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The TWEAK-Fn14 Pathway: A Potent Regulator of Skeletal Muscle Biology in Health and Disease

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Abstract

TNF-like weak inducer of apoptosis (TWEAK), a TNF superfamily ligand, and its bona fide receptor, the TNF receptor superfamily member fibroblast growth factor-inducible protein 14 (Fn14), represents a pivotal axis for shaping both physiological and pathological tissue responses to acute or chronic injury and disease. In recent years significant advances have been made in delineating the prominent role of this axis in regulating skeletal muscle mass and metabolism. Also emerging from the broad study of tissue injury in skeletal muscle and other organs is the role of the TWEAK-Fn14 pathway in promoting fibrosis. This review article highlights recent advancements towards understanding how the TWEAK-Fn14 pathway regulates the response to various skeletal muscle insults and more broadly, engages multiple mechanisms to drive tissue fibrosis.

Keywords

Skeletal muscle atrophy; Regeneration; NF-kappa B; Tissue injury; Fibrosis

1. Introduction

Skeletal muscle comprises approximately 40% of total body mass which provides structural support, enables the body to maintain posture, and ensures basic functions such as locomotion, respiration, energy storage, and whole body metabolism. Skeletal muscle also demonstrates high level of plasticity in response to various physiological stressors such as weight bearing and exercise. In response to environmental demands, skeletal muscle remodels by activating multiple signaling pathways to reprogram gene expression to sustain

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muscle mass, energy metabolism, and performance. By contrast, chronic diseases or prolonged inactivity leads to loss of skeletal muscle mass and function commonly known as skeletal muscle atrophy or wasting [1]. Furthermore, degeneration of myofibers and their eventual replacement by fibrotic tissues is a major pathological feature in many genetic muscle disorders such as muscular dystrophy [2].

TNF-like weak inducer of apoptosis (TWEAK), a TNF superfamily ligand, and its only known signaling receptor, the TNF receptor superfamily member fibroblast growth factor inducible 14 (Fn14), have emerged as a pivotal axis for shaping tissue responses in response to acute and chronic tissue injury and disease [3]. Usually dormant due to the relatively low levels of Fn14 expressed in normal healthy tissues, the TWEAK-Fn14 axis is activated as a consequence of the highly induced local expression of Fn14 in injured and diseased tissues. Fn14 can be induced in various architectural tissue cell types, including epithelial, vascular, and other mesenchymal and stromal cell types in response to growth factors and proinflammatory cytokines as well as profibrotic cytokines [4]. Fn14 can also be expressed in tissue progenitor cells. The ligand, TWEAK, which is constitutively and fairly ubiquitously expressed in all types of leukocytes and some non-hematopoietic cell types, is initially synthesized as a Type II transmembrane protein, but then secreted as a soluble cytokine due to efficient furin-mediated cleavage [4]. Thus, the TWEAK-Fn14 axis becomes specifically engaged in contexts of tissue injury and disease, triggering the activation of various downstream signaling pathways and thereby shapes the tissue response to injury through the induction of cellular responses including proinflammatory and proangiogenic responses, and the regulation of cell survival, migration and differentiation, and progenitor cell fate.

Recently, significant advances have been made in delineating the prominent role of the TWEAK-Fn14 axis in regulating skeletal muscle biology, with respect to mature muscle fiber atrophy, mitochondrial function, and muscle regeneration through its control of myoblast proliferation and differentiation [5]. Also emerging from the studies of skeletal muscle injury is the role of the TWEAK-Fn14 pathway in driving a number of skeletal muscle pathologies including tissue fibrosis. In this focused review, we continue to explore the paradigm and mechanisms of how the TWEAK-Fn14 pathway shapes tissue response in contexts of health, injury and disease [5, 6]. Firstly, we review recent advances in our understanding of TWEAK-Fn14 pathway-mediated tissue responses to skeletal muscle injury and atrophic stimuli, including differential utilization and regulation of multiple signaling pathways. Secondly, we discuss the evidence for an emerging role for the TWEAK-Fn14 axis in promoting tissue fibrosis as a pathological outcome of tissue injury and disease, highlighting multiple underlying mechanisms.

2. TWEAK-Fn14: A key regulator of skeletal muscle biology

Emerging evidence suggests that TWEAK-Fn14 signaling axis is a major regulator of skeletal muscle atrophy, regeneration, and metabolic function. In the following section, we discuss the role of TWEAK-Fn14 system in skeletal muscle health and disease.

2.1. TWEAK and Fn14 regulate myogenesis

Skeletal muscle formation or myogenesis is a highly regulated process that involves the lineage determination of multipotential mesodermal cells to give rise to myoblasts, exit of these myoblasts from the cell cycle, and their differentiation into muscle fibers. This process is required not only for the embryonic development of skeletal muscle but also for postnatal growth and regeneration of myofibers after injury [7]. Myogenesis is regulated by sequential expression of several myogenic regulatory factors (MRFs), a group of basic helix-loop-helix transcription factors, which include Myf5, MyoD, myogenin, and MRF4. During myogenesis, fusion of myoblasts into multinucleated myotubes is the terminal step of differentiation after which no further mitotic divisions occur within the myotubes or myofibers [8]. The extra nuclei required for muscle growth or repair are provided by satellite cells (i.e. adult muscle stem cells), which are located under the basal lamina of the muscle fiber [7, 9].

The TWEAK receptor, Fn14, is constitutively expressed in muscle progenitor cells such as satellite cells and myoblasts [10, 11]. Accumulating evidence suggests that TWEAK-Fn14 axis affects multiple steps in the process of myogenesis (Figure 1). Initial studies demonstrated that addition of exogenous TWEAK to cultured myoblasts augments proliferation but inhibits their differentiation into multinucleated myotubes through suppressing the expression of MyoD and myogenin and reducing the stability of MyoD protein in differentiating myoblasts [11, 12]. TWEAK stimulates mitogen-activated protein kinase (MAPK) and canonical and non-canonical nuclear factor-kappa B (NF- κ B) signaling pathways in myoblasts [10, 12, 13]. One of the mechanisms by which TWEAK inhibits myogenesis is through the activation of canonical NF- κ B signaling. Activation of canonical NF- κ B signaling is also responsible, at least in part, for the enhanced degradation of MyoD in TWEAK-treated myoblasts [12]. Consistent with the inhibitory effects of TWEAK in myogenesis, a recent study has demonstrated that TWEAK is a negative regulator of human mesoangioblast differentiation and TWEAK-Fn14 axis is dysregulated in sporadic inclusion-body myositis (IBM) muscle. Compared to controls, IBM mesoangioblasts have increased levels of Fn14 protein, produce significantly higher amounts of TWEAK in culture medium, and show reduced differentiation capacity. Importantly, inhibition of TWEAK using Fn14-Fc chimera or short interfering RNA technique improved myogenic differentiation in IBM mesoangioblasts providing initial evidence that dysregulated TWEAK-Fn14 axis interferes with myogenic differentiation in IBM [14].

While the TWEAK-Fn14 axis can promote myoblast proliferation and impede their differentiation, a distinct role of TWEAK and Fn14 in myogenesis is supported by the findings that primary myoblasts from Fn14-knockout (KO), but not TWEAK-KO mice, display reduced proliferative capacity and slightly aberrant myotube formation upon induction of differentiation [11]. Although the exact mechanisms remain poorly understood, knockdown of Fn14 impairs the activation of RhoA GTPase and serum response factor (the positive regulators of myogenesis) in cultured myoblasts, providing a potential mechanism by which Fn14 regulates myogenic differentiation [10, 15]. Consistent with published reports suggesting that increased expression of Fn14 alone is sufficient to induce various cellular responses including tumor cell migration and invasion [16–18], these findings imply

that Fn14 receptor can signal independently of TWEAK and produces distinct biological responses. While it remains a formal possibility that a yet to be identified ligand other than TWEAK can bind and signal through Fn14, a more intriguing scenario is that it might be evolutionarily advantageous for Fn14 to retain some ability to trigger ligand-independent signaling under certain biological conditions. For example, in settings of acute skeletal muscle injury, the significantly increased Fn14 expression could potentially be conducive to TWEAK-independent signaling, thus ‘jumpstart’ the proliferation of myogenic progenitors required for repair before abundant TWEAK becomes available from the infiltrating leukocytes. Thus, it is likely that in absence of TWEAK, Fn14 predominantly activates promyogenic signaling and stimulates myotube formation. However, when the levels of TWEAK are elevated as observed during chronic tissue injury and inflammation, it binds to Fn14 and causes the activation of specific signaling pathways such as MAPKs and canonical NF- κ B which promote proliferation but inhibit myogenesis by blocking the withdrawal of myoblasts from the cell cycle and inducing degradation of MyoD protein (Figure 2).

Recent studies have also highlighted that while relatively high levels (100 ng/ml) of TWEAK induce proliferation and inhibit differentiation, low amounts (10 ng/ml) of TWEAK can produce distinct responses in muscle progenitor cells. Myoblast fusion is a critical step during myogenesis. It is now increasingly clear that coordinated interaction between different signaling pathways regulates fusion of myoblasts during myogenesis [8]. Enwere et al recently reported that non-canonical NF- κ B signaling, which gets activated during myogenesis, stimulates myoblast fusion during myogenesis. This study also showed that low levels of TWEAK predominately activate non-canonical arm of NF- κ B signaling to promote myoblast fusion [13]. While low levels of exogenous TWEAK may transiently enhance myoblast fusion, it is notable that chronic presence of even low levels of TWEAK can lead to deleterious effects on nascent myotubes. The amount of TWEAK (i.e. 10 ng/ml), which induces myoblast fusion, also causes atrophy and reduces the survival of differentiated myotubes [19]. Furthermore, chronic presence of even low levels of TWEAK, especially in the conditions where the expression of Fn14 is also increased in myogenic cells, may be sufficient to inhibit myogenic differentiation. This possibility is supported by studies in IBM mesoangioblasts which produce relatively low levels (~ 0.8 ng/ml) of TWEAK; however, neutralization of TWEAK is sufficient to increase myotube formation in IBM mesoangioblasts cultures [14]. Similarly, it has found that neutralization of endogenously produced TWEAK using Fn14-Fc chimera increases myogenic differentiation in C2C12 myoblasts [10] and cultured primary myoblasts from TWEAK-KO mice show increased myotube formation upon induction of differentiation [20].

Self-renewal of satellite cells is an essential process to maintaining the robustness of skeletal muscle regenerative capacity in adults [9]. We have recently reported that low levels (i.e. 10 ng/ml) of exogenous TWEAK significantly reduces the number of Pax7⁺/MyoD⁻ (self-renewing satellite cells) and increases the proportion of Pax7⁻/MyoD⁺ (cells committed to myogenic lineage) on myofiber explants [21]. TWEAK inhibits satellite cell self-renewal through the activation of canonical NF- κ B and repression of Notch signaling pathway [21]. Collectively, these reports suggest that while chronic presence of TWEAK inhibit myogenic differentiation, transient low levels of TWEAK are sufficient to inhibit satellite cell self-renewal, promote formation of myoblasts, and potentially cause premature fusion of

myoblasts [13, 21]. Since TWEAK activates canonical NF- κ B in satellite cells (to inhibit their self-renewal) and non-canonical NF- κ B signaling in myoblasts (to promote fusion), there appears to be a signaling cross-talk between canonical and non-canonical NF- κ B pathways as satellite cells differentiate into myoblasts in the presence of TWEAK [13, 21]. Future studies will identify the exact mechanisms by which TWEAK affects the activation of canonical and non-canonical arms of NF- κ B in muscle progenitor cells.

Another potential mechanism by which TWEAK inhibits myogenesis is through affecting the expression of certain microRNAs. Recently, a few muscle-specific microRNAs (miRs) such as miR-1, miR-133a, miR-133b, and miR-206 (also called myomiRs) have been identified which are essential for muscle progenitor cell proliferation, differentiation, and maintenance [22]. Forced expression of miR-1 and miR-133a in embryonic stem cells and other non-muscle cell types promotes their differentiation into the skeletal muscle lineage. TWEAK inhibits the expression of levels of miR-1, miR-133a, miR-133b, and miR-206 in myotubes [23]. Furthermore, it has also been reported that myogenic transcription factors myocyte-specific enhancer factor 2C (MEF2c) and MyoD control the expression of myomiRs in skeletal and cardiac muscles [22]. TWEAK diminishes the levels of both MEF2c and MyoD in myogenic cells [12, 23]. Altogether, these findings suggest that elevated levels of TWEAK blocks myogenic differentiation through reducing the levels of MEF2c and MyoD and potentially repressing the expression of various myomiRs.

2.2. TWEAK inhibits skeletal muscle regeneration

Although skeletal muscle is a terminally differentiated tissue, it has remarkable ability to regenerate upon injury. In response to injury, satellite cells are rapidly activated to reenter in the cell cycle, undergo several rounds of proliferation, and differentiate into myocytes which eventually fuse with each other or pre-existing myofibers to complete the repair process [7]. Impairment in the regeneration process is a critical determinant for the loss of skeletal muscle mass in many diseases [2, 5].

The role of TWEAK in skeletal muscle regeneration has been investigated using both TWEAK-KO and muscle-specific TWEAK-transgenic (Tg) mice. As has been observed in many other tissues, the expression levels of TWEAK and Fn14 are significantly increased within 3–5 days following intramuscular injection of cardiotoxin (CTX), a known trigger for satellite cell-driven skeletal muscle regeneration [11, 24]. The number of myofibers which stained positive for embryonic myosin heavy chain (eMyHC, a marker for regenerating myofibers) was improved and the regenerating myofibers of TWEAK-KO mice appeared larger in diameter whereas myofibers of TWEAK-Tg mice expressed less eMyHC and were significantly smaller in size compared with wild-type mice [24]. TWEAK inhibits muscle regeneration by reducing the pool of satellite cells evident by the findings that the number of satellite cells is significantly increased in regenerating myofibers of TWEAK-KO mice [21]. Furthermore, TWEAK exaggerates inflammatory response in injured myofibers. The inflammatory response, which starts within hours of injury and lasts up to seven days, involves infiltration of neutrophils and macrophages in injured muscle which help in removing tissue debris through phagocytosis. These infiltrating phagocytes in injured skeletal muscle also produce a number of cytokines and chemokines which can directly

affect the proliferation and differentiation of myoblasts and cause extracellular matrix remodeling. While initial inflammatory response is critical for efficient regeneration of injured muscle, chronic inflammation and elevated levels of inflammatory cytokines interfere with the process of skeletal muscle regeneration and lead to fibrosis [2, 25, 26]. The activation of NF- κ B, levels of many inflammatory molecules, and interstitial fibrosis were all found to be reduced in injured skeletal muscle of TWEAK-KO mice and increased in TWEAK-Tg mice compared to wild-type mice [24]. It has been consistently observed that the activation of canonical NF- κ B signaling inhibits skeletal muscle regeneration in response to injury [1, 26]. More recently, it has been reported that the membrane-bound form of TWEAK is more potent than its soluble variant with respect to activation of the canonical NF- κ B pathway, potentially due to higher degree of receptor crosslinking and signaling [27]. Interestingly, it has been reported that degenerating myofibers of IBM patients show increased membrane TWEAK immunoreactivity [14]. Thus it is possible that sustained TWEAK signaling or increased expression of membrane-bound TWEAK in disease conditions such as IBM diminishes skeletal muscle regeneration through the activation of canonical NF- κ B signaling.

It is also notable that the role of TWEAK and Fn14 in muscle regeneration is quite similar to their distinct roles in differentiation of cultured myoblasts. Fn14-KO mice displayed delayed myofiber regeneration after injury [11]. Deferred muscle regeneration in Fn14-KO mice could be attributed to diminished inflammatory response potentially due to reduced expression of certain chemokines such as monocyte-specific chemokine 3 [11]. Certainly, more investigations are required to understand why TWEAK and Fn14 differentially regulate adult skeletal muscle regeneration. However, the available literature suggests that elevated levels of TWEAK inhibit skeletal muscle regeneration in both healthy and disease muscle.

2.3. TWEAK-Fn14 system mediates skeletal muscle atrophy

Skeletal muscle atrophy or wasting is a major human morbidity and contributes to mortality in a variety of clinical settings [1, 28]. Skeletal muscle wasting occurs due to accelerated degradation of muscle protein. It has been consistently observed that ATP-dependent ubiquitin-proteasome system (UPS) plays a prominent role in myofibril proteolysis [29]. In almost all catabolic conditions, the expression of two E3 ubiquitin ligases (i.e. MuRF1 and MAFBx), which label the target proteins for degradation by 26S proteasome, is highly up-regulated. Muscle wasting is considerably inhibited in mice null for *MuRF1* or *MAFBx* compared to wild-type mice in response to a wide variety of stimuli suggesting that these ubiquitin ligases are the master drivers of muscle proteolysis [29]. In addition to UPS, the autophagy-lysosomal system (ALS) also plays an important role in degradation of muscle protein in atrophy conditions. Autophagosome formation and elevated expression of several genes of ALS have been observed in skeletal muscle of mice in response to starvation and denervation [30]. Furthermore, there is also evidence that some other proteolytic systems such as caspases function upstream of UPS and ALS to induce degradation of muscle proteins in catabolic states [30, 31]. Moreover, recent studies also suggest that the changes in the mitochondrial content, integrity, and function play a critical role in promoting muscle wasting [32, 33].

Studies in our laboratory have identified TWEAK as a potent mediator of skeletal muscle wasting in mice [19]. Even low levels (i.e. 10 ng/ml) of TWEAK cause significant atrophy in cultured myotubes and induce degradation of specific muscle proteins such as myosin heavy chain (MyHC) and creatine kinase. Activation of canonical NF- κ B pathway causes skeletal muscle atrophy in response to multiple stimuli primarily through augmenting the expression of the components of UPS including MuRF1 [1, 26, 34]. Incidentally, NF- κ B is also involved in TWEAK-induced expression of MuRF1 and proteolytic degradation in cultured myotubes [19]. Furthermore, it has been found that TWEAK induces the expression of the components of ALS and activate caspases (especially caspase3) in cultured myotubes suggesting that multiple proteolytic pathways are involved in TWEAK-induced atrophy in cultured myotubes [35].

TWEAK also causes muscle wasting *in vivo*. Chronic administration of TWEAK protein in mice led to significant reduction in body weight, skeletal muscle mass, and fiber cross-sectional area [19]. Furthermore, muscle-specific transgenic (Tg) overexpression of relatively high levels of TWEAK (>16 fold increase) in mice caused profound loss of skeletal muscle mass and neonatal lethality. Founder TWEAK-Tg mice were smaller in size and exhibited impairment in breathing and locomotion due to profound muscle loss [19]. However, comparatively lower levels of overexpression of TWEAK (4–6 fold) did not cause lethality in mice but showed progressive atrophy and interstitial fibrosis in skeletal muscle [36]. Muscle-specific transgenic overexpression of TWEAK or systemic treatment with TWEAK protein led to the activation of NF- κ B, expression of MuRF1, and ubiquitination of MyHC in skeletal muscle [19, 36].

Although TWEAK is a proinflammatory cytokine, it was interesting to find that TWEAK-Fn14 system mediates muscle atrophy in response to denervation and starvation, which generally do not involve any noticeable inflammatory response [36–38]. In these catabolic conditions, the expression of Fn14 in skeletal muscle is dramatically induced. It is notable that the expression of TNF- α (a well-known muscle-wasting cytokine) or its receptors remained unaffected in denervated skeletal muscle of mice [36]. The role of TWEAK-Fn14 system in muscle atrophy is evident by the findings that denervation- or starvation-induced loss of skeletal muscle mass is rescued in TWEAK-KO mice [36, 37]. By contrast, TWEAK-Tg mice showed increased muscle atrophy and fibrosis upon denervation compared with littermate wild-types [36]. Consistent with studies in cultured myotubes, TWEAK-Fn14 signaling stimulates NF- κ B and increases the expression of *MuRF1* in denervated skeletal muscle [36].

Peroxisome proliferator-activated receptor-gamma (PPAR- γ) coactivator 1 α (PGC-1 α) plays a key role in preserving skeletal muscle mass and mitochondrial content in catabolic states [39]. Although the exact mechanisms remain poorly understood, our recent studies have demonstrated that TWEAK represses the expression of PGC-1 α in cultured myotubes and in denervated skeletal muscle of mice [40, 41]. Overexpression of PGC-1 α rescues TWEAK-induced atrophy and activation of NF- κ B and the components of UPS in cultured myotubes. Furthermore, progressive muscle atrophy in TWEAK-Tg mice is significantly attenuated in TWEAK-PGC-1 α double Tg mice suggesting that PGC-1 α plays an important role in TWEAK-induced muscle atrophy [40]. Furthermore, our studies have provided

evidence that a reciprocal interaction exists between TWEAK-Fn14 and PGC-1 α in denervated muscle. While TWEAK-Fn14 signaling represses the expression of PGC-1 α , transgenic overexpression of PGC-1 α prevents the expression of Fn14 in denervated skeletal muscle of mice [40].

It is notable that all the effects of TWEAK on various signaling pathways or proteolytic systems in cultured myotubes are not recapitulated in denervated muscle. In cultured myotubes, TWEAK inhibits the activity of PI3K/Akt pathway; however, there was no significant difference in level of phosphorylation of Akt kinase in denervated muscle of wild-type, TWEAK-Tg and TWEAK-KO mice [19, 36]. Furthermore, while the denervation led to increased expression of several autophagy-related genes (such as LC3B, Beclin1, Atg5, Atg12, and Gabarap11) and caspase3 in skeletal muscle, this induction was comparable between wild-type, TWEAK-Tg, and TWEAK-KO mice [36]. We believe that denervated muscle may contain certain factors which neutralize some of the actions of TWEAK or endogenous negative feedbacks within the Fn14 signaling pathway that are slow acting in nature and are not recapitulated in cell culture. It is likely that TWEAK also mediates muscle atrophy in many yet to be identified conditions and in those conditions TWEAK stimulates the activation of ALS and caspases as well (Figure 3). Indeed, a recent study has demonstrated that the activity of TWEAK-Fn14 signaling is increased in non-burn human skeletal muscle in response to severe burn injury suggesting that this receptor-ligand dyad may also mediate muscle atrophy after burn injury [42].

2.4. TWEAK inhibits skeletal muscle oxidative phosphorylation capacity

Skeletal muscle contains four types of fibers: I, IIA, IIX, IIB based on the myosin heavy chain (MyHC) that is primarily expressed. MyHC type I fibers are “slow oxidative” that have high mitochondrial content, favor fatty acid oxidation, and have slower contraction/relaxation profile. MyHC type II fibers are called “fast glycolytic” fibers. Type IIA and type IIX fibers are fast oxidative/glycolytic fibers that have intermediate biophysical properties but tend to be also oxidative and rich in mitochondria [43, 44]. Transition from slow-to-fast type and vice-versa is an important component of skeletal muscle plasticity [45]. Studies in the past decade have suggested that PGC-1 α is a key player in regulating skeletal muscle fiber composition, mitochondrial content, and oxidative metabolism [46, 47]. Transgenic mice overexpressing PGC-1 α in skeletal muscle have increased proportion of fibers expressing MyHC I and IIA [48]. These effects of PGC-1 α are mediated, at least in part, through regulating the activity of MEF2 transcription factor, a known inducer of type I fibers [49].

We analyzed fiber-type composition in skeletal muscle of TWEAK-Tg and TWEAK-KO mice. A significant reduction in type I fibers with a concomitant increase in type II fibers was noticeable in both soleus and extensor digitorum longus muscles of TWEAK-Tg mice [36]. Furthermore, hind limb muscle of the founder TWEAK-Tg mice, which did not survive beyond neonatal age, appeared pale compared to more reddish hind limb muscle of wild-type littermates [19]. By contrast, skeletal muscle of TWEAK-KO mice showed increased proportion of type I fibers [36]. Moreover, progressive age-associated loss in skeletal muscle oxidative capacity was found to be rescued in TWEAK-KO mice [41].

Furthermore, subsarcolemmal and intermyofibrillar mitochondrial content was considerably increased in skeletal muscle of 5-month old TWEAK-KO mice compared to wild-type mice. Consistent with increased number of oxidative muscle fibers and mitochondria content, adult TWEAK-KO mice showed improved exercise capacity compared to wild-type mice [41]. One of the important mechanisms by which TWEAK causes slow-to-fast-type fiber transformation is through repressing PGC-1 α levels in skeletal muscle. Transcript levels of PGC-1 α and several other molecules involved in oxidative metabolism were considerably higher in skeletal muscle of 5-month old TWEAK-KO mice [41].

Treatment with TWEAK also reduced the expression of PGC-1 α and several mitochondrial genes and decreased mitochondrial respiratory capacity in cultured myotubes [41]. Consistent with these findings, TWEAK also represses PGC-1 α in cardiomyocytes through the activation of canonical NF- κ B pathway [50]. Furthermore, there are also reports that TNF- α , a structural homologue of TWEAK, inhibits PGC-1 α in skeletal muscle and other cell types [51, 52]. Taken together, these studies clearly suggest that the repression of PGC-1 α is an important mechanism by which TWEAK reduces mitochondrial content and oxidative phosphorylation capacity in skeletal muscle and in other cell types (Figure 3).

2.5. Epigenetic events regulate the expression of Fn14 in denervated skeletal muscle

The expression of Fn14 is a rate-limiting step in TWEAK-induced muscle atrophy. However, the underpinning mechanisms leading to the increased expression of Fn14 remain less understood. Previous *in silico* analysis has suggested that both human and mouse Fn14 promoters lack a typical TATA box which is generally required for the expression of mammalian genes. However, Fn14 promoter contains consensus sequence for many transcription factors which regulate gene expression in response to growth factors and cytokines [53]. Recent studies in our laboratory have shown that epigenetic regulators and MAPK signaling are involved in the inducible expression of Fn14 in denervated muscle (Tajrishi et al; unpublished observation). Both mouse and human Fn14 promoters contain a CpG island close to transcription start site. Fn14 promoter also contains multiple consensus DNA sequence for activator protein 1 (AP1) and specificity protein 1 (SP1) transcription factors. Our studies suggest that denervation causes hypomethylation at specific CpG sites in mouse Fn14 promoter leading to the expression of Fn14 in skeletal muscle. Coincidentally, the levels of DNA methyltransferase 3a (Dnmt3a) are repressed in denervated skeletal muscle of mice. The role of Dnmt3a in Fn14 expression has been validated by our findings that overexpression of Dnmt3a inhibits the expression of Fn14 and attenuates muscle atrophy upon denervation. Furthermore, we have found that denervation increases the activation of MAPK and AP1 and SP1 which stimulate the expression of Fn14 in skeletal muscle.

Alteration of the acetylation status of histones or other proteins through histone deacetylases (HDACs) is another key epigenetic mechanism that controls gene transcription and protein function [54]. Class II HDACs such as HDAC4 and HDAC5 mediate neurogenic muscle atrophy through augmenting the expression of muscle-specific E3 ubiquitin ligases such as MuRF1 [55]. Recently, it has been found that HDAC4 causes activation of AP1 through deacetylation and activation of a key MAP3 kinase, MEKK2 in denervated muscle [56]. We

have initial evidence that HDAC4 is involved in the increased expression of Fn14 in denervated muscle potentially through activation of MAPK signaling cascades (unpublished observation). These novel findings are suggestive that the complex epigenetic and signaling mechanisms are involved in the inducible expression of Fn14 in denervated muscle. Whether similar mechanisms also regulate the expression of Fn14 in other cell-types and in response to injury remain to be investigated.

3. TWEAK-Fn14: An emerging player in the promotion of skeletal muscle fibrosis

As discussed in the preceding sections, the TWEAK-Fn14 axis has emerged as pivotal player in skeletal muscle biology through its regulation of key pathways controlling myofiber mass, mitochondrial metabolism, and skeletal muscle regeneration. Current data also support a model in which the TWEAK-Fn14 pathway productively contributes to tissue repair and regeneration when Fn14 is transiently upregulated after acute tissue injury, and the array of TWEAK-Fn14 pathway-induced activities, including proinflammatory, angiogenic and fibrogenic responses, in addition to its effects on myofibers and their progenitors, are then appropriately orchestrated. However, when exaggerated and dysregulated in contexts of robust or chronic injury and disease, sustained TWEAK-Fn14 activation drives these responses towards a pathological remodeling process which leads to progressive tissue damage [3]. In chronically injured or diseased skeletal muscle, normal tissue is often progressively replaced by adipose and fibrotic tissue, which is essentially an excessive accumulation of extracellular matrix (ECM), resulting in functional impairment of the affected muscles [2]. In this section, we synthesize recent advances supporting this model based on the experimental study of skeletal muscle, with a particular focus on emerging evidence for TWEAK-Fn14 as a driver of skeletal muscle fibrosis, and the underlying mechanisms whereby it mediates this outcome.

Tissue injury triggers the response of injured tissue resident cell types to deal with the stress and damage. This tissue response occurs quickly after acute injury and involves a well-orchestrated series of events including the production of growth factors and cytokines that promote cell survival and growth and recruit inflammatory cells to the site of injury. Importantly, the latter act both to remove cellular and foreign debris and activate progenitor cells, thereby promoting tissue repair and regeneration. Also featured are the fibrogenic responses, involving the activation of fibroblasts to produce new and temporary ECM to stabilize and guide the remodeling of the tissue. Skeletal muscle has a particularly remarkable ability to regenerate after acute insult. However, robust acute, repetitious, or chronic insult perturbs the finely regulated orchestration of these events, posing a challenge for normal repair and regeneration. Thus pathological outcomes may ensue including functional tissue loss due to cell death, degeneration, atrophy, metabolic dysfunction, and tissue fibrosis. The latter entails excessive fibrogenic and impeded progenitor cell responses tipping the balance to myofibroblasts, the major collagen-producing cell type in injured tissue, and the excessive accumulation of ECM replacing the lost tissue. Skeletal muscle fibrosis is a common pathological outcome of skeletal muscle injury and disease that

impedes skeletal muscle regeneration and compromises muscle function, and is therefore a significant medical issue [2, 57].

Several lines of evidence support a role for the TWEAK-Fn14 pathway in regulating the response of skeletal muscle to injury and disease. Firstly, the expression of Fn14 is highly induced locally in many contexts of skeletal muscle injury, including denervation, immobilization, starvation, and cardiotoxin-induced injury in mice [24, 36, 58]. Importantly, this pattern of Fn14 upregulation in injury and disease is conserved in humans, as reported in various forms of inflammatory myositis [14] and young patients with Duchenne muscular dystrophy (GEO dataset: GSE6011). Further analysis of emerging expression profiling datasets is needed to expand our knowledge in this regard. It is also notable that at the molecular level, Fn14 is induced by a host of mediators featured in tissue injury and fibrosis, such as fibroblast growth factor 1 (FGF1), platelet-derived growth factor (PDGF)-BB, transforming growth factor- β 1 (TGF- β 1), interleukin-1 β (IL-1 β), endothelin-1 (ET-1), angiotensin II and thrombin [4, 59, 60]. In addition, supporting a beneficial, physiological role for this pathway in the repair and regeneration of acutely injured skeletal muscle, Fn14-KO mice exhibit reduced inflammation and delayed regeneration kinetics [11]. This paradigm is also illustrated in the context of acute liver injury, where Fn14-KO mice exhibit delayed progenitor cell expansion along with reduced inflammatory and fibrogenic responses, and was recently reviewed in detail [3]. More recently, the pathophysiological role of the TWEAK-Fn14 pathway in skeletal muscle, including its promotion of fibrosis, has been validated in denervated skeletal muscle and after cardiotoxin-induced injury in mice [24, 36]. This role was demonstrated using both muscle-specific TWEAK-Tg mice and loss of function approaches employing TWEAK-KO mice or wild-type mice exposed to TWEAK blocking monoclonal antibodies (mAbs) as summarized in Table 1. These results complement the data supporting TWEAK-Fn14 as a pathophysiological, profibrotic mediator emerging in other organs systems [3, 61].

There are multiple mechanisms whereby TWEAK-Fn14-mediated signaling may promote skeletal muscle fibrosis, including through its regulation of mature skeletal muscle fibers, chronic tissue inflammation, myofibroblasts and their precursors, tissue enzymes balancing ECM accumulation, and progenitor cell fate. The role of TWEAK-Fn14 in these underlying mechanisms is discussed as follows.

The TWEAK-Fn14 pathway's profound effect on mature skeletal muscle loss through myofiber atrophy (reviewed in section 2.3) is accompanied by increased fibrosis in the muscle-specific TWEAK Tg mice after denervation, or simply with age [36]. In addition, the regeneration myofibers were smaller in diameter, and fibrosis increased in TWEAK-Tg mice after cardiotoxin injury [24]. Conversely, skeletal muscle atrophy was reduced in TWEAK-KO mice after denervation or starvation, and in denervated mice treated with a TWEAK-blocking monoclonal antibody (mAb), and fibrosis correspondingly reduced where studied in the denervation and cardiotoxin injury models [24, 36, 37]. Interestingly, while TWEAK-Fn14 pathway has been shown to contribute to fibrogenic response after tissue injury in other tissues by directly inducing death of various primary cells types, there is so far no evidence pointing to TWEAK-Fn14 induced mature skeletal myofiber death. Rather, TWEAK-Fn14 mediated skeletal myofiber atrophy appears to form the basis for reduced

skeletal muscle mass post injury and the subsequent replacement of the incurred tissue space loss by ECM. Since the tissue environment of skeletal muscle after injury is a dynamic mixture of Fn14-responsive cell types including myofibers, supporting vascular and stromal cells, and all of their progenitors, as well as TWEAK-producing immune infiltrates, the TWEAK-Fn14 axis is perfectly positioned to further promote tissue fibrosis of the affected muscles by altering the crosstalks between these cell types in settings of robust and/or chronic injury. In addition to its effect on mature myofiber mass, recent studies have shown that the TWEAK-Fn14 regulates myofiber function by modulating mitochondrial content and oxidative phosphorylation capacity (reviewed in section 2.4). While this may normally be a means to adapt muscle to functional demands, its sustained and dysregulated modulation by TWEAK-Fn14 axis may promote myofiber metabolic dysfunction and stress. It is likely that this forms an additional cue for pathological tissue remodeling including fibrosis as with other stressed cell types that promote fibrotic pathways [62].

In addition to providing the potential cues for skeletal muscle fibrosis by effecting mature myofiber atrophy and stress, TWEAK-Fn14 signaling broadly activates proinflammatory pathways in many cell types and may thereby promote tissue fibrosis, a common pathological outcome of chronic inflammation [59, 60, 63, 64]. TWEAK-Fn14 induced proinflammatory activities include the production of cytokines and chemokines, angiogenesis, and the expression of vascular adhesion molecule and MMPs, all of which participate in orchestrating the inflammatory response locally within injured and diseased target tissues [3, 63, 65, 66]. With respect to skeletal muscle, TWEAK induces chemokine and adhesion molecule gene expression in mesenchymal lineage progenitors, including human bone marrow mesenchymal stem cells and C2C12 myoblasts [11]. Some contexts of skeletal muscle stress and injury, such as those resulting in inactivity, generally do not involve a noticeable inflammatory response. However, in the model of cardiotoxin-induced injury, TWEAK overexpression in muscle-specific Tg mice exaggerates the inflammatory response in the injured myofibers. Conversely, chemokine expression and macrophage infiltration was delayed in Fn14-KO mice, and cytokine and chemokine responses reduced TWEAK-KO mice [11, 24]. Thus the TWEAK-Fn14 axis may contribute to progressive tissue fibrosis through its promotion of sustained inflammation in chronic injury and disease settings. Macrophages are a major cell type recruited to injured and diseased tissue and a heterogeneous population of cells, with apparently distinct activities that are normally controlled with respect to kinetics, duration and intensity for productive wound healing. However, if imbalanced, unrestrained wound healing and fibrosis result and this is most commonly associated with an M2 macrophage subset [60]. Interestingly, recently it was shown that TWEAK is more highly expressed in macrophages of the M2 type [64]. In addition to macrophages, functionally dichotomy in the T helper (Th) cell responses plays into the outcome in chronic tissue injury and disease, with CD4⁺ Th2-type cells tipping the balance to profibrotic pathways. Recently it was shown that TWEAK collaborates with Interleukin-13 (IL-13), a key Th2-type profibrotic cytokine, to promote Th2 type adaptive immunity and fibrosis in a model of chronic intestinal injury [67]. Altogether, these studies suggest the TWEAK-Fn14 axis may promote fibrosis by chronically perturbing the nature of the immune response in injured and diseased tissues, including skeletal muscle.

Recent evidence supports that the TWEAK-Fn14 promotes fibrogenic activities through its direct action on stromal cell types, and thereby the accumulation of myofibroblasts, the major collagen-producing cell type in injured and fibrotic tissues. Myofibroblasts may derive from multiple cellular sources including stromal cells [62] and are defined by the de novo expression of α -smooth muscle actin (α -SMA) and its incorporation into organized actin stress fibers. For example, several groups have shown that TWEAK promotes the proliferation of fibroblasts [67–70], and one group demonstrated that TWEAK signaling through Fn14 directly promoted their differentiation to myofibroblasts [71] providing the basis for the reduced fibroblast proliferation observed in Fn14-KO mice in the pulmonary arterial banding-induced model of cardiac fibrosis of the right ventricle [71] and reduced numbers of myofibroblasts and proliferating myofibroblasts in the TWEAK-KO mice after persistent kidney injury in experimental unilateral ureteral obstruction model [70]. Thus, the ability of the TWEAK-Fn14 pathway to directly promote fibroblast expansion is likely a general mechanism whereby TWEAK-Fn14 pathway promotes fibrosis, irrespective of the injury-type or -target tissue. Notably, TWEAK also cooperates with the key fibrotic cytokines, namely IL-13 and TGF- β 1 to promote Fn14-dependent fibroblast proliferation as shown with cultured mouse embryonic fibroblasts [67] and therefore may engage these collaborative pathways depending on the fibrotic tissue milieu.

Although these mechanisms have not been studied in the context of skeletal muscle, muscle is intimately linked to connective tissue stromal cells, including fibroblasts, adipocytes, and fibro/adipogenic progenitors (FAP), bipotential cells that can become either fibroblasts or adipocytes. These cell types play important roles in muscle regeneration but if unchecked contribute to fibrosis [72]. Since Fn14 is expressed on all mesenchymal lineage cells examined so far and can potently inhibit their terminal differentiation in vitro [11], it would be of great interest to examine how TWEAK/Fn14 signaling may influence their fate decision in vivo, especially for bipotential FAP cells. In addition, the Fn14 expressing myoblasts can also differentiate into myofibroblastic cells in response to TGF- β [73] and it would be interesting to determine if TWEAK will modulate TGF- β -induced transdifferentiation of these cells, as there have been conflicting reports on whether TWEAK synergizes or antagonizes TGF- β -induced activities in cultured fibroblasts [74, 75].

The TWEAK-Fn14 pathway has also been shown to directly regulate collagen gene expression, a key ECM type that excessively accumulates in fibrotic tissue. Excessive accumulation of collagen followed by collagen crosslinking increased tissue stiffness, thereby impeding organ function. TWEAK-induced collagen expression was shown using reporter assays for the Col1a1 and Col1a2 genes [69] and by the increased mRNA for several collagen types in TWEAK-treated 3T3 fibroblasts (our unpublished observation). Contrary to these observations, however, TWEAK-Fn14 signaling decreased collagen and fibronectin protein levels in cultured renal fibroblasts [70]. These apparently disparate results may depend on the culture conditions and nature of the fibroblasts used in each study. Presently, it is not clear how they relate to the physiological situation. Regardless, the net effect of the TWEAK-Fn14 pathway appears to increase fibrosis in vivo. This was shown in the denervated skeletal muscle model, where Col1a2 levels were increased in muscle-specific TWEAK Tg mice and decreased in TWEAK-KO mice, mirroring the level of skeletal muscle fibrosis [36]. Likewise, myofibroblast numbers and collagen

accumulation in TWEAK-KO mice in the renal injury model [70], α -SMA, collagen genes, and ECM deposition were all reduced in Fn14-KO mice in the cardiac fibrosis model, and α -SMA expression reduced in wild-type mice treated with a TWEAK blocking mAb in a model of liver injury with fibrosis [69, 76].

Beyond ECM gene expression, the TWEAK-Fn14 axis regulates the expression of tissue enzymes that balance ECM deposition and degradation in tissues. The accumulation of ECM is normally tightly regulated by the expression and activity of a variety of proteases and their specific inhibitors. As in other tissues, muscle-associated proteolytic molecules include the family of MMPs that degrade collagens and other noncollagenous substrates, and their inhibitors, TIMPs, as well as components of the plasminogen activator (PA) system. Appropriate regulation of these enzymatic systems is crucial for the required migration of tissue resident cell types and infiltrating leukocytes, release of ECM-associated cytokines and growth factors, activation of myogenic programs, and appropriate ECM deposition for productive tissue repair. Thus excessive ECM deposition, the very definition of tissue fibrosis, can result from imbalances in these systems [60, 62]. Numerous studies have demonstrated that the TWEAK-Fn14 pathway regulates the expression of MMPs and TIMPs, including in injured skeletal muscle. TWEAK promotes MMP-9 mRNA and protein expression in skeletal muscle cells and tissues and induced MMP-9-dependent myopathy in mice, with degradation of the skeletal muscle basement membrane, inflammation, myofiber atrophy and necrosis [66]. Furthermore, increased MMP-9 expression correlates with the increased skeletal muscle fibrosis in muscle-specific TWEAK Tg mice after cardiotoxin-induced injury [24], and with the increased fibrosis in these mice and reduced fibrosis in TWEAK-KO mice after denervation [36]. On this protease inhibitor side, although not studied in the context of skeletal muscle, TWEAK increased the expression of TIMP-1 and -2 without affecting MMP-2 or 9 in a model of liver injury. In addition, recently it was shown that TWEAK upregulates the expression of plasminogen activator inhibitor (PAI-1), an inhibitor of the PA proteolytic system, in vascular smooth muscle cells [77]. Thus TWEAK-Fn14-induced changes in ECM proteases and their inhibitors may shift the balance to excessive ECM deposition, and thereby be a mechanism contributing to skeletal muscle fibrosis.

Finally, the ability of TWEAK-Fn14 pathway to regulate the fate of myogenic progenitor cells (reviewed in section 2.1) may also have potential implications in the fibrotic response in chronically injured/diseased skeletal muscle tissues. Current results support a model in which TWEAK signaling through acutely induced Fn14 promotes the expansion of endogenous tissue progenitor cells that differentiate and regenerate normal tissue as Fn14 expression subsides to basal levels. However, since TWEAK signaling through sustained Fn14 expression in settings of repeated insult or chronic disease may impede endogenous tissue progenitor cell differentiation, as supported by TWEAK's inhibition of myotube formation in human mesangioblast cultures from sIBM patients [14], this would likely shift the balance to expanded but undifferentiated populations of tissue progenitor cells and the accumulation of myofibroblasts, and thereby the replacement of normal tissue with scar tissue. However, few studies have thus far addressed the impact on tissue progenitor cells when TWEAK-Fn14 pathway engagement is prolonged and thereby dysregulated in severely or chronically injured tissues. Interestingly, in a model of skeletal muscle injury

induced by cardiotoxin, muscle-specific TWEAK Tg mice exhibited reduced muscle regeneration and increased fibrosis, and conversely, TWEAK-KO mice showed increased skeletal muscle regeneration and reduced fibrosis [24]. Thus the TWEAK-Fn14 pathway may promote fibrosis by impeding normal tissue regeneration programs.

4. Conclusions

The understanding of the role and mechanisms of activation of TWEAK-Fn14 system in skeletal muscle development, atrophy, metabolism, and regeneration has taken a quantum leap in recent years. Studies summarized above indicate that elevated levels of TWEAK causes many deleterious effects on skeletal muscle. Furthermore, emerging evidence suggests that TWEAK is a major mediator of fibrosis not only in injured tissues but elevated levels of TWEAK is sufficient to cause fibrosis in naïve tissues. At the molecular level, significant progress has also been made on the differential signaling potential of the TWEAK-Fn14 axis, increasing appreciation of its complexity and calling for a more thorough investigation of its effects on various cell types, especially in the context of sustained TWEAK/Fn14 signaling so as to better understand its pathogenic roles in settings of chronic injury and disease. Taken together, these studies have highlighted the potential of inhibiting the TWEAK-Fn14 system as a novel therapeutic approach to improve tissue regeneration and to prevent the formation of scar after injury, such as in the skeletal muscle tissues.

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NON-standard Abbreviations

ALS	autophagy-lysosomal system
α-SMA	α-smooth muscle actin
Dnmt	DNA methyltransferase
ECM	extracellular matrix
Fn14	fibroblast growth factor-inducible 14
HDAC	histone deacetylase
IBM	inclusion-body myositis
KO	knockout
PGC-1α	peroxisome proliferator-activated receptor γ coactivator 1α
MEF2C	myocyte-specific enhancer factor 2C
MMP	matrix metalloproteinase
MyHC	myosin heavy chain
miR	micro RNA

NF-κB	nuclear factor-kappa B
PA	plasminogen activator
SP1	specificity protein 1
Tg	transgenic
TIMP	tissue inhibitors of metalloproteinase
TWEAK	TNF-like weak inducer of apoptosis
UPS	ubiquitin-proteasome system

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Biographies

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Dr. Timothy S. Zheng is currently a Director of Molecular Discovery in the Department of Immunology at Biogen Idec, Cambridge, MA. Dr. Zheng received his Ph.D. in the laboratory of Richard Flavell at Yale where he made important contributions to the role of mammalian caspases using genetically engineered mice, including demonstrating for the first time that mammalian caspases were indeed critically important for mediating apoptosis *in vivo*. Since joining Biogen in 1999, Dr. Zheng has focused his research activities on members of the TNF superfamily such as TWEAK/Fn14 and is particularly interested in how their activation and inactivation govern cell fate decision making process and how such knowledge can be applied to developing novel therapeutics for disease conditions with unmet medical needs such as autoimmunity and fibrosis. Dr. Zheng has published over 50 peer-reviewed original papers, reviews and book chapters.

Figure 1. Schematic diagram illustrating effect of TWEAK in different steps of myogenesis

Progenitor cells undergo myogenic commitment to produce myoblasts. TWEAK decreases self-renewal of progenitor cells and increases their progression in myogenic lineage resulting in formation of more myoblasts. Elevated levels of TWEAK inhibit differentiation of myoblasts. Whereas high amounts of TWEAK inhibits, low levels of TWEAK promote myoblast fusion. Upon injury and in disease conditions, TWEAK attenuates regeneration of damaged myofibers. Specific proteins expressed at various stages of myogenesis are shown in bottom boxes.

Figure 2. Mechanisms of action of TWEAK in myogenesis

Inhibition of myomiRs by TWEAK inhibits myogenesis. TWEAK activates canonical NF- κ B pathway resulting MyoD degradation which causes inhibition of myogenesis. TWEAK also activates non-canonical NF- κ B pathway to promote myoblast fusion. TWEAK inhibits satellite cell self-renewal (Pax⁺/MyoD⁻) through inhibiting Notch signaling pathway. myomiR, muscle-specific micro RNA.

Figure 3. Mechanisms of action of TWEAK-Fn14 system in skeletal muscle atrophy and fibrosis

Binding of trimeric TWEAK to Fn14 receptor leads to the recruitment of cIAPs and various TRAFs resulting in the activation of multiple downstream signaling cascades. TWEAK stimulates the NF- κ B pathway which causes translocation of NF- κ B into the nucleus and expression of several genes such as MuRF1 which activate UPS system. TWEAK also activates MAPK signaling cascades resulting in the activation of the AP1 transcription factor and gene expression. The activated genes also include cytokines, chemokines and MMPs which contribute to collagen remodeling and fibrosis. TWEAK-Fn14 system inhibits Akt signaling and thus the activation Foxo family transcription factors leading to proteolytic degradation through enhanced expression of MAFBx and MuRF1. TWEAK also induces the expression of the components of autophagy lysosomal system and activates caspases in cultured myotubes to induce atrophy. TWEAK inhibits the expression of PGC1 α which results in the inhibition of mitochondria biogenesis. TRAF, TNF receptor-associated factor; cIAP; cellular inhibitor of apoptosis; AP1, activator protein-1; FOXO, Forkhead box O.

TABLE 1

Effects of overexpression, deficiency or blockade of TWEAK/Fn14 pathway in murine models of skeletal muscle injury or disease featuring fibrosis.

Model	Muscle-specific TWEAK-Tg	KO mice or blocking mAb	Effect as compared to control mice	References
Naïve 6 month old mice	✓		<ul style="list-style-type: none"> • Muscle atrophy (reduced myofiber cross-sectional area) • Reduced % of Type 1 (slow-twitch, oxidative) myofibers • Increased levels of collagen fibers • Reduced mitochondria content 	[36, 40]
		TWEAK-KO	<ul style="list-style-type: none"> • Increased myofiber cross-sectional area • Increased % of Type 1 myofibers • Increased mitochondria content 	[36,41]
Denervation	✓		<ul style="list-style-type: none"> • Increased muscle atrophy • Increased fibrosis 	[36]
		Anti-TWEAK mAb or TWEAK-KO	<ul style="list-style-type: none"> • Reduced muscle atrophy • Reduced fibrosis • Preserved muscle absolute force production 	[36]
Starvation		TWEAK-KO	<ul style="list-style-type: none"> • Reduced muscle atrophy 	[37]
Cardiotoxin-injury	✓		<ul style="list-style-type: none"> • Reduced myofiber regeneration • Increased inflammation • Increased fibrosis 	[24]
Cardiotoxin-injury		Fn14-KO	<ul style="list-style-type: none"> • Reduced inflammation • Delayed regeneration kinetics 	[11]
Cardiotoxin-injury		TWEAK-KO	<ul style="list-style-type: none"> • Increased myofiber regeneration • Reduced inflammation • Reduced fibrosis 	[24]