1995; 8: 27-34.
 69. 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: 2897-904.
 70. Ortiz A, Lorz C, Catalán MP, Danoff TM, Yamasaki Y, Egido J, Neilson EG. Expression of apoptosis regulatory proteins in tubular epithelium stressed in culture or following acute renal failure. *Kidney Int* 2000; 57: 969-981.
 71. Goes N, Urmson J, Ramassar V, Halloran PF. Ischemic acute tubular necrosis induces an extensive local cytokine response. Evidence for induction of interferon-gamma, transforming growth factor-beta 1, granulocyte-macrophage colony-stimulating factor, interleukin-2, and interleukin-10. *Transplantation* 1995; 59: 565-72.
 72. Lorz C, Ortiz A, Justo P, González-Cuadrado S, Duque N, Gómez-Guerrero C, Egido J. Proapoptotic Fas ligand is expressed by normal kidney tubular epithelium and injured glomeruli. *J Am Soc Nephrol* 2000; 11: 1266-77.
 73. Janes KA, Albeck JG, Gaudet S, Sorger PK, Lauffenburger DA, Yaffe MB. A systems model of signaling identifies a molecular basis set for cytokine-induced apoptosis. *Science* 2005; 310: 1646-53.
 74. Khwaja A, Tatton L. Resistance to the cytotoxic effects of tumor necrosis factor alpha can be overcome by inhibition of a FADD/caspase-dependent signaling pathway. *J Biol Chem* 1999; 274: 36817-23.
 75. Metz-Kurschel U, Kurschel E, Wagner K, Aulbert E, Graben N, Philipp T. Folate nephropathy occurring during cytotoxic chemotherapy with high-dose folic acid and 5-fluorouracil. *Ren Fail* 1990; 12 (2): 93-7.
 76. Ortega A, Ramila D, Ardura JA, Esteban V, Ruiz-Ortega M, Barat A, Gazapo R, Bosch RJ, Esbrit P. Role of parathyroid hormone-related protein in tubulointerstitial apoptosis and fibrosis after folic acid-induced nephrotoxicity. *J Am Soc Nephrol* 2006; 17: 1594-603.
 77. Girgenrath M, Weng S, Kostek CA, Browning B, Wang M, Brown SA, Winkles JA, Michaelson JS, Allaire N, Schneider P, Scott ML, Hsu YM, Yagita H, Flavell RA, Miller JB, Burkly LC, Zheng TS. TWEAK, via its receptor Fn14, is a novel regulator of mesenchymal progenitor cells and skeletal muscle regeneration. *EMBO J* 2006; 25: 5826-39.
 78. Schwartz N, Su L, Burkly LC, Mackay M, Aranow C, Kollaros M, Michaelson JS, Rovin B, Putterman C. Urinary TWEAK and the activity of lupus nephritis. *J Autoimmun* 2006; 27: 242-50.