

## Insulin-like growth factor 1 and muscle growth: implication for satellite cell proliferation

Shuichi Machida<sup>1</sup> and Frank W. Booth<sup>1,2\*</sup>

<sup>1</sup>Department of Biomedical Sciences and <sup>2</sup>Department of Medical Pharmacology and Physiology and the Dalton Cardiovascular Center, University of Missouri-Columbia, E102 Veterinary Medical Building, 1600 East Rollins Road, Columbia, MO 65211, USA

Insulin-like growth factor 1 (IGF-1) has been shown to rescue the aging-related or inactivity-induced loss of muscle mass through the activation of satellite cells. However, the signalling pathways and the mechanism by which IGF-1 affects satellite cells have not been completely identified. The purpose of the present review is to provide current understanding of the cellular and molecular events underlying IGF-1 induced proliferation of satellite cells.

### Insulin-like growth factor 1: Satellite cells

During the early stage of skeletal muscle development myoblasts fuse to form myotubes, which become innervated and develop into muscle fibres. Thereafter, skeletal muscle myonuclei are terminally post-mitotic and are unable to divide (O'Neill & Stockdale, 1972). However, during postnatal growth and muscle hypertrophy, additional myonuclei are acquired via satellite cell fusion to the muscle fibre (Rosenblatt & Parry, 1992). Satellite cells are small mononucleated cells that are located between the basal lamina and sarcolemma of muscle fibres. In adult skeletal muscles these cells are mitotically quiescent, but are activated and then proliferate in response to a number of stimuli, including mechanical loading, exercise and damage (Hawke & Garry, 2001). Insulin-like growth factor 1 (IGF-1) is a potent mitogen, which is probably produced locally during muscle hypertrophy and can induce proliferation of satellite cells (Adams & Haddad, 1996; Adams & McCue, 1998). IGF-1 and satellite cells have been shown to play an essential role in the process of muscle hypertrophy (Rosenblatt & Parry, 1992; Adams & Haddad, 1996).

#### Mechanical loading and insulin-like growth factor 1

Mechanical loading, such as compensatory hypertrophy by muscle ablation (DeVol *et al.* 1990; Adams & Haddad, 1996), stretch (Yang *et al.* 1997) and eccentric contraction (Yan *et al.* 1993) induce production of IGF-1 within the

skeletal muscle. DeVol *et al.* (1990) have demonstrated a 3-fold increase in total IGF-1 mRNA levels in the rat soleus and plantaris muscles after tenotomy-induced hypertrophy. Bamman *et al.* (2001) have extended the observation to human skeletal muscle, where 48 h after a single resistance training bout muscle IGF-1 mRNA increases. These reports suggest a relationship between local stimulation of skeletal muscle growth and IGF-1 expression. Skeletal muscle hypertrophy is regulated by at least three major molecular processes: (1) satellite cell activity; (2) gene transcription; (3) protein translation. IGF-1 can influence the activity of all these mechanisms, including increases in satellite cell proliferation, skeletal  $\alpha$ -actin mRNA expression and protein synthesis (Florini *et al.* 1996; Chakravarthy *et al.* 2000a). Thus, increased IGF-1 expression plays an important role in mediating muscle hypertrophy induced by mechanical loading (Adams & Haddad, 1996; Adams & McCue, 1998).

#### Roles for insulin-like growth factor 1 and satellite cells in load-induced muscle hypertrophy

Since myonuclei are post-mitotic, the hypertrophying skeletal muscle must rely on an alternative source for additional myonuclei (Rosenblatt & Parry, 1992). Satellite cells have a tremendous proliferative capacity and are thought to be tissue-specific progenitor cells that are important in the hypertrophy and regeneration of skeletal

**Abbreviation:** IGF-1, insulin-like growth factor 1; PI3K, phosphatidylinositol 3-kinase.

**\*Corresponding author:** Dr Frank W. Booth, fax +1 573 884 6890, email boothf@missouri.edu

muscle (Hawke & Garry, 2001). The necessity for satellite cells for muscle hypertrophy was first demonstrated by Rosenblatt & Parry (1992) in an experiment that prevented satellite cell proliferation by exposing the muscle to low-level  $\gamma$ -irradiation, with a resultant failure to produce full hypertrophy in response to functional overload. Thus, satellite cell proliferation is believed to be necessary for the full increase in skeletal muscle mass induced by overload. During the process of load-induced muscle hypertrophy, satellite cells are thought to proliferate, differentiate and then fuse with existing myofibres (Schultz & McCormick, 1994). IGF-1 has been shown to stimulate these myogenic processes in skeletal muscles (Florini *et al.* 1996; Hawke & Garry, 2001). For example, Adams & Haddad (1996) have reported a positive correlation between muscle IGF-1 expression and the increase in muscle nuclear DNA content in the overloaded muscle. In addition, Adams & McCue (1998) have found that localized infusion of IGF-1 into the skeletal muscles of rats *in vivo* results in hypertrophy and that DNA:protein of the hypertrophied muscles is unchanged from that of controls. These results indicate that the elevated muscle IGF-1 induced by loading may be contributing to the hypertrophy response, in part, by stimulating the proliferation of satellite cells. Recent data suggest that a specific IGF-1 isoform is expressed in muscle during overload hypertrophy (McKoy *et al.* 1999). This isoform, termed 'mechano growth factor' (i.e. IGF-1 Eb) by Geoffrey Goldspink, has been shown to be markedly up regulated in response to stretch and electrical stimulation, accompanied by an up-regulation of the liver form of IGF-1 (i.e. IGF-1 Ea) mRNA (McKoy *et al.* 1999). On the other hand, the same group (Owino *et al.* 2001; Hameed *et al.* 2003) has recently reported that mechanical overload and high-resistance exercise induces the expression of mechano growth factor mRNA, while no changes are observed in the level of IGF-1 Ea mRNA.

#### Effect of physical exercise on satellite cell proliferation

An increased level of physical activity, such as running or resistance training, can also stimulate satellite cell mitotic activity (McCormick & Thomas, 1992) and result in elevated satellite cell numbers (Kadi & Thornell, 2000). Exercise training by progressive treadmill running results in the activation of satellite cells, in conjunction with morphological changes indicative of ongoing muscle fibre injury and repair (McCormick & Thomas, 1992). Although local production of IGF-1 in skeletal muscle has been shown to increase after exercise (Hellsten *et al.* 1996), the cellular mechanism(s) linking exercise to increased IGF-1 is unclear.

#### Insulin-like growth factor 1 can rescue sarcopenia

Sarcopenia is the involuntary loss of skeletal muscle mass and strength that occurs with aging, resulting in physical frailty. One reason for sarcopenia may be that older skeletal muscles fail to respond to mechanical overload. For example, the gastrocnemius muscle of old rats fails to

regrow after atrophy by limb immobilization (Chakravarthy *et al.* 2000b). Further, the mechanical loading-induced up-regulation of mechano growth factor mRNA is attenuated in old muscles of human subjects and rats (Owino *et al.* 2001; Hameed *et al.* 2003). However, Barton-Davis *et al.* (1998) have demonstrated that IGF overexpression, using recombinant adeno-associated virus, rescues age-related muscle loss between the ages of 23 and 27 months in mice. Chakravarthy *et al.* (2000b) have extended the Barton-Davis *et al.* (1998) observation from normal-aged muscle to aged muscle forced to atrophy. They found that direct IGF-1 administration onto an atrophied muscle promotes an enhancement of satellite cell proliferation in culture and regrowth of skeletal muscle from limb immobilization in 30-month-old rats. These results suggest that satellite cells in skeletal muscle of 30-month-old rats are in sufficient quantity, but inactive as a result of lack of some endogenous growth factors, possibly including IGF-1. Thus, IGF-1 rescues muscle from sarcopenia, in part, through the proliferation of satellite cells.

#### The mechanism by which insulin-like growth factor 1 stimulates satellite cell proliferation

Unlike other growth factors, IGF-1 stimulates both myoblast proliferation and differentiation (Engert *et al.* 1996; Florini *et al.* 1996). In proliferating myoblasts IGF-1 increases the expression of the cell-cycle progression factors (Engert *et al.* 1996). After withdrawal of myoblasts from the cell cycle IGF-1 promotes muscle differentiation by inducing the expression or activity of myogenic regulatory factors (Musaro & Rosenthal, 1999). The proliferative *v.* the differentiating functions of IGF-1 appear to be mediated by distinct intracellular signalling pathways (Coolican *et al.* 1997). Previous studies using immortalized myogenic cell lines such as L6A1 (Coolican *et al.* 1997), MM14 (Jones *et al.* 2001) and L8 (Tamir & Bengal, 2000) have suggested that the mitogen-activated protein kinase mediates cellular proliferation, whereas the phosphatidylinositol 3-kinase (PI3K) pathway is activated during differentiation. However, although it is not clear what role the PI3K pathway plays in differentiation, recent evidence demonstrates a key role for the PI3K pathway in primary satellite cell proliferation (Chakravarthy *et al.* 2000a; Machida *et al.* 2003). Chakravarthy *et al.* (2000a) have demonstrated that IGF-I-stimulated proliferation of primary satellite cells isolated from transgenic mice overexpressing IGF-1 is associated with the activation of the PI3K/Akt signalling pathway, the up-regulation of a cyclin-dependent kinase 2 kinase activity and the down-regulation of the cell-cycle inhibitor p27<sup>Kip1</sup> (Chakravarthy *et al.* 2000a). Ectopic expression of p27<sup>Kip1</sup> has been shown to block the IGF-I-induced increase in satellite cell proliferation (Chakravarthy *et al.* 2000a). Thus, p27<sup>Kip1</sup> has been proposed to be a key regulatory factor, particularly in its ability to regulate satellite cell cycle progression. Machida *et al.* (2003) have recently reported that IGF-1 represses p27<sup>Kip1</sup> transcriptional activity through phosphorylation of Akt and forkhead transcription factor FOXO1,

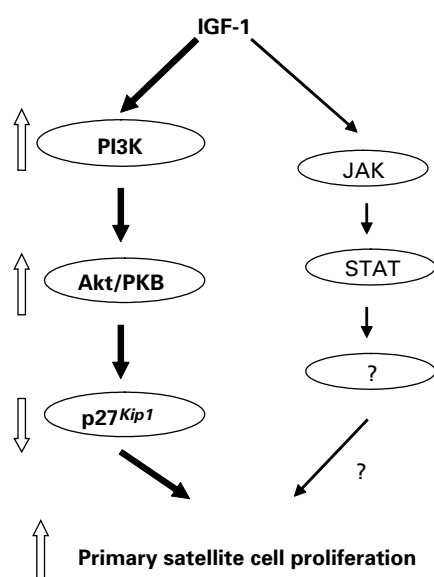
implying that FOXO1 may be an intermediary signal between Akt phosphorylation and p27<sup>Kip1</sup> promoter activity in primary satellite cells of skeletal muscle.

### The other signal pathways contributing to insulin-like growth factor 1-induced satellite cell proliferation

IGF-1 stimulates primary satellite cells to proliferate by increasing the phosphorylation of Akt/protein kinase B and FOXO1, down regulating p27<sup>Kip1</sup>, which in turn releases inhibition of cyclin-dependent kinase 2, increasing phosphorylation of pRb, allowing the cell cycle past the restriction point into the S phase (Chakravarthy *et al.* 2000a; Machida, 2003). IGF-1 also signals the janus kinase/signal transducers and activators of transcription pathway, but not the mitogen-activated protein kinase pathway in primary satellite cells (S Machida and FW Booth, unpublished results).

### Summary

There is now an increasingly aged population. IGF-1 has been shown to be able to rescue aging-related or inactivity-induced loss of muscle mass through the activation of satellite cells. The present review has compiled the current knowledge relating to the cellular and molecular events underlying IGF-1-induced proliferation of satellite cells. IGF-1 enhances satellite cell proliferation by decreasing the cell-cycle inhibitor p27<sup>Kip1</sup> protein through the PI3K/Akt pathway (Fig. 1). Thus, p27<sup>Kip1</sup> has been proposed to be a key regulatory factor, particularly in its ability to regulate satellite cell cycle progression.



**Fig. 1.** Diagram of the signal transduction pathways by which insulin-like growth factor 1 (IGF-1) stimulates satellite cell proliferation.  $\leftarrow$ , Changes in activity or status;  $\rightarrow$ , flow of the IGF-1 signalling pathway. PI3K, phosphatidylinositol 3-kinase; Akt/PKB, Akt/protein kinase B; JAK, janus kinase; STAT, signal transducers and activators of transcription.

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