Introduction: The novel cytokine Tumor Necrosis Factor (TNF)-like weak inducer of apoptosis (TWEAK) was identified. TWEAK is a member of the TNF-alfa superfamily of cytokines and is primarily expressed as a type II transmembrane protein. TWEAK can be cleaved to generate a soluble factor with many biological activities, including stimulation of cell proliferation, migration, angiogenesis, differentiation, and the expression of proinflammatory cytokines. TWEAK acts on responsive cells via binding to a small, 14 kDa cell surface fibroblast-like growth factor-inducible protein, Fn14. Fn14 is a member of the TNF receptor superfamily. TWEAK binding to Fn14 activates the nuclear factor-kappa B (NF-kappa B) pathway. Fn14 is highly up-regulated in the context of tissue injury, regeneration, and inflammatory responses.

A recent report identified a specific role of TWEAK in the promotion of arthritis in a mouse collagen-induced arthritis (CIA) model (Perper SJ et al.). The level of TWEAK in serum was dramatically elevated and inhibition of TWEAK with a neutralizing antibody significantly reduced clinical severity. The authors of that study concluded that TWEAK contributes to rheumatoid arthritis pathogenesis for the following reasons. First, the production of TWEAK by macrophages promotes joint inflammation by stimulating synovial fibroblasts to produce various cytokines, including MIP-1, lymphotoxin, IFN-gamma-inducible protein 10 (IP-10), MCP-1, and RANTES. Second, TWEAK directly triggers damage of cartilage and bone via enhancement of MMP activity. Third, TWEAK contributes to joint tissue pathology through synovial angiogenesis. Finally, TWEAK can hinder endogenous repair by blocking the differentiation of osteoblastic and chondrocytic precursors. Thus, TWEAK may also play a role in inflammation, neovascularization, and degradation in intervertebral disc tissues.

Materials and Methods: Mice.

Homozygous wild type C57BL/6J, TNF receptor 1-null B6.129-Tnfrsf1atm1Mak/J, and TNF receptor 2-null B6.129S2-Tnfrsf1btm1Mwm/J mice were used in these studies. The experimental protocols were approved by the Institutional Animal Care and Use Committee of our university.

Cultures of intervertebral discs.

For organ culture assays, microscopy was used to harvest coccygeal intervertebral disc tissues completely free from endplates. When suppression of aggrecan synthesis was assessed, anatomically preserved intervertebral disc tissues containing endplates were used to examine intervertebral disc tissues histologically after stimulation by recombinant mouse (rm) TNF-alfa or TWEAK. For real-time RT-PCR studies, whole intervertebral disc tissues (10 discs/35mm dish) were cultured in 1 mL of DMEM containing 0.1% FBS, 50 microg/ml penicillin and streptomycin in the presence or absence of 100 ng/mL rmTWEAK or 10 ng/mL of rm TNF-alfa for 3 days in a humidified environment of 5% CO2 at 37o C. For the detection of MMP-3 expression by ELISA, three discs per 12-well plate were cultured in 1 mL of DMEM containing 0.1% FBS in the presence or absence of 10, 100, 300 ng/mL rm TWEAK or 10 ng/mL of rm TNF-alfa for 3 days in a humidified environment of 5% CO2 at 37o C. In other experiments, three discs were cultured in the presence or absence of 100 ng/mL of rm TWEAK for 1, 3, or 5 days. For some experiments, rm TWEAK receptor (TNFRSF12)/Fc chimera or anti-mouse TWEAK neutralizing antibody was added to the culture medium. Culture media were clarified by centrifugation and processed for determination of MMP-3 by ELISA.

Results: The expression of both TWEAK and its receptor, Fn14, in discs was confirmed by immunohistochemistry and quantitative real time PCR. TWEAK induced disc cells to generate MMP-3 in a dose- and time-dependent manner. This induction was strongly inhibited in the presence of a neutralizing antibody to TWEAK or a chimeric Fn14/Fc fusion protein. In disc tissues derived from TNF-alfa receptor 1- or TNF-alfa receptor 2-deficient mice, recombinant TWEAK modestly induced MMP-3. In contrast, in disc cultures lacking TWEAK, tissues from wild type mice or receptor-deficient mice failed to express MMP-3. Furthermore, aggrecan expression was potently abrogated in a time-dependent manner in the presence of recombinant TWEAK.

Discussion: This is the first report to confirm expression of TWEAK and its receptor Fn14 in murine intervertebral disc tissues. Based on them, we conclude that TWEAK may be a novel proteoglycan-degrading mediator in intervertebral disc degeneration as observed in osteoarthritis or rheumatoid arthritis. TWEAK contributes inhibitory effects on cartilage metabolism through both the induction of matrix degrading enzymes such as MMP-3 and the impediment of endogenous cartilage repair through down-regulation of aggrecan synthesis. Therefore, novel therapeutic approaches for targeting TWEAK expression or function may slow down or halt the process of intervertebral disc degeneration.


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Novel function of TWEAK in inducing intervertebral disc degeneration

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