Type I insulin-like growth factor receptor signaling in skeletal muscle regeneration and hypertrophy

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Abstract

Skeletal muscle is able not only to increase its mass as an adaptation to mechanical loading generated by and imposed upon muscle but also to regenerate after damage, via its intrinsic regulation of gene transcription. Both cellular processes, muscle regeneration and hypertrophy, are mediated by the activation, proliferation and differentiation of muscle satellite cells and appear to be modulated by the mitotic and myogenic activity of locally produced insulin-like growth factor 1 (IGF-1), which functions in an autocrine/paracrine mode. Differentiation of satellite cells into myoblasts involves the regulation of skeletal muscle-specific proteins belonging to the family of myogenic regulatory factors (MRFs). The endocrine, autocrine and paracrine functions of IGF-1 are mediated through binding to the type I IGF receptor (IGF-1.R), which is a ligand-activated receptor tyrosine kinase. The binding of IGF-1 to IGF-1.R induces its autophosphorylation, which recruits specific cytoplasmic molecules containing the Insulin Receptor Substrate Proteins (IRS). The recruitment of IRS proteins by IGF-1/IGF-1.R binding is a critical level at which the proliferative and differentiative actions of IGF-1 diverge. Specific signaling pathways downstream of IGF-1, potentially involved in the mitogenic and myogenic responses and mediating skeletal muscle protein synthesis and hypertrophy following exercise-induced muscle overloading and damage, are discussed. A potential alternative activation of different signaling pathway(s) via a different receptor remains to be demonstrated.

Keywords: IGF-1, Muscle Satellite Cells, Regeneration, Adaptation, Hypertrophy, Autocrine/Paracrine Actions

Introduction

Various activity models, such as increased loading, eccentric exercise (where the activated muscle is lengthened) or stretch, have been shown to lead to increased muscle protein synthesis and muscle growth or hypertrophy, whereas mostly eccentric (lengthening) muscle contractions result also in muscle damage¹⁻³. According to the popping sarcomere hypothesis proposed by Morgan⁴, lengthening of active muscle does not occur by uniform lengthening of all sarcomeres in muscle fiber, but by a non-uniform distribution of sarcomere length change, causing some weak sarcomeres to over-extend ("pop") beyond filament overlap, thus resulting in damage. An adaptation of skeletal muscle to lengthening contraction-induced muscle damage is an long-term shift of the length-tension curve⁵⁻⁶, which was proposed to be caused by the addition of sarcomeres in series in the damaged myofibres.

This suggestion is a corollary of the "overstretched" sarcomeres hypothesis described by Morgan⁴, who argued that an increase in the number of sarcomeres in series (sarcomere-ogenesis) would allow muscle fibres to operate at longer lengths in order to avoid the descending limb of the length-tension curve, which is the region of sarcomere length instability and damage. The overstretching effect of lengthening contractions and the consequent adaptation to an increased functional muscle length is known to be associated with increased muscle protein synthesis².

Muscle overload and muscle stretch are major factors in activating protein synthesis and hypertrophy, and their combination, as it occurs in lengthening contractions, has an addi-
tive effect. It has been suggested that increases in muscle size and the hypertrophic response may be regulated mainly at the level of gene translation rather than at the level of gene transcription. It could probably be a result of a rise in translational efficiency due to an increase in synthesis rate per unit RNA or in the number of ribosomes. The increased mass of the activated adult skeletal muscle, as an adaptation to overloading, is achieved mainly by an increase in the volume of the individual myofibres. Because skeletal muscle is a post mitotic tissue, the enlarged myofibres expand only with the addition of nuclei from mitotically active cells, so as to a constant ratio of nuclei to cytoplasmic volume to be maintained during the hypertrophic response. The subsequent increased rate of protein synthesis enables new contractile filaments to be added to the pre-existing muscle fibres. New myotubes are accompanied by accumulation of actin and formation of nuclear rings within the body of hypertrophic myofibres, indicating a re-organization of cytoskeleton.

In addition, myofiber regeneration following local muscle damage involves a dynamic restructuring of the muscle’s intermediate filament system (3-7 days following damage), the formation of new multinucleated myotubes, which begin to be formed 3-4 days after damage co-instantaneously with the appearance of fibres expressing embryonic myosin heavy chain (3-10 days following damage), and consequently the replacement of the damaged muscle fibres.

Myofiber regeneration and hypertrophy processes are both dependent on the proliferative activation of satellite cells and their subsequent myogenic differentiation into myoblast-like cells. These potentials of satellite cells enable regeneration and hypertrophy of existing myofibres or the generation of new myofibres.

Skeletal muscle regeneration and adaptation

Skeletal muscle fibres are characterized by high plasticity that can constantly adapt to the external stimuli, such as mechanical overload or damage, by changing their size, length and qualities. This cellular level of adaptation could simply be explained by regulatory mechanisms that should reside in the adapted muscle, since in the case of a central mechanism-induced adaptation of a specific muscle, it would require a downregulation of the non-target tissues response to the central (e.g., circulating) signal, so as only the impact muscle to adapt. As it was mentioned above, the contractile system of muscle fibres sustains damage during lengthening contractions and muscle fibres have to obtain extra nuclei for supporting the repair process, early enough to avoid cell death and the subsequent functional deficit of the muscle.

Damaged muscle fibres constitute the site of mobilization of the small, mononucleated satellite cells, which are located between the basal lamina of the muscle and the sarcolemma of myofibres. Their study became much simpler after the discovery of proteins such as M-cadherin, that expressed only by satellite cells and not by myonuclei in postmitotic myofibres. Their study was also facilitated by the development of new methods of preparing viable isolated myofibres with their attendant satellite cells and no other cell type present. Satellite cells near the injury site are impelled to proliferate and migrate, whereas there is evidence that they approach the injury from other sites within the muscle, even from contiguous muscles. Based on functional evidences, satellite cells appear to be a heterogeneous population.

Following skeletal muscle damage, some activated satellite cells do not replicate prior to fusion with damaged muscle fibres and it has been hypothesized to be more committed precursor cells. Others proliferate prior to differentiating into myoblasts or giving rise to more satellite cells. Finally, there is a small sub-population of stem cell-like muscle precursor cells that are radiation resistant, they are awakened in response to extreme trauma and are thought to be a deeply quiescent satellite cells. Satellite cells are the main, if not the only, cell type that contributes to muscle regeneration. It has been reported that muscle regeneration could also occur by other cell types (e.g., bone marrow cells and hematopoietic stem cells) or by de-differentiation of mature muscle fibres in some amphibian species; however, it remains an open question if such de-differentiation could occur in damaged mammalian skeletal muscle in vivo. Moreover, it was found that satellite cells could accomplish muscle regeneration without a need for a contribution from other cell types. Satellite cells begin the repair process by a proliferative response, in which some or all of the activated cells undergo multiple mitotic cycles. After this initial phase, some of the activated satellite cells or their progeny subsequently differentiate into myoblast-like cells, which, next, become incorporated with the damaged segments of myofibres, providing the extra set of genes required for the increased protein synthesis during the repair process. Alternatively, these differentiat-ed myoblast-like cells can fuse with the overloaded myofibres, providing extra nuclei for the adaptation process (hypertrophy), or they can fuse with each other to form multinucleated young muscle cells, the myotubes, which then undergo further differentiation and innervation before they form new, fully functional myofibres (hyperplasia). However, one of the most interesting issues to be resolved in muscle biology is the mechanisms involved in regulating the longitudinal growth of skeletal muscle (serial sarcomerogenesis) that occurs, e.g., because of chronic static stretch.

The mechanotransduction pathways mediating the different response of skeletal muscle to overloading or to chronic stretch, i.e., responses such as (a) the addition of new contractile filaments and sarcomeres in parallel as a hypertrophic response to overloading and (b) the addition of sarcomeres in series following chronic stretch], remain yet poorly understood. Currently, it is not known whether satellite cell activation is a pre-requisite for the longitudinal growth of skeletal muscle. The experimental elimination of the proliferating capacity of satellite cells, e.g., via irradiation, inhibits the regeneration and hypertrophy process, although there is evidence suggesting that muscle hypertrophy can proceed, at least to a certain extent, by the myonuclei transcriptions.
regulation without satellite cell proliferation41.

Activation of satellite cells occurs under regenerating and adapting conditions in response to diverse stimuli, such as skeletal muscle overloading, stretching or injury45. Repair process following skeletal muscle damage involves fiber degeneration and a subsequent influx of leukocytes into the site of damage46. Then, regeneration begins after the inflammatory cells have cleared necrotic tissue. Muscle regeneration is completed successfully, when the infiltration of inflammatory cells is followed by muscle repair and growth, which processes involve activation, proliferation and terminal differentiation of satellite cells49. Co-ordination between inflammation and regeneration is crucial for muscle recovery following damage. Tissue regeneration depends on a balance between pro-inflammatory and anti-inflammatory factors that determine whether the damage will be resolved with muscle cell replacement or with scar formation47,48. Insulin-like growth factor 1 (IGF-1, more specifically IGF-1 Ea isoform), reduces the chronic inflammatory response and promotes muscle regeneration and repair40. The molecular mechanisms involved in the regulation of satellite cells activation include the inflammatory response and the release of certain growth factors. Although the actual stimulus for satellite cells activation in vivo has to be defined, however, leukocytes, polymorphonuclear lymphocytes, macrophages, interleukin 6 (IL-6) and several growth factors have been implicated in the activation of satellite cells (for review, see41). Nevertheless, there is a growing interest in the literature in the specific importance of IGF-1-induced actions on skeletal muscle satellite cells, as a potential mediator of both the skeletal muscle regeneration and hypertrophy process46. Vector-mediated49 or transgenic40 overexpression of IGF-1 in skeletal muscle appears to stimulate muscle regeneration and hypertrophy via the activation of satellite cells. Locally produced IGF-1 was found to increase following acute skeletal muscle damage, the abundance of MRFs increases markedly31,60. Because MRFs’ increased expression temporally coincide with satellite cell proliferation, it was hypothesized that this increase occurs by satellite cells41. However, MRFs increases have been found also at a time before satellite proliferation would have been expected59,62,63, which typically occurs 24-48 hours following the physiological stimulus, e.g., skeletal muscle overload or damage57,64.

Besides, it was found that satellite cells proliferation, which had been eliminated by irradiation, was not a pre-requisite for MRF mRNA expression and for the acute muscle hypertrophy following stretch-overloading of guaia’s skeletal muscles and that satellite cells contribute only to myogenin mRNA increases, following 3 days of stretch-overload44. These observations indicated that newly formed myotubes or myonuclei also increase MRFs following the physiological stimulus. Besides, the contribution of satellite cell proliferation in the strength recovery after eccentric contraction-induced muscle injury was found to be approximately half of the total65. The authors attributed the satellite cells-dependent portion of strength recovery to the restoration of the contractile proteins, which requires satellite cell proliferation and fusion with the injured myofibrils.

The expression program of MRFs during satellite cell activation, proliferation and differentiation involves the rapid upregulation of MyoD, already within 0 to 12 hours following muscle overloading or injury, i.e., before the initiation of satellite cell proliferation31,32,66. Within 24 hours of satellite cell activation, 98% of them contain MyoD77. MyoD is associated with cell determination and is often found to be highly expressed in actively proliferating myoblasts, whereas it may be critical for proliferation following skeletal muscle damage31,68. It has been reported that MyoD is only observed in cells that already are expressing Myf529. Typically, either Myf5 or MyoD is expressed first, followed by co-expression of Myf5 and MyoD. These two skeletal muscle-specific transcription factors are key factors for the acquisition of myogenic identity and are rapidly upregulated in activated satellite cells and myoblasts66,67. Following the synchronous activation of myogenic regulatory factor(s) and satellite cells, their daughter myoblasts proliferate with a doubling time of 16-18 hours in culture29 or of 18-21 hours during skeletal muscle regeneration27. Previous studies on satellite cells cultured on the surface of their parent myofiber have shown that satellite cells expressed markers of terminal differentiation by 2-3 days in culture27, whereas there are reports that
dividing myoblasts were still detected 96-120 hours following muscle damage, suggesting a potential prolongation of the proliferation phase, with up to five cell divisions without differentiating. Myogenin and MRF4 are expressed following satellite cell proliferation and during their differentiation program. Myogenin appears to be expressed last, during the committed stages of satellite cell differentiation and fusion. Nevertheless, there is evidence for a different expression profile of myogenin and MRF4 and an earlier response (within 2 to 24 hours) following resistance exercise of human skeletal muscle, or within 3-48 hours following eccentric or isometric resistance exercise in rats.

In an attempt to elucidate the signaling pathways that activate the myogenic differentiation of satellite cells, the pivotal role of endogenously derived factors regulated by shear or stress forces have been highlighted. Many growth factors are involved in the chemotaxis, proliferation and differentiation of satellite cells. Among them, IGF-1 intracellular and extracellular signaling especially has been an interesting context in muscle biology, since IGF-1 has been implicated in promotion of chemotaxis of satellite cells and induces both proliferation and differentiation of myoblasts and satellite cells. Moreover, it has been proposed that these cellular processes of proliferation and differentiation, and the increased protein synthesis required for muscle repair or hypertrophic adaptation, are regulated by a differential expression and by the distinct roles of IGF-1 isoforms via IGF-1 signal transduction pathways.

**IGF-1.R signal transduction pathways in skeletal muscle pathophysiology**

The endocrine, autocrine and paracrine functions of IGF-1 are mediated through binding to the IGF-1 receptor (IGF-1.R), which is a ligand-activated receptor tyrosine kinase. From the two characterized IGF receptors (IGF-R), type 1 and type 2, IGF-1.R binds IGF-1 with high affinity and with lower affinity binds IGF-2 and insulin. Therefore, it appears that IGF-1 acts via IGF-1.R and the IGF-1.R-activated intracellular processes can affect cell proliferation, apoptosis and differentiation (for review see excellent work by Denley et al.).

It is well documented that various types of exercise induce increases in IGF-1, IGF-1 receptors and IGF-1-activated signaling pathways in skeletal muscles. More specifically, increased IGF-1.R mRNA and protein levels have been found in response to both acute aerobic and chronic resistance or endurance exercise, attributed to an increase in IGF-1.R transcription or to changes in IGF-1 receptor mRNA and/or protein stability. An increased IGF-1.R binding capacity has also been reported after a single acute bout of treadmill running or chronic activity, while different responses between acute and chronic exercise appear to be elicited in terms of IGF-1.R affinity, since only the chronic exercise led to a large increase in affinity. It is interesting to point out that these changes in IGF-1.R could be contributing to an enhanced IGF-1 action in skeletal muscle observed in aged animals after exercise. Although the aged muscle appears to be characterized by an IGF-1 insensitivity or by an impaired ability to upregulate IGF-1.R, these deficiencies can be reversed, since it has been shown that the old muscle retains a plasticity for IGF-1.R so as to restore its ability to respond to the anabolic actions of IGF-1.

IGF-1.R is a transmembrane protein consisting of two extracellular α-subunits, which contain the IGF-1 binding site, and two transmembrane β-subunits that have a cluster of three tyrosine residues, which undergo phosphorylation and so activation upon IGF binding (Figure 1). The binding of IGF-1 to IGF-1.R induces its autophosphorylation, which recruits specific cytoplasmic molecules containing the Insulin Receptor Substrate proteins (IRS). The recruitment of IRS proteins by IGF-1/IGF-1.R binding is a critical level at which the proliferative and differentiative actions of IGF-1 diverge (see review). More specifically, from the six members of the IRS family (IRS-1 – IRS-6), which appear to be expressed differentially in varying tissues and to play a more specific and distinct but potentially overlapping role in signal transduction, IRS-1 serves as a mediator and docking protein for other downstream signaling molecules in skeletal muscle (Figure 1). In studies involving muscle cell lines and satellite cell cultures, it has been found that activation of the IGF-1.R initiates intracellular signaling cascades involved in mitogenic and myogenic responses. Depending on timing and intracellular conditions, IGF-1 can stimulate both cellular proliferation and differentiation.

Only recently a considerable amount of progress has been made in the understanding of the specific signaling pathways downstream of IGF-1, mediating skeletal muscle protein synthesis and hypertrophy (Figure 1). Two primary signal pathways have been proposed to be associated with the activation of the IGF-1.R. One pathway, activated by IGF-1, involves phosphorylation of IRS-1 leading to the activation of phosphatidylinositol 3-kinase (PI3K). PI3K activation is involved in cellular processes such as protection from apoptosis via AKT activation and an alteration in intracellular calcium levels via the inositol phosphate cascade, whereas it is also involved in increased translation. The initiation of translation occurs via alterations in the phosphorylation state of the p70 S6 kinase and of eukaryotic initiation factor 4 binding protein.

Anabolic effects, such as enhancement of the translation of mRNA ribosomal proteins and elongation factors, which are integral factors of the protein synthesis, are associated with p70 S6 kinase activation. Indeed, it seems that the hypertrophic response to muscle overloading is modulated by the activation of intracellular kinases that control the RNA transcription/translation rate. A strong positive relationship between activation of p70 S6 kinase and the long-term increase in muscle mass has been observed in rat skeletal muscle following resistance training. Moreover, portions of the PI3K activation signaling cascade appears to be critical for the differentiation of cultured muscle cell.
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Figure 1. Type I insulin-like growth factor receptor (IGF-1.R)-activated signaling pathways in skeletal muscle.
It should be recommended to evaluate all these findings in parallel with the proposed interactions, between IGF-1 and satellite cell activation, proliferation and differentiation and with the time course of such interactions during the processes of skeletal muscle regeneration and/or the hypertrophic adaptation (see subsection: Gene transcription during skeletal muscle regeneration and adaptation). The outcomes of the two pathways described above are based on complex interactions that require comprehensive identification. In some cell types, ERK and PI3K appear to act in concert, e.g., both ERK and PI3K are possibly required for the differentiation of myoblasts. Nevertheless, the cellular proliferation and differentiation processes are thought to be mutually exclusive, and there are studies reported that activity of one pathway might inactivate portions of the other.

Another signaling pathway that has been implicated in differentiation and hypertrophy processes of myocytes is the calcium-dependent pathway of calcineurin. There is an increasing body of evidence that there are interactions between IGF-1 signaling pathways and calcineurin in skeletal muscle that co-ordinate myogenic differentiation of satellite cells. It has been demonstrated that IGF-1 induces the calcineurin-nuclear factor of activated T cells signaling pathway, resulting in the activation of a transcription factor (GATA-2) whose upregulation is associated with myofiber hypertrophy.

Apart from the myogenic and anabolic aspects of the IGF-1 signaling, this growth factor also appears to be associated with cellular calcium homeostasis and to modulate components of the excitation-contraction (E-C) coupling mechanism. Failure of this mechanism, along with contractile protein loss and physical disruption of muscle fibers, is the major component that contributes to the strength loss following muscle damage. L-type calcium channels in skeletal muscle include dihydropyridine receptors (DHPR) that detect depolarization in the transverse tubular system and activate directly the calcium release channels in the sarcoplasmic reticulum. The slow inward conduction of Ca\(^{2+}\) regulated by DHPR might affect the long-term intracellular calcium signaling. From both, in vitro and in vivo studies, it was shown that IGF-1 induces a significant increase in DHPR receptor concentration in skeletal muscle cells, indicating the potential impact of IGF-1 on the E-C coupling mechanism and also on the functional recovery of skeletal muscle following damage.

Moreover, the intracellular signaling pathways initiated by the activation of the IGF-1.R may interact with signaling via G-protein receptors or other mediators, modulating some responses. There are also reports that some cytokines may interact with intracellular IGF-1.R signaling. IGF-1.R signaling appears to be sensitive to inhibition by factors of pro-inflammatory cytokine intracellular signaling pathways. There have been reports that intense exercise could result in significant increases in circulating levels of pro-inflammatory cytokines, such as interleukin-6 and interleukin-1\(\beta\). Sensitivity of the skeletal muscle IGF-1 system to pro-inflammatory cytokines may have an additive effect to the anti-anabolic or catabolic action of these cytokines on skeletal muscle. Supporting the idea of the close interaction between IGF-1 and the inflammatory response, it was reported that chronic inflammation, indicated by the synthesis of interleukin-6, inhibits local skeletal IGF-1 expression leading to muscle functional impairment. Nevertheless, it has been shown that pro-inflammatory cytokines such as interleukin-6 and TNF-\(\alpha\) stimulate satellite cells or myoblast proliferation in vitro, suggesting a specific role of the inflammatory response to muscle regeneration and adaptation. Moreover, IGF-1 Ea (mIGF-1) isoform appears to reduce the chronic inflammatory response and promotes muscle regeneration and repair following muscle damage.

With respect to the concept of satellite cell activation and proliferation during muscle regeneration and adaptation processes, another potential regulation pathway has been also proposed. The cell cycle is regulated in part by the cyclin-dependent kinases, which in turn are regulated by the absence or presence of various cyclins and inhibitory proteins, such as p21 and Cyclin D1. Cyclin D1 has been reported as a generalized or non-skeletal muscle cell-specific marker of potential proliferation. An increase in the cyclin D1 mRNA has been reported in the overloaded skeletal muscles of rats and humans 12 hours post-exercise, but not 24 hours post-exercise. Cyclin D1 overexpression indicates that some cells within the muscle were becoming mitotically active in response to the exercise stimulus. The upregulation of cyclin D1 was thought to be mediated by mitogenic factors and it has been reported that IGF-1 may also play a role in the regulation of cyclin D1 levels.

Finally, it should be pointed out that, although skeletal muscle is characterized by abundant expression of the IGF-1.R, nevertheless, a potential alternative activation of different signaling pathway(s) via different receptor(s), proposed for IGF-1 isoforms specific actions, remains to be demonstrated.

Conclusions and prospects

An extensive body of evidence indicates that IGF-1 operates in an autocrine/paracrine mode as an intrinsic mediator of skeletal muscle repair and adaptation, by increasing the proliferation potential of satellite cells, promoting their differentiation, enhancing muscle regeneration and increasing muscle mass. Specific signaling pathways downstream of IGF-1 have been proposed to be involved in the mitogenic and myogenic responses following exercise-induced muscle overloading and damage. However, it remains a challenge not only to elucidate and identify the specific stimuli and the mechanotransduction signaling mechanisms by which IGF-1 synthesis is modulated at skeletal muscle cellular level, but also to demonstrate a potential alternative activation of different IGF-1 signaling pathway(s) via different receptor(s).
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