



Getting the Skinny on Follistatin and Fat

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Obesity is a chronic disease that develops as a result of a long-term imbalance between energy intake and energy expenditure. Maintaining a healthy diet and staying physically active are proven strategies to prevent weight gain; however, the increasing incidence of obesity suggests an urgent demand to develop novel therapeutic strategies. White adipose tissue (WAT) and brown adipose tissue (BAT) are two distinct fat depots found in mammals (1). WAT consists of adipocytes with large unilocular lipid droplets that function to store excess energy as triglycerides and release fatty acids depending on organismal needs (2, 3). In contrast, BAT consists of multilocular adipocytes with large numbers of mitochondria that uncouple respiration through the expression of the BAT-enriched protein uncoupling protein 1 (UCP1) and contribute to nonshivering thermogenesis via energy dissipation and heat release (4, 5). Emerging evidence supports the existence of a third class of adipose cell, beige adipocytes (also called “brite” cells), that are present within WAT. These adipocytes can be stimulated to exhibit features reminiscent of brown adipose tissue in that they express UCP1 and possess thermogenic capacity (6). This so-called browning process (*i.e.*, converting adipocytes from white to beige) can be increased by exposure to cold or pharmacologic agents. Therefore, an exciting new strategy gaining traction to combat obesity is the purposeful induction of thermogenesis to enhance energy utilization (7–9).

It is important to note that the developmental origins of WAT and BAT are distinct. BAT and skeletal muscle are derived from Myf5⁺ precursors (10). In contrast, white adipocytes and beige cells are thought to originate primarily from Myf5⁻ precursors (3, 6), although it has been suggested that subsets of WAT can also originate

from Myf5⁺ precursors (11). The distinct developmental origins suggest that there are at least two general pathways that can be targeted for obesity. Thermogenesis can be increased by expanding the BAT progenitor cell pool and/or enhancing transdifferentiation of white adipose cells to beige cells (6, 12, 13).

Numerous signaling pathways have been implicated in the development and differentiation of white, beige, and brown fat (1). The transforming growth factor- β (TGF- β) serine/threonine kinase receptors and downstream Smad3 transcription factor pathway is one example (13). High TGF- β 1 levels are associated with obesity in mice and humans (13–15), whereas suppression of TGF- β /Smad3 signaling has been shown to promote a white to brown adipocyte transition by increasing mitochondrial biogenesis (16). Inhibition of TGF- β signaling promotes increased expression of BAT-specific and muscle-specific markers in white adipose tissue, suggesting that TGF- β signaling basally suppresses the differentiation of adipocytes at the progenitor level (13, 16).

Follistatin (Fst) is a secreted glycoprotein that functions to bind and inhibit the activity of members of the TGF- β superfamily and is a well-known promoter of skeletal muscle growth (13, 17, 18). Fst is widely expressed, and previous work has shown that Fst levels are induced during brown adipocyte differentiation in mice (19). Mouse embryonic fibroblast (MEF) cultures from follistatin knockout (Fst KO) mice were found to have decreased expression of genes involved in lipid and energy metabolism as well as BAT-specific markers compared with wild-type (WT) MEFs when undergoing adipogenic differentiation (19). Treatment of Fst KO MEFs with recombinant follistatin (rFst) was able to restore UCP1 gene and protein expression and increase

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Abbreviations: BAT, brown adipose tissue; ERK, extracellular signal-regulated kinase; Fst, follistatin; Fst KO, follistatin knockout; Fst-Tg, follistatin-transgenic; MAPK, mitogen-activated protein kinase; MEF, mouse embryonic fibroblast; rFst, recombinant follistatin; TGF- β , transforming growth factor- β ; UCP1, uncoupling protein 1; WAT, white adipose tissue; WT, wild-type.

mitochondrial respiration (19). In this issue of *Endocrinology*, Singh *et al.* (20) explore the *in vivo* actions of Fst on adipose tissue using follistatin-transgenic (Fst-Tg) mice and determine that Fst promotes the production of both beige and brown adipose tissue via distinct molecular mechanisms.

The mice used by Singh *et al.* (20) express Fst under a muscle-specific promoter. These mice have 1.5-fold greater circulating Fst levels compared with WT mice and exhibit increased muscle and BAT mass. The expression levels of BAT signature genes and proteins important for differentiation, mitochondrial biogenesis, and fatty acid oxidation were elevated in interscapular BAT and gonadal epididymal and inguinal subcutaneous WAT of Fst-Tg mice compared with WT mice (20). The brown adipose tissue marker UCP1 and beige adipocyte-specific marker CD137 were induced in both WAT depots, with greater changes observed in subcutaneous WAT compared with epididymal WAT. These depot differences are consistent with previous literature in which epididymal WAT was found to be less responsive to browning than subcutaneous WAT (21). Collectively, the findings implicate Fst as a browning agent for WAT (19, 20).

The authors demonstrate that the actions of Fst on WAT and BAT are mechanistically distinct. In epididymal and subcutaneous WAT, Fst promotes increased phosphorylation of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) 1/2 (Fig. 1) (20). Activation of p38 MAPK and increased UCP1 expression was observed in 3T3-L1 preadipocyte cells treated with rFst, whereas UCP1 levels were decreased when rFst was dosed in the presence of p38 MAPK and ERK inhibitors (20). In BAT and differentiated mouse BAT cells, Fst increased Myf5 protein expression (Fig. 2). Moreover, Myf5 knockdown abolished rFst-mediated increases in UCP1. Additionally, the authors show that rFst is able to rescue Myf5 gene and protein levels in Fst KO MEFs, reinforcing the importance of Myf5 expression downstream of Fst signaling.

TGF- β activates the noncanonical p38 MAPK pathway in addition to the canonical Smad pathway; thus, how these signals become selectively transduced by Fst remains unclear. One possibility may be that Fst acts as a selective inhibitor of the activin/Smad3 signaling cascade while sparing other TGF- β -activated pathways. Fst has high affinity for binding and inhibiting activin with lower affinities to other TGF- β superfamily ligands, including myostatin, inhibin, and bone morphogenic proteins (22, 23). Fst does not bind to TGF- β 1 and, therefore the p38 MAPK signaling arm is likely to stay intact even in the presence of Fst, which inhibits activin/Smad3 signaling (24, 25). Smad3 was previously found to be essential for TGF- β -mediated inhibition of myogenic transcription

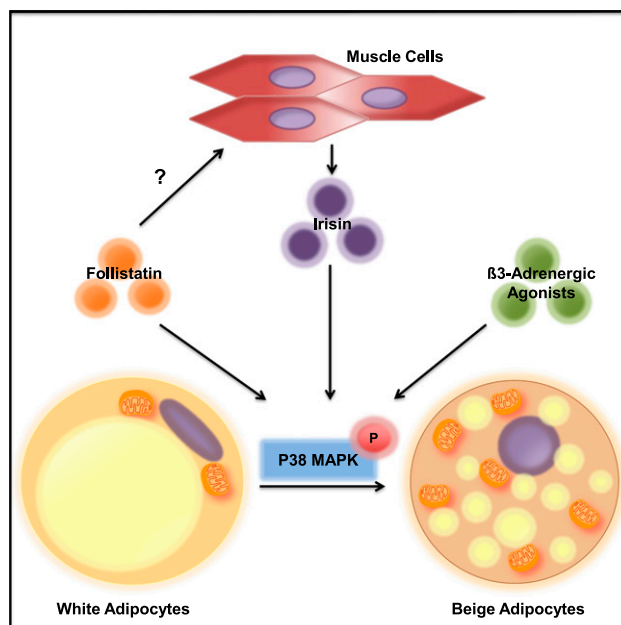


Figure 1. Schematic illustrating some pathways known to promote browning of white adipocytes into beige adipocytes through p38 MAPK signaling. β -Adrenergic agonists and secretion of irisin by skeletal muscle are two mechanisms that induce phosphorylation and activation of p38 MAPK signaling. Follistatin also induces phosphorylation and activation of the p38 MAPK pathway, leading to browning of WAT, as shown by Singh *et al.* (20). Evidence for the direct effect of follistatin on adipocytes is provided but does not preclude the possibility that indirect effects of follistatin—for example, via regulation of other factors such as irisin—may be contributing to p38 MAPK signaling *in vivo*.

factors, including Myf5 in skeletal muscle (26, 27). Given that BAT and skeletal muscle share the same Myf5-expressing progenitor cells, it can be proposed that Fst promotes BAT activation and muscle growth through the induction of Myf5 expression, resulting in expansion of the precursor cell pool (Fig. 2) (3, 9, 13, 20). Thus, Fst induces Myf5 expression in BAT/Myf5⁺ progenitors to promote classical BAT activation whereas Fst enhances the phosphorylation of p38 MAPK/ERK1/2 in WAT to promote browning (20).

The *in vitro* studies reported by Singh *et al.* (20) support a direct role for Fst in white adipocytes, but they note that this does not preclude the possibility that other extrinsic factors may be contributing to p38 MAPK activation by Fst *in vivo*. A potential candidate contributing to this phenomenon may be irisin, a myokine encoded by the *Fndc5* gene. Secretion of irisin by skeletal muscle in response to exercise was shown to induce phosphorylation of p38 MAPK/ERK1/2, leading to browning of WAT (28). Fst secretion is also increased following exercise (29, 30). Furthermore, a recent study found that TGF- β /Smad3 signaling suppresses *Fndc5* gene expression in skeletal muscle (31). In line with this, rFst treatment elevated *Fndc5* gene expression in muscle (29), and thus it remains to be determined to what degree Fst is

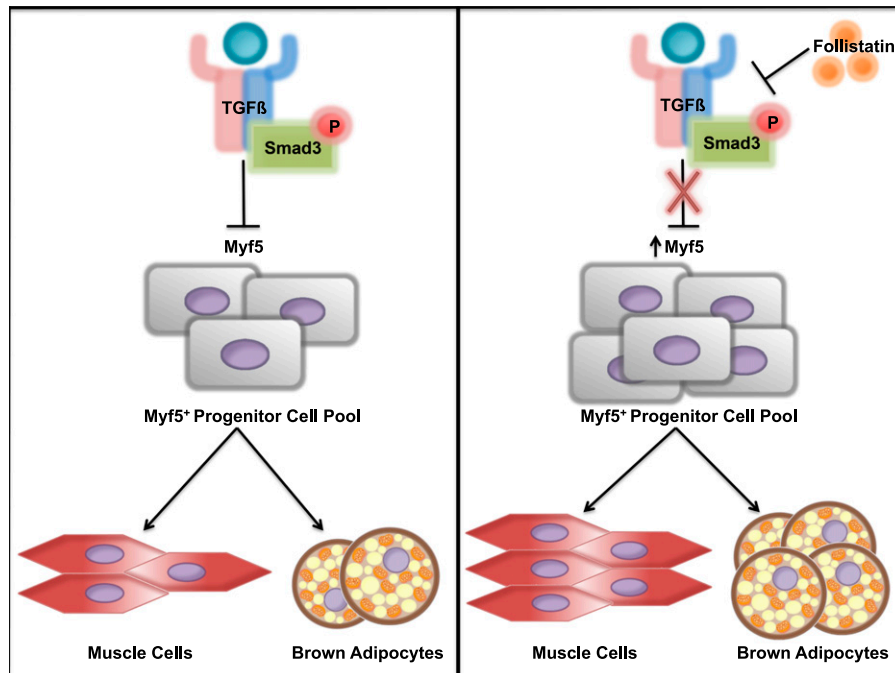


Figure 2. Schematic illustrating the effects of follistatin on TGF- β -mediated signaling in the Myf5⁺ progenitor cell pool. In the absence of follistatin, baseline activation of the TGF- β /Smad3 pathway represses Myf5 expression in progenitor cells and results in a basal level of muscle and brown adipocyte differentiation. In the presence of follistatin, the TGF- β /Smad3 pathway is inhibited, increasing Myf5 expression in progenitor cells, which promotes increased differentiation of these cells into skeletal muscle and brown adipocytes.

indirectly affecting p38 MAPK/ERK1/2 activation via irisin *in vivo*.

β 3-Adrenergic receptor signaling is a well-defined regulator of p38 MAPK activation that enhances browning in WAT and nonshivering thermogenesis in BAT (32–34). The authors tested whether treatment with the β 3 agonist CL 316,243 would enhance BAT activation and browning potential more in Fst-Tg mice compared with WT mice. Indeed, β 3-adrenergic activation in Fst-Tg mice was able to elicit an additive response in UCP1 levels in WAT and BAT compared with WT mice (20). Future studies could test whether cold exposure or exercise has the ability to exert additive or synergistic effects in Fst-Tg mice and whether the loss of Fst will diminish these responses.

Overall, the work by Singh *et al.* (20) is the first to demonstrate the action of Fst in targeting both browning of WAT and activation of classical BAT through distinct mechanisms. A growing body of evidence has identified Fst as a nonsteroidal anabolic agent to induce skeletal muscle development and growth (35). The data provided by Singh *et al.* (20) now add to the therapeutic potential of Fst by demonstrating that its actions extend directly to both beige and brown adipocytes and thus may find utility for the treatment of obesity.

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