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The Hippo Signaling Pathway in the Regulation of Skeletal Muscle Mass and Function

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Abstract

The Hippo signaling pathway regulates the activity of the proteins Yes-associated protein (Yap) and transcriptional co-activator with PDZ-binding motif (Taz) to control tissue growth in many different cell types. Previously, we demonstrated Yap is a critical regulator of skeletal muscle mass. We hypothesize that alterations in Yap and Taz activity modulate the anabolic adaptations of skeletal muscle to resistance exercise.

Summary:

Yes-associated protein (Yap) and transcriptional co-activator with PDZ-binding motif (Taz) may modulate resistance exercise-induced skeletal muscle hypertrophy.

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Keywords

Hippo signaling pathway; Yes-associated protein (Yap); Transcriptional co-activator with PDZ-binding motif (Taz); skeletal muscle; exercise-induced hypertrophy

Introduction

The integrative response of intracellular signaling pathways to changes in the external environment is essential for tissues to maintain homeostasis, and to adapt appropriately to stresses, including exercise (1, 2). While many of the critical signaling pathways that govern satellite cell activation, and skeletal muscle fiber growth have been explored in the context of exercise including the Transforming Growth Factor-Beta (Tgf- β), Mitogen-Activated Protein Kinase (Mapk) and Insulin-PI3K-Akt-mTOR signaling pathways, there remains an incomplete understanding of the underlying mechanisms that occur in tissues in this setting (2, 3). Recently, we and others demonstrated a role for key elements of the Hippo signaling pathway, an essential mediator of tissue growth in a number of epithelial cell types, during adult skeletal muscle fiber growth and atrophy (4–7). These findings significantly advance our understanding of the mechanisms underlying alterations in muscle mass, and extend the evidence supporting the Hippo pathway as a critical element in both muscle fibers and muscle satellite cells. However, the physiological stimuli that control the activity of this pathway in skeletal muscle remains unclear. Studies in non-muscle cells demonstrate that the Hippo pathway is influenced by mechanical, hormonal and metabolic stimuli (8). Given that these stimuli also play roles in muscle fiber anabolic signaling, and satellite cell activation in the adaptation of muscle to exercise (1, 9, 10), we hypothesise that the Hippo signaling pathway may play a crucial role in this setting, and that at least some elements of the pathway are likely responsive to, and effectors of, the hypertrophic adaptive response to resistance exercise. In this review, we will summarize: a) the key signaling elements that constitute the Hippo pathway and how these function to regulate the core effectors of this pathway, the transcriptional co-activators Yes-associated protein (Yap) and transcriptional co-activator with PDZ-binding motif (Taz), b) the current evidence of changes in Yap and Taz expression in skeletal muscle under various conditions, c), the evidence generated to date that supports a vital role for Yap and Taz in satellite cell activity and muscle fiber growth and, d) outline the potential significance of this pathway to exercise-induced hypertrophy. We will limit our discussion to the literature describing a role for elements of the Hippo pathway in satellite cells and muscle fibers unless otherwise stated.

The Hippo signaling pathway

The Hippo signaling pathway is a powerful regulator of biological function across species (8). While a number of proteins have been implicated as members of this diverse and highly redundant pathway, the precise elements that control Hippo signaling activity are context-dependent. These include diffusible molecules with significant roles in cell growth and adaptation, such as estrogen, insulin and lipids, changes in the mechanical properties of the extra-cellular matrix and actin cytoskeleton, and vital metabolic molecules, such as glucose (8, 11). Despite the context-dependent nature of these stimuli, most of these inputs converge

at a common level to activate or inhibit the core Hippo pathway kinases, Mammalian Ste-20 like kinase 1 and 2 (Mst1/2) and/or mitogen-activated protein kinase kinase kinases (Map4k) 1–4, 6 and 7 (11) (Fig 1). The activation of these kinases by phosphorylation is critical for their increased catalytic activity and, in the case of MST1/2, for the formation of a complex with the adaptor protein WW-domain containing 1 (Sav1) (12–15). When active, Map4k 1–4, 6 and 7 and/or Mst1/2 phosphorylates a C-terminal motif of the NDR family kinases Large Tumour Suppressor kinase 1 and 2 (Lats1/2) and/or the related kinases NDR1/2 (12, 14–16). Mst1/2 also binds the adaptor proteins Mob1A/1B (Mob) leading to phosphorylation of Mob on two N-terminal residues, Thr12 and Thr35. Mst1/2 and Map4k1–4, 6, 7 phosphorylation results in activation of Mob and a conformational change that favours Mob binding to Lats or NDR kinases (17). When induced, the Lats/MOB and/or NDR/MOB complexes suppress the activity of Yap and Taz, typically by phosphorylation at critical serine residues (Ser61, 109, 127, 164 and 381 in human YAP; Ser66, 89, 117 and 311 in human TAZ) (18, 19). In addition to direct inhibition by the core Hippo pathway kinases, Lats1/2 and NDR 1/2, the activity of Yap and Taz can be influenced by physical retention, disruption of protein-protein interactions and a number of post-translational modifications (8).

When active, Yap and Taz localise to the nucleus of the cell where they interact with the Tead family of transcription factors (Tead1–4) to control gene expression (18–20). While Yap and Taz can bind to other transcription factors, disrupting the interaction with Tead by mutation of a critical serine residue (Ser94 in human Yap/Ser89 in human Taz) is sufficient to account for the biological activity of Yap and Taz in most settings, suggesting that Teads are the primary interacting partners of Yap and Taz (20). Yap-Tead and Taz-Tead complexes bind mainly to enhancer regions and, to a lesser extent, the promoter regions of target genes, where they can influence transcription by activating, de-repressing or directly repressing target genes (21–24).

Yap and Taz expression in skeletal muscle

Yap protein is highly expressed in foetal mouse fast-twitch muscle and progressively declines throughout post-natal maturation (4). During this time, the inhibitory Lats1/2-mediated phosphorylation of Yap at Ser112 (equivalent to Ser127 in human YAP) followed the same pre- and post-natal pattern as total Yap protein, with no change in the phospho/total ratio over this period, suggesting that Hippo pathway activity also declines during this time (4). It has also been reported that the expression of the upstream Lats1/2 activator, Mst1, essentially follows a similar post-natal decrease as total Yap and Ser112 phosphorylation in mouse striated muscles (25). This suggests the possibility of a coordinated regulation of Yap and Mst1 levels, and Lats1/2 activation and the relatively tight control over Yap activation in skeletal muscle. These findings are consistent with recent reports demonstrating that Yap and Taz activity is controlled by negative feedback mechanisms through transcriptional control of upstream regulators, including Lats2 (26). Yap levels have also been demonstrated to increase during chronic mechanical overload-induced hypertrophy in mouse skeletal muscle (27), suggesting a possible anabolic role. Again, similar to the post-natal development period, there was also a parallel increase in Yap^{Ser112} phosphorylation levels (27). Further supporting a potential anabolic role for Yap, muscle hypertrophy induced by

inhibition of the Tgf- β ligands Myostatin and Activin was associated with an upregulation of total Yap and Yap^{Ser112} phosphorylation (28). Total Yap levels and Yap^{Ser112} phosphorylation were also upregulated in atrophic denervated muscle, and in a neurogenic atrophy model of amyotrophic lateral sclerosis, the SOD1^{G93A} mouse, but not in innervated muscles that had undergone tenotomy-induced atrophy (4). These data suggest that Yap activity is partially influenced, not under atrophic conditions *per se*, but as a consequence of a loss of the nerve-muscle interaction. Further, the levels of total Yap and Yap^{Ser122} phosphorylation are also elevated in the *mdx* mouse model of Duchenne muscular dystrophy, supporting a role for Yap in the setting of skeletal muscle regeneration (28). These findings are of particular interest given recent studies linking Hippo signalling to Agrin, an essential element regulating stability and organisation of the neuromuscular junction and the Dystroglycoprotein complex in cardiac tissue (29–31). Finally, very little is known about YAP expression in human skeletal muscle; however, a recent proteomics study using single-muscle fibers has reported that YAP protein expression in slow-twitch muscle fibers is ~ 2-fold higher than in fast-twitch 2A fibers from young subjects (32). In the same study, it was also shown that YAP is ~50% lower in both these muscle fiber types in aged subjects compared to younger controls (32). These data suggest possible fiber type-dependent differences in the regulation of YAP, and that a reduction in YAP could play a role in the age-dependent loss of skeletal muscle mass (i.e. sarcopenia).

Taz is detectable at the protein level in human and mouse myoblasts, and mouse skeletal muscle (33). Unlike Yap, Taz was unresponsive to acute and chronic Myostatin/Activin inhibition, and was unchanged in muscles from Myostatin knock-out mice (34). To date, no studies have investigated the mechanism underlying the regulation of Taz protein in human or mouse skeletal muscle under basal, or different hypertrophic or atrophic, conditions.

The functional role of Yap and Taz during satellite cell activation

The best studied role of Yap and Taz in skeletal muscle is as a regulator of myoblast proliferation and terminal differentiation; first demonstrated in the mouse myoblast cell line, C2C12 (35, 36). These observations have since been validated in both satellite cells derived from isolated mouse muscle fibers and in regenerating myoblasts *in vivo* (37–39). In sum, these studies collectively demonstrate that Yap and Taz activity are increased as satellite cells/myoblasts proliferate and that further increasing their activity, by over-expression of mutant Yap/Taz proteins that cannot be inhibited by Lats1/2, results in an enhanced rate of myoblast proliferation (33, 35, 37, 38). In addition, sustained Yap activation is sufficient to inhibit the terminal differentiation program necessary for the appropriate fusion and formation of myofibers (35, 38). Consequently, the sustained activation of Yap in this setting *in vivo* leads to the formation of embryonic Rhabdomyosarcoma-like tumours (38). Importantly, cessation of the transgene driving active Yap leads to a complete reversal of this phenotype, suggesting that the transient activation of the protein, either by physiological or pharmacological means, may enhance satellite cell proliferative dynamics, while still permitting the differentiation of the myoblast to support skeletal muscle growth and adaptation.

Interestingly, while Yap and Taz function in a similar manner in myoblasts to positively regulate proliferation, sustained activation of Taz actually enhances the terminal differentiation of the myoblast *in vitro* and *in vivo* (33, 36, 40). The underlying reasons for this difference in function are still unclear, although studies comparing Yap and Taz single gene knock-in/knock-out models suggest that the interacting proteins and target genes of these proteins differ (40). While such findings may explain these effects in part, studies comparing the effects of modulating Yap and Taz in isolation, and in combination, at physiologically relevant levels will be required to understand the true function and interaction network of these proteins in skeletal muscle during terminal differentiation.

Yap and Taz promote muscle growth via Tead in Post-natal muscle fibers

Consistent with its role during satellite cell/myoblast activation, Yap also functions as a positive regulator of post-natal muscle fiber mass (4, 5); however, the underlying mechanisms driving this effect remain elusive. When activated in the post-natal muscle fiber, Yap is sufficient to increase cell size, with no effect on cell number, and concurrent increases in the rates of protein synthesis (4). Yap promotes muscle mass via Tead transcription factors since mutation of the Tead binding domain in Yap, or the co-expression of a dominant negative Tead2 protein, prevented muscle hypertrophy (4). Recent chromatin immunoprecipitation-sequencing (ChIP-seq) studies in adult muscle fibers using Tead4 knock-out mouse models supports the described data and shows that Teads regulate the expression of muscle structural and identify genes (41). While the essential target genes responsible for the anabolic effects of Yap have not been identified, it appears that Yap and Tead stimulate protein synthesis and muscle fiber hypertrophy via a mechanism independent of rapamycin-dependent mammalian target of rapamycin (mTOR) signaling (4, 5).

In the post-natal muscle, Yap is required for the maintenance of muscle mass, since expression of an adeno-associated viral vector, encoding short hairpin RNA targeting endogenous Yap in the adult myofiber, lead to a reduction in muscle mass, protein synthesis and myofiber cross sectional area (4). In contrast, however, a recent study that crossed mice expressing human skeletal actin (HSA) promoter-driven CRE (causes recombinase protein) recombinase with mice in which Yap exons 1 and 2 are flanked by loxP sites to generate a skeletal muscle-specific Yap knockout model, reported no difference in muscle fiber size in the absence of Yap compared to controls (42). The reason for the different results of these studies remains to be determined but may be due to the timing/developmental stage of the YAP knockdown/knockout (i.e. embryonic muscle vs mature adult muscle), or to changes in food intake and activity in the tissue specific knockout model that would not influence the findings when comparing to a contralateral muscle within the same animal. Despite this, mice lacking skeletal muscle expression of Yap display reduced force generating capacity, due to alterations in the formation and regeneration of the neuromuscular junction, suggesting distinct consequences may occur upon loss of Yap in skeletal muscle depending on the development stage assessed (42).

The functional role of Taz in post-natal skeletal muscle fibers is less clear than Yap; however, it is likely that Taz also functions to promote muscle mass under certain conditions since pharmacological stimulation of Taz is sufficient to partially prevent muscle atrophy in

mice treated with the corticosteroid, dexamethasone, by stimulating protein synthesis (7). Consequently, the mechanisms regulating Yap and Taz activity in skeletal muscle, and how Yap and Taz responsive genes are altered in settings of growth, atrophy or adaptation remain to be fully explored.

While the upstream regulatory elements that control Yap/Taz activity remain unclear, the inhibition of Mst1 is also sufficient to prevent atrophy in denervated, fast-twitch muscles, suggesting that physiological stimuli that result in anabolic/catabolic responses in skeletal muscle would likely influence Yap and Taz via the canonical Hippo pathway kinases (6). Exploring the therapeutic potential of targeting the Hippo pathway kinases using recently developed Mst1/2 kinases inhibitors to activate Yap and Taz in such settings may therefore offer potential to treat such catabolic conditions (43).

Conclusions

Here, we present evidence supporting an important role for the Hippo signaling pathway in skeletal muscle cells. Given the significant role played by key elements of this pathway in the adaptation of muscle during regeneration, post-mitotic cell growth, and in settings of neuromuscular disease, we propose that modulation of the Hippo pathway effectors Yap and Taz may, in part, provide a mechanistic explanation for the hypertrophic effects of resistance exercise through changes in the rates of protein synthesis and satellite cell activity (Fig 2). Resistance exercise affects metabolic (e.g. glucose and AMP-kinase (AMPK)), hormonal (e.g. glucocorticoids and estrogen) and mechanical responsive elements (e.g. mechanotransduction and the dystroglycoprotein complex); all mediators of Yap and Taz activity in epithelial cells. We hypothesise that some, or all, of these inputs will also alter Yap and Taz activity in skeletal muscle during resistance exercise (Fig 2). Manipulation of the metabolic, hormonal or mechanical pathways engaged during this setting may provide insight into the mechanisms regulating Yap and Taz activity in skeletal muscle that could be exploited for therapeutic benefit in isolation, or combination, with exercise-based interventions.

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Key points

- The maintenance of skeletal muscle mass is controlled by a complex signaling network that functions in concert to maintain homeostasis.
- The Hippo signaling pathway controls skeletal muscle growth and wasting in a number of conditions.
- The activity of the Hippo signaling pathway is influenced by metabolic, mechanical and hormonal stimuli, all essential mechanisms underlying the hypertrophic adaptation to resistance exercise in skeletal muscle.
- The Hippo signaling pathway in skeletal muscle is likely response to, and required for, exercise-induced muscle fiber hypertrophy.

Fig 1. Schematic depicting the core elements of the Hippo signaling pathway in mammals. The core elements of the mammalian Hippo signaling consist of the Sterile-20 kinases mammalian Ste-20 like kinase 1 and 2 (Mst1/2) and mitogen-activated protein kinase kinase kinase kinases (Map4k) 1–4, 6 and 7 and the adaptor protein WW-domain containing 1 (Sav1). When active, these kinases phosphorylate the Large Tumour Suppressor kinase 1 and 2 (Lats1/2) and the related kinases NDR1/2 leading to association with the MOB1A/1B adaptor protein (Mob). Active Lats1/2 or NDR1/2 phosphorylate the transcriptional co-activators Yes-associated protein (Yap) and transcriptional co-activator with PDZ-binding motif (Taz) to limit their activity through cytoplasmic retention and protein degradation. When active, under conditions of low Hippo-activity, Yap and Taz bind the Tead transcription factors to regulate gene expression. Factors that limit growth are shown in white; factors that promote growth in grey.

Fig 2. Schematic depicting the proposed hypothesis that resistance exercise-induced adaptations may require the Hippo pathway effectors YAP and TAZ.

Resistance exercise activates mechanical (blue), hormonal (green) and metabolic (red) elements to induce anabolic responses in skeletal muscle. These diverse inputs have all been demonstrated to influence the phosphorylation and activity of the Hippo pathway core kinases, adaptor proteins and effectors (YAP and TAZ) in epithelial cells. We hypothesise that in skeletal muscle during resistance exercise, these external stimuli may act through the core Hippo pathway kinases (Mst1/2, Map4k1–4, 6, 7, Lats1/2 and NDR1/2), to regulate the activity of Yap, Taz and Tead to promote satellite cell activation, protein synthesis and myofiber growth. Factors that limit growth are shown in white; factors that promote growth in grey.

Abbreviations: Mammalian Ste-20 like kinase 1 and 2 (Mst1/2), mitogen-activated protein kinase kinase kinase (Map4k) 1–4, 6 and 7, Large Tumour Suppressor kinase 1 and 2 (Lats1/2) and the related kinases NDR1/2, WW-domain containing 1 (Sav1) and

MOB1A/1B (Mob), Yes-associated protein (Yap) and transcriptional co-activator with PDZ-binding motif (Taz).

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