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GROWTH FACTOR ENHANCEMENT OF MUSCLE REGENERATION: A CENTRAL ROLE OF IGF-1

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THE MOLECULAR BASIS OF MYOGENEIS: AN OVERVIEW

The achievement of biological process such as development, proliferation, differentiation involves the activation of a specific program of gene expression and the protein machinery regulating its performance.

The complex program of skeletal muscle development involves an orderly progression of molecular signals that induce primordial muscle precursor cells to be committed in a myogenic fate and subsequently to differentiate into mature muscle. Identification of myogenic transcription factors responsible for the induction of the skeletal muscle phenotype has represented a significant breakthrough in our understanding of the molecular pathways underlying skeletal muscle differentiation (1, 19). The four myogenic regulatory factors (MRFs) MyoD, myogenin, myf5, and MRF4 share about 80% homology within a 70 amino acid basic Helix-Loop-Helix motif (bHLH), and each is able to orchestrate an entire program of muscle specific gene expression when ectopically expressed in non-muscle cell types. During muscle development the myogenic proteins exhibit distinct expression patterns, suggesting that they play specific roles in the establishment of skeletal muscle cell commitment and differentiation.

In the last decades transgenic animals have been become a powerful and exciting research models to study the molecular mechanisms underlying the cellular and physiological processes, such as cell growth, differentiation, apoptosis, and the regulation of specific gene expression. In the context of skeletal muscle development, transgenic mice and gene-targeting approaches have led to the definition of specific roles for Muscle Regulatory Factors (MRFs) during embryogenesis (1).

In addition, this experimental approach has been demonstrated that the activation of the muscle regulatory gene is dependent upon signals derived from axial structures, such as neural tube and notochord and from dorsal ectoderm. In particular, Tajbakhsh and co-workers (20) showed that axial structures activate myf-5 expression via Wnt1 signal, whereas dorsal ectoderm, expressing Wnt7a, activates MyoD and therefore the hypaxial muscle development. The endpoint of muscle develop-

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ment is the generation of a population of heterogeneous fibers, conferring upon muscle tissue a considerable degree of plasticity.

THE CELLULAR AND MOLECULAR BASIS OF MUSCLE REGENERATION

Regeneration represents one of the most important homeostatic processes in adult tissues, including skeletal muscle, which after development maintains the capacity to regenerate in response to injury activating the classical stem cell compartment known as satellite cells. Satellite cells are population of quiescent mononucleated myoblasts, localized between the basal lamina and sarcolemma of myofibers (13).

The remarkable adaptive ability of postnatal skeletal muscle to physiological demands such as normal growth, growth after intensive exercise, and regeneration after injury, is attributable to the efficacy of satellite cell activation, proliferation, differentiation and fusion (5). In response to myotrauma, signals from immune cells and the extracellular matrix stimulate conversion of satellite cell from a quiescent to an activated state. This induces an asymmetric cell division that results in replacement of the quiescent cell and production of an activated muscle precursor, both expressing Pax7 (6, 21). The muscle precursor cell migrates to the site of injury, differentiates, and fuses to existing muscle fibres with concomitant down-regulation of Pax7 and up-regulation of muscle determination genes (11, 18). It is believed that satellite cells have a finite capacity for self-renewal (4); in pathological cases where the demand for fiber repair is ever-present such as in Duchenne Muscular Dystrophy, the satellite cell pool becomes exhausted and the muscle eventually fails to regenerate, resulting in loss of muscle function and death (10).

More recently it has been reported that that hematopoietic stem cell (HCS) migration into sites of injury may be a mechanism by which damaged tissues are repaired (7, 9, 12). However this seems a rare event and presents limitations for an efficient tissue repair.

In fact it has been reported that the poor recruitment of HSC into the dystrophic muscle of the mdx mouse is the major obstacle for muscle regeneration and therefore for the rescue of the genetic disease (8).

How to increase stem cell recruitment and improve muscle regeneration?

THERAPEUTIC STRATEGY TO IMPROVE MUSCLE REGENERATION AND TO ATTENUATE MUSCLE WASTING

Although in recent years considerable evidence has been accumulated regarding the physiopathology of senescent and pathological muscle, it is still an open question what molecular mechanisms might regulate the phenotypic changes leading to the pathological pattern of muscle aging and diseases. The decrease in the production and activity of the growth hormone/IGF-I, the decline in the proliferative capa-

city of satellite cells and the autocrine/paracrine signals which skeletal muscle is exposed all have been postulated as contributing factors to the pathological pattern of muscle diseases.

Among trophic factors, Insulin-like Growth Factor-1 (IGF-1) plays a critical role in muscle regeneration. It promotes the proliferation and differentiation of satellite cells in the muscle, enabling them to fuse to existing muscle fibers and to repair damaged fibers (16).

In addition, IGF-1 activates specific signal transduction pathways promoting muscle growth, differentiation, and hypertrophy and inhibiting muscle protein breakdown, a critical step for myofibers survival.

These observations make IGF-1 a good candidate for gene therapy approaches.

However, the generation of appropriate experimental models is important to develop therapeutic strategies to attenuate muscle wasting and promotes myofibers survival.

The fact that IGF-1 can act either as a hormone or as a local growth factor, has complicated the analysis of animal models in which transgenic IGF synthesized in extrahepatic tissues was released into the circulation (16), therefore determining pathological side effects.

By the light of these evidences, restricting the action of supplemental mIGF-1 to the tissue of origin by choice of the appropriate isoform allowed us to study its autocrine/paracrine role in skeletal muscle throughout the lifespan of the animal, exclusive of possible endocrine effects on other tissues.

We recently generated transgenic mice in which an alternate isoform of IGF-1 driven by MLC promoter (MLC/mIGF-1) exhibit sustained muscle hypertrophy, producing pronounced increases in muscle mass and strength with no undesirable side effects (15). Expression of the mIGF-1 transgene safely enhanced and preserved muscle fiber integrity even at advanced ages (15), suggesting that the MLC/mIGF-1 transgene acts as a survival factor by prolonging the regenerative potential of younger muscle. This is supported by preliminary evidence demonstrating reactivation of myoblast proliferation following terminal differentiation of MLC/mIGF-1 primary myocyte cultures so that mIGF-1 produced by post-mitotic myocytes appears to promote regeneration by extending stem cell proliferative capacity as well.

The capacity of the mIGF-1 transgene to attenuate the structural and functional consequences of muscle aging was independent of its action during embryogenesis or early postnatal life, since local delivery of mIGF-1 in individual mouse muscles by AAV virus mediated gene transfer also permanently blocked age-related loss of muscle size and strength, presumably by improving regenerative capacity (3) through increases in satellite cell activity. Because it is clear that IGF-1 can prevent aging-related loss of muscle function (3, 15), it is possible that IGF-I can prevent or diminish muscle loss associated with disease.

More recently we demonstrated that muscle-specific expression of IGF-1 counters muscle wasting in mdx dystrophic mice, improving muscle mass and strength and elevating pathways associated with muscle survival and regeneration (2).

These data suggest that IGF-1 is critical in mediating muscle growth and its loss appears central to muscle atrophy in muscle pathologies. The anabolic effects of IGF-1 may be due in part to stimulation of activation of satellite cells that have a precocious ability to form myotubes compared to those isolated from wild-type littermates. It is not known whether in transgenic animals, the satellite cells have an increased ability for self-renewal or whether there is an increased recruitment of non-satellite cells.

Our recent experimental evidences indicate that IGF-1 promotes the two suggested pathways which can be considered two temporally separated events of the same biological process (17). In this study, the effects of mIGF-1 on the enhancement of stem cell-mediated muscle regeneration were analyzed using a combination of SP analysis and expression of CD45, c-Kit and stem cell antigen-1 (Sca-1), which represent haematopoietically relevant cell surface markers (14).

We demonstrated that upon muscle injury, stem cells expressing c-Kit, Sca-1, and CD45 antigens increased locally and the percentage of the recruited cells were conspicuously enhanced by IGF-1 expression (17).

FACS profiles of tissues from wild type and MLC/mIGF-1 transgenic mice, whose muscles were injured with cardiotoxin, revealed a consistent increase of SP cells in the bone marrow, compared to non-injured controls which percentage increase in MLC/mIGF-1 transgenic mice. In contrast, the number of SP cells remained unchanged in other tissues, such as spleen and liver of both wild type and MLC/mIGF-1 transgenic mice after muscle injury.

Thus, humoral signals emanating from the injured muscles were sufficient to induce stem cell proliferation in the bone marrow, but not in other tissues. Indeed, treatment of injured mice with 5-fluorouracil (5-FU), a cytotoxic agent which depletes cycling stem cells, was sufficient to block proliferation of bone marrow SP and expansion of the CD45+/Sca-1+ population in injured muscle (17).

In addition, to definitely demonstrate the recruitment of bone marrow-stem cells into the site of muscle injury and the role of mIGF-1 in this process, we performed a bone marrow transplantation of both wild type and MLC/mIGF-1 transgenic mice using bone marrow-SP cells of c-kit/GFP transgenic mice. FACS analysis revealed the presence of c-kit/GFP positive cells in the injured area of skeletal muscle and confirms the effect of mIGF-1 in the enhancement of circulating stem cells into the site of muscle injury.

These results establish mIGF-1 as a potent enhancer of stem cell-mediated regeneration and provide a baseline to develop strategies to improve muscle regeneration in muscle diseases.

SUMMARY

The prolongation of skeletal muscle strength in aging and neuromuscular disease has been the objective of numerous studies employing a variety of approaches. In the last decade, dramatic progress has been made in elucidating the molecular

defects underlying a number of muscle diseases. With the characterization of mutations responsible for muscle dysfunction in several inherited pathologies, and the identification of novel signaling pathways, subtle alterations in which can lead to significant defects in muscle metabolism, the field is poised to devise successful strategies for treatment of this debilitating and often fatal group of human ailments. Yet progress has been slow in therapeutic applications of our newly gained knowledge. The complexity of muscle types, the intimate relationship between structural integrity and mechanical function, and the sensitivity of skeletal muscle to metabolic perturbations have impeded rapid progress in successful clinical intervention. The relatively poor regenerative properties of striated muscle compound also the devastating effects of muscle degeneration.

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