Skeletal muscle regeneration: a brief review

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ABSTRACT: This review article aims to concise the main aspects concerning the skeletal muscle tissue regenerative process and to describe some of the most important scientific evidences about its possible underlying mechanisms. Muscle regeneration has been the object of many scientific studies throughout the years. Many modulating factors, as well as a particular cell population named muscle satellite cells, are of extreme importance for the efficiency of the skeletal muscle repair. Other adult stem cells may also play an important role in this process. The discovery of new small molecules, such as reversine, a recent synthetic purine-derivated small molecule capable of promoting the dedifferentiation of lineage-committed cells into progenitor-type cells, promises new insights and possible applications for muscle regeneration.

KEYWORDS: Skeletal muscle, regeneration, satellite cells, stem cells, reversine.

REGENERATION

The term “regeneration” is physiologically used to designate the renewal or repair of damaged or destroyed cells/tissues, with the potential to restore its initial functional activity (1).

Skeletal muscle is susceptible to injury after direct trauma (e.g., lacerations, intensive physical activity and resistance training) or indirect causes (e.g., innate genetic defects, neurological dysfunction). These lesions, if left unrepaired, may lead to muscle mass loss, locomotive deficit and even lethality. In order to maintain a functional skeletal musculature, this tissue has a notable ability to initiate a rapid and extensive repair process. In fact, after muscle injury, a complex set of cellular responses is activated, leading to the regeneration of a well-intervated, totally vascularized and contractile muscle apparatus (2).

The advances of cellular and molecular biology research related to the role of muscle satellite cells, signaling factors, molecular pathways, multipotent stem cells capable of myogenic differentiation and other components involved in the muscle regenerative process are important for its better understanding and may also be useful for the development of therapeutic strategies for pathological muscle conditions (2). Although many evidences are available, the complex muscle regenerative process still remains to be fully characterized.

MYOGENESIS

Vertebrate skeletal muscles (except the head muscles) are derived from mesodermal precursor cells originated from the somites (3). The specification of mesodermal precursor cells to the myogenic lineage is regulated by positive and negative signals from surrounding tissues, during embryonic development. This process requires the upregulation of MyoD and Myf5, which are transcriptional activators of the myogenic regulatory factor family (MRF). Proliferative MyoD and/or Myf5 positive myogenic cells are named myoblasts (cells committed to the myogenic lineage). After this, upregulation of the secondary MRFs – myogenin and MRF4 – induces terminal differentiation of myoblasts into myocytes. These terminally differentiated myocytes express the MRFs mentioned above and consequently muscle-specific genes – myosin heavy chain and muscle creatine kinase. At last, mononucleated myocytes fusion originates multinucleated synctium, which will mature into contracting muscle fibers and form a stable muscle tissue (2). During the later phase of embryonic myogenesis, a distinct subpopulation of myoblasts derived from satellite cells fails to differentiate and fuses to existing myofibers enabling myofiber growth. Some satellite cells remain in a quiescent undifferentiated state. Although the embryonic origin of satellite cells remains to be defined, it is known that Pax7 expression is essential for the specification/expansion of the satellite cell population. Muscle regeneration appears to recapitulate to some degree the muscle embryonic developmental process (2).

SKELETAL MUSCLE REGENERATION

REGENERATION PHASES

Mammalian adult skeletal muscle tissue has a very little turnover of nuclei, under normal circumstances (4,5). Minor lesions in adult mammalian skeletal muscle that are caused by daily injuries produce a slow turnover of
In vitro, the muscle regeneration is characterized by two phases: the degenerative phase and the regenerative phase (2).

The degenerative phase is initiated by necrosis of the damaged muscle tissue. This occurrence is generally triggered by the disruption of the myofiber sarcolemma, which is reflected by the increase of serum muscle proteins levels (e.g., creatine kinase), leading to an increase of myofiber permeability (2). Some authors suggest that after sarcolemmal and sarcoplasmic reticulum damage, the augmented calcium influx results in a loss of calcium homeostasis and in a calcium-dependent proteolysis that leads to tissue degeneration (6-8). Certain calcium-activated proteases, named calpains, which have the capacity to cleave myofibrillar and cytoskeletal proteins, are also implicated (8-10). What determines if the autolysis of disrupted myofibers is total or focal is the degree of the muscle damage (2).

This degenerative phase has another event that consists in the activation of an inflammatory response. Mononucleated cells are activated, mainly inflammatory and myogenic cells. Some studies mention that the damaged muscle release factors which activate inflammatory cells present in the muscle and these provide the chemotactic signals to inflammatory cells that are in circulation (11,12). Within the site of injury, neutrophils are the first inflammatory cells to invade de damaged muscle (13,14) and after approximately 48 hours the predominant cells are macrophages (12,14). The macrophages’ function is to phagocyte cellular debris and it is suggested that they activate myogenic cells (15-18). Other studies, that demonstrate the stimulation of peritoneal macrophages after intensive physical exercise suggest that a systemic factor is released after muscle damage, inducing an inflammatory response (19-21). There are known many mediating factors involved in the activation of inflammatory response and there is still a further need to characterize their role in the muscle regenerative process (12).

In conclusion, the main morphological characteristics of the early event upon muscle lesion are the muscle fiber necrosis and the increased number of nonmuscle mononucleated cells involved in the inflammatory response (2).

The regenerative phase takes place after the muscle degeneration. Cellular proliferation is a critical event for muscle regeneration. The expansion of myogenic cells offers a reasonable source of new myonuclei for muscle repair (22-24). After this proliferation, the myogenic cells differentiate and fuse, leading to new myofiber formation (25-28). The histological study of muscle cross-sections reveals newly formed myofibers with small caliber and centrally located myonuclei. They are frequently basophilic (due to high protein synthesis) and express embryonic forms of myosin heavy chain which reflects the novel muscle fiber formation (2,29,30). The longitudinal sections of muscle tissue and isolated muscle fibers reveal central myonuclei in discrete portions of regenerating fibers or along the entire new fiber. This suggests that cell fusion is focal to the site of injury (31). Another event observed in muscle regeneration is the fiber splitting or branching, probably due to the incomplete fusion of fibers regenerating within the same basal lamina (31-33). When the fusion is concluded, the new myofibers increase in size and myonuclei move to a peripheral position. In normal conditions, the regenerated muscle tissue demonstrates similar morphological and functional characteristics to the healthy muscle (2).

**MUSCLE SATELLITE CELLS**

Muscle satellite cells are undifferentiated mononuclear myogenic cells that can be found in skeletal muscles of various animals, including mammals (34-36). In vitro, these cells demonstrate specific properties that distinguish them from embryonic and fetal myoblasts (37-39). They can be identified by electron microscopy because of their unique location within the basal lamina that surrounds individual myofibers, juxtaposed between the cytoplasmatic membrane of the fiber and the basement membrane. They also exhibit an elevated nuclear-to-cytoplasmic ratio, low organelle content and a smaller nucleus with augmented heterochromatin (2).

Thus, the muscle satellite cells are in a quiescent state and transcriptionally less active than myonuclei (40,41). The satellite cell population decreases with age and is more predominant in slow muscle fibers (2,42). Satellite cells can be activated with muscle injury (e.g., mechanical trauma, direct injury or disease) (22-24). Different stimuli have been suggested as activators of the satellite cells: compounds released from damaged fibers, soluble factors from connective tissue and molecules released from the macrophages that invade the damaged site (16,43-45). Several trophic factors have been described to have the ability to balance the growth and differentiation of satellite cells (24,46). Some examples are the members of the fibroblast growth factor (FGF) family (potent activators of the myogenic precursor cells’ proliferation and inhibitors of their differentiation), transforming growth factor-beta (TGF-beta) family (cytokines that regulate cell growth and modulate myoblasts’ activity by inhibiting the proliferation and differentiation (47-51)), insulin-like growth factor (IGF) family (they regulate growth and development of various tissues, alter MRFs expression and promote the proliferation and differentiation/fusion of myoblasts (48,49,52-57)), hepatocyte growth factor (HGF) (one of the most important growth factors for organ regeneration due to its mitogenic and motogenic properties (58), it is also the primary muscle factor which has the ability to induce quiescent muscle satellite cell activation (59), and many others as interleukine-6 (IL-6) family of cytokines, tumor necrosis factor-alpha (TNF-alpha), neural-derived factors, nitric oxide and adenosine-5’-triphosphate (ATP).

In the process of muscle regeneration, satellite cells start proliferating and after several rounds, they differ-
satellite cells (73,74). Pax7 behavior. On the other hand, the MDSC and muscle stem cells may have different actions in muscle regeneration – MyoD enhances the progression of satellite cells to terminal differentiation, while Myf5 promotes satellite cells’ self-renewal (2).

It is possible to proceed to the culture of muscle satellite cells and transplant them into regenerating muscle tissue, where they enhance myofiber formation and the satellite cell pool for later rounds of regeneration (2,27,31,70-72). Scientific studies suggest that in Pax7(-/-) mice, which lack muscle satellite cells, the skeletal muscle regeneration is significantly reduced (2). Pax7 is a gene that is strictly expressed in cultured satellite cells and in quiescent and activated in vitro satellite cells (73,74). Pax7 expression is increased in regenerating muscles and it is also associated with central nuclei of newly regenerated muscle fibers. These findings propose that recently activated and fusing satellite cells express Pax7 (74). Thus it is suggested that Pax7 has a crucial role in lineage determination, mainly in the specification of myogenic progenitors to the satellite cell lineage (2).

The renewal of the satellite cell pool depends not only on the satellite cell compartment but also on other adult stem cells (derived from bone marrow (75-78), adult musculature (77,79-82), neuronal compartment (83,84) and many mesenchymal tissues (85,86)) that are able to undergo myogenic differentiation and contribute to that pool after transplantation (2).

EXPERIMENTAL STUDIES

The phenomenon of muscle regeneration, its components and pathways, have been the target of a great diversity of experimental studies throughout the years. Some of the main results and discoveries are described next.

Floss et al. (1997) investigated the importance of the fibroblast growth factor-6 (FGF-6) in skeletal muscle regeneration. FGF-6 is part of a family of cytokines that control various events: cell proliferation, cell differentiation and morphogenetic. It is known that FGF-6 has a restricted expression profile, mainly in the myogenic lineage. This team of investigators inactivated the FGF-6 gene in mice and obtained different results in different mice populations. Their main results were: wild-type mice upregulated FGF-6 after skeletal muscle injuries and were able to fully regenerate experimentally damaged skeletal muscle; FGF-6(-/-) mutant mice exhibited a severe regeneration defect with enhanced fibrosis and myotube degeneration; the number of activated satellite cells that expressed MyoD and Myogenin, after injury, were significantly reduced in regenerating muscle tissue of mutant mice, probably due to a lack of activation or proliferation; the skeletal muscles of FGF-6(-/-)/mdx double mutant mice expressed myotube degeneration, large amounts of mononuclear cells, fibrosis and attenuated hypertrophy; MyoD expression was upregulated in skeletal muscles of mdx mice, but not in FGF-6(-/-) and FGF-6(-/-)/mdx mice. These studies suggested that FGF-6 is an essential component of mammalian muscle regeneration, possibly through the stimulation or activation of satellite cells and the expression of MyoD (87).

Qu-Petersen et al. (2002) isolated three populations of myogenic cells from normal mouse skeletal muscle, based on their adhesion and proliferation properties: early preplate (EP) cells, late preplate (LP) cells and long time proliferating cells (MDSC). The EP cells represented the main population of myogenic cells derived from skeletal muscle, while the LP population only represented approximately 1% of the satellite cells. It was difficult to evaluate the relative number of cells in the MDSC population within the muscle cell cultures. The EP and LP cells represented two populations of satellite cells, based on their patterns of myogenic marker expression and in vitro behavior. On the other hand, the MDSC exhibited unique characteristics, commonly associated with noncommitted progenitor cells. The MDSC are capable of self-renewal in vitro, as it was demonstrated by the detection of similar phenotypes in these populations and by the maintenance of that phenotype for a long time in vitro. These cells could also be expanded in vitro for over 30 passages, preserving a normal karyotype, and were unable to produce tumors in immunodeficient mice. Other results demonstrated that the MDSC are capable of self-renewal in vitro and have a high capacity for long-term proliferation in vitro and in vitro. The investigators also detected donor-derived cells in myofibers, peripheral nerves and blood vessels within the MDSC-injected muscle, which demonstrates their multipotent nature in vitro, when properly stimulated with growth factors. It was ultimately confirmed that MDSC was a novel population of muscle stem cells with a great potential for muscle regeneration and enhancement of muscle cell-mediated therapies (88).

Musaro et al. (2004) investigated the mechanism by which the expression of a transgene encoding a locally acting isoform of insulin-like growth factor 1 (mIGF-1) may enhance the repair of skeletal muscle damage. This group of investigators had already demonstrated that the postmitotic expression of mIGF-1 induces myocyte hypertrophy, increases the mass and strength of postnatal muscle and preserves the regenerative capacity of the senescent and dystrophic mice muscles. In this study, they observed that the enhancement of muscle regeneration was associated with the increased recruitment of marked, transplanted bone marrow stem cells to the sites of muscle injury after lethal irradiation. In nonirradiated MLC/mIGF-1 transgenic mice, muscle injury expanded the side population compartment in the bone marrow. The transgenic animal models had elevated levels of cells that coexpressed markers of the stem cells lineage and myogenic commitment, at sites of muscle damage. When these cells were isolated from regenerating muscles, they demonstrated an accelerated myogenic differentiation in culture and induced muscle-specific markers...
in cocultured bone marrow cells. These authors concluded that mIGF-1 is a powerful regenerative agent that mediates the recruitment of bone marrow cells to sites of muscle damage, increases bone marrow and local stem cells’ pools, enhances local repair mechanisms and provides an explanation for the effects of supplemental MLC/mIGF-1 transgene expression on muscle mass and integrity, in vitro and in vivo (89).

Vadivelu et al. (2004) focused their attention on the tetracosanoyl phorbol acetate-induced sequence 7 gene (tis7). The mouse tis7 (PC4) gene had been identified as an immediate-early gene that was specifically induced by tetracosanoyl phorbol acetate, epidermal growth factor and fibroblast growth factor in Swiss 3T3 mouse cells and cultured rats astrocytes (90,91). The investigators described the generation and analysis of mice lacking the tis7 gene. They observed the following results: the disruption of the tis7 gene by homologous recombination delayed the muscle regeneration and modified the iso- metric contractile properties of skeletal muscles after muscle crush damage in TIS7(-/-) mice; cultured primary stem cells from TIS7(-/-) mice demonstrated marked reductions in differentiation potential and fusion index in a cell-autonomous type only; the loss of tis7 caused the downregulation of muscle-specific genes (as those for MyoD, myogenin and laminin-alfa2); the fusion potential in TIS7(-/-) muscle stem cells could be rescued by tis7 expression or laminin supplementation. Based on their results, these investigators suggested that tis7 could play an essential role in muscle stem cell fusion and differentiation in vitro during adult muscle regeneration. In early phases, the tis7 protein expression was transiently increased and localized to the central myonuclei of the regenerating muscle fibers in the TIS7(-/-) soleus muscle. After muscle crush damage, some cytoplasmic factors were released and stimulated the proliferation of the muscle stem cells, largely located in the basal lamina. They generally fuse to form myotubes and a normal muscle fiber. It was also demonstrated that SKMc15, the tis7 homologue present in the earliest stages of development, could rescue TIS7(-/-) mice from embryonic lethality. That homology could account for the less severe phenotype in TIS7(-/-) mice during adult muscle regeneration. Apparently, both tis7 and SKMc15 are present in TIS7(-/-) mice during muscle regeneration, which may enhance muscle stem cells differentiation in vitro. It was concluded that although tis7 was not essential for muscle development, it played a novel regulatory role in adult muscle regeneration (92).

Zhao et al. (2005) based their studies on the premise that fibroblast growth factors (FGFs) and their receptors (FGFRs) are essential for the development of most cell types. This and other groups of investigators had already demonstrated that the major receptor expressed in the muscle is FGFR4 and the major ligand is FGF6. It had also been shown that the inhibition of FGFR4 leads to the arrest of muscle progenitor differentiation in chick embryo, with reduced expression of Myf5, MyoD, embryonic myosin heavy chain and significant loss of limb muscles, which was independent of myoblast proliferation. Previous studies showed that Fgfr4 null mice had a normal development, without any evident muscle defects. These investigators studied staged muscle regeneration in vitro and suggested that FGFR4 may be required for effective muscle regeneration, which was tested using staged degeneration/regeneration Fgfr4 null mice. It was observed that Fgfr4 was induced in a strong but transient manner in muscle regeneration, when the myoblast-myotube transition occurs. At this point the proliferating myoblasts leave the cell cycle, fuse with neighboring cells and transiently differentiate into multinucleated myotubes. The muscle regeneration in Fgfr4 was highly abnormal, with poorly differentiated myotubes at day 7 and extensive replacement of muscle tissue by fat and calcifications by day 14. After studying these data, this group of investigators designed a transcriptional pathway upstream of Fgfr4 and showed that one of the TEA domain transcriptional factors, Tead2, was induced at day 3 of regeneration. Tead2 regulated the Fgfr4 promoter through the M-CAT motif (CATTCCT). They also demonstrated that MyoD binded directly and activated Tead2 first intron. With these results, it was possible to define a MyoD-Tead2-Fgfr4 pathway that is important for effective muscle regeneration (93).

Sasaki et al. (2007) studied the effects of resting intervals in mice muscle regeneration. They knew that causing unrelenting damage to the muscles occasionally induces prolonged muscle fatigue, soreness and underperformance. Thus, rest is critical in regular muscle training in order to avoid the accumulation of muscle damage. With this background they proposed that differences in the resting intervals between two periods of exercise could result in histological differences in muscle regeneration. The investigators used 10-week-old male C57BL mice and designed an eccentric contraction model of the mouse gastrocnemius muscle, using percutaneous electrical stimulation. The mice received eccentric exercises once or twice with resting intervals of 0, 12, 24 hours, 2 and 3 days. Untreated mice were used as control and were allowed free cage activity between or after exercises. The centronuclear cell ratio (CNCR) is the ratio of myofibers with central nuclei to whole myofibers and was histologically analyzed at 14 days after the second exercise, as an index of muscle regeneration. It was observed that the myofibers in each fascicle fitted closely together with little variation in size and shape in all groups. In the control group, the nuclei of the myofibers were relatively inconspicuous and peripheral. No myofibers with central nuclei were found in this group. It was possible to observe myofibers with central nuclei in all sections of every exercise groups and could easily be distinguished from those whose nuclei were peripherally shifted. No disrupted or inflammatory cells were found. The myofibers that had central nuclei were more frequent in the 12 and 24 hour groups. The CNCR of the single-exercise group was 29.5%. In the twice exercised group it increased from 31.8% in the 0 hour group to a peak of 43.9% in the 24 hour group and then decreased to 32.8% in the 3 day group. The CNCRs of
the 12 and 24 hour groups were statistically higher than those of the single-exercise, 0 hour, 2 day and 3 day groups. With these data it was possible to conclude that the resting interval between two periods of eccentric exercises affected the histological properties of muscle regeneration. The quantity of muscle damage and/or the recovery process of damaged muscles should vary according to the length of the resting interval between intense exercises. Thus, an appropriate resting interval is essential to avoid the accumulation and further muscle damage in mice (94). Deasy et al. (2007) executed various studies in order to understand the broad heterogeneity in phenotype and performance that has been described for muscle-derived stem cells (MDSCs) and other muscle stem cell populations. They had already demonstrated that the transplantation of MDSCs into diseased muscle resulted in a large amount of regenerated myofibers (88,95). It had also been observed a high degree of variability in the ability of different MDSCs populations to regenerate skeletal muscle. While investigating the MDSCs population's heterogeneity and properties that make possible an efficient in vitro skeletal muscle regeneration, this authors found that cell sex, a rarely considered variable, had a significant effect on the in vitro outcome. They identified various sex-related differences as factors in MDSCs variability in skeletal muscle regeneration. The male (M) and female (F) MDSC populations that were isolated by the preplate technique shared stem cells characteristics. Extensive in vitro screening showed that only 2/10 male populations had an in vitro regenerating index (RI) near 200. Sixty percent of the 15 female populations had an RI higher than the mean RI of M-MDSCs (RI=95) and 40% of the F-MDSCs had an RI higher than the maximal male RI (RI=203). After transplantation into the skeletal muscle of dystrophic mice, F-MDSCs transplanted into the host of either sex, consistently regenerated more dystrophin-positive myofibers than M-MDSCs transplanted into hosts of either sex. Using microarray analysis, it was possible to observe trends in sex differences in genes related with apoptosis, hypoxia, oxidative stress and general cell stress response. With these results, the authors concluded: F-MDSCs regenerated skeletal muscle more efficiently; despite using additional isolation techniques and cell cloning, it was not possible to obtain a male subfraction with a regenerating capacity similar to the one of the female populations; the difference in MDSCs’ regeneration potential could be derived from innate sex-related differences in the cells’ stress responses; M-MDSCs had an increased differentiation after exposure to oxidative stress induced by hydrogen peroxide, which could lead to an in vitro donor cell depletion and a proliferative advantage for the F-MDSCs that would eventually increase the muscle regeneration. This study brought to consideration the implications of the cell sex variable in experimental works (96). Pelosi et al. (2007) studied the role of a muscle-restricted insulin-like growth factor 1 (mIGF-1) in muscle regeneration. They had the following background knowledge on which they based their study: muscle regeneration following injury is characterized by myonecrosis, local inflammation, activation of satellite cells and repairmen of injured fibers; the resolution of the inflammatory response is essential so that muscle regeneration may occur, since the persistence of inflammation often renders the damaged muscle incapable of sustaining an efficient muscle regeneration. The investigators observed that, at a molecular level, mIGF-1 expression significantly downregulated proinflammatory cytokines (e.g., TNF-alfa, IL-1beta) and modulated the expression of CC chemokines that were involved in the recruitment of monocytes/macrophages. When analyzing the underlying molecular mechanisms, they discovered that the rapid restoration of injured mIGF-1 transgenic muscle was associated with connective tissue remodeling and a fast recovery of its functional properties. They concluded that supplemental mIGF-1 created a qualitatively different environment for sustaining a more efficient muscle regeneration and repair, by modulating the inflammatory response and limiting fibrosis (97).

Giacinti et al. (2008) investigated a diversity of aspects concerning muscle regeneration. They structured their studies based on the knowledge that satellite cells repair myofibers after injury, as they exit their normal quiescent state, proliferate, activate MyoD and Myf-5 expression and finally differentiate and fuse in order to reconstitute the injured muscle architecture. In previous works, this group of investigators had already demonstrated that cdk9 is necessary for myogenesis in vitro through the activation of MyoD-dependent transcription. The MyoD then recruits cdk9 on the chromatin of muscle-specific regulatory regions, at the same time as the recruitment of chromatin-modifying enzyme and phosphorylation of cdk9-specific target residues at the carboxyl-terminal domain of the ribonucleic acid (RNA) polymerase II occur. In their later studies, they referred that cdk9-55, a second cdk9 isoform, had a critical role in muscle regeneration and differentiation in vitro. They also reported that cdk9-55 was specifically induced in injured myofibers and its activity was strictly required for the achievement of the muscle regeneration process (98). There are many more studies beyond these and it would be impossible to describe every single one of them. The main idea that must remain is that, although it has already been discovered a great deal of underlying mechanisms and components of muscle regeneration, much information remains unknown as the studies executed worldwide seem endless.

**REVERSINE AND MUSCLE REGENERATION**

Reversine is a 2,6-disubstituted purine derivative that was discovered in 2003. It is the first synthetic, low-molecular-weight and permeable compound that can act as an external signal and induce lineage-committed mammalian cells to become multipotent progenitor cells (99,100). The objective of Ding and Schultz’s study (2004) was to identify small molecules that could induce
true dedifferentiation of C2C12 myoblasts, which are myogenic lineage committed myoblasts. Using a high throughput screening of many small chemicals, they identified reversine as the molecule that demonstrated those properties. It was concluded that reversine inhibited myotube formation and treated myoblasts continued to grow to form a confluent culture of mononucleated cells, which could redifferentiate into osteoblasts and adipocytes when exposed to appropriate differentiation conditions (99). All of their observations suggested that reversine acted as a dedifferentiation-inducing agent rather than simply enriching certain progenitor cells by selectively killing myoblasts (101,102).

More recently, Anastacia et al. (2006) suggested that reversine treatment transforms primary murine and human dermal fibroblasts into myogenic-competent cells both in vitro and in vivo. It was the first study that demonstrated the plasticity changes that arise in primary mouse and human cells following reversine exposure. It had already been shown that fibroblasts from different sources have the ability to differentiate into skeletal muscle, but with a very low frequency, below that of possible therapeutic efficacy. These authors data showed that primary murine and human fibroblasts treated with reversine could be induced to differentiate into skeletal muscle at high frequency both in vitro and in vivo. The mechanism by which reversine exerted its effects on fibroblasts remained unknown. Reversine itself did not activate MyoD or Myf5 in fibroblasts and, in fact, spontaneous myogenic differentiation of treated cells did not occur. Thus, the authors could only speculate that reversine reprogrammed somatic cells to a state of increased plasticity so that further stimuli, such as cell-cell interactions, could activate differentiation at high frequency (103). Thus, reversine may play an important role in the future understanding and application of muscle regeneration.

**CONCLUSION**

The phenomenon of muscle regeneration has been the main theme of a great variety of scientific studies. Nonetheless, many mechanisms and components involved still remain undiscovered, which will provide a continuous source of research. Regeneration corresponds to the renewal or repair of injured cells or tissues so that its initial function can be restored.

Skeletal muscle’s injuries may occur by direct trauma or indirect causes. In order to prevent muscle dysfunction, this tissue is capable to initiate a complex repair mechanism, which involves satellite cells, signaling factors, molecular pathways, multipotent stem cells and other components. Their interactive process regards better understanding.

The myogenic lineage development event requires the upregulation of MyoD and Myf5 and later the upregulation of myogenin and MRF4. The terminally differentiated myocytes express these MRFs and also muscle-specific genes – myosin heavy chain and muscle creatine kinase.

Some satellite cells remain in a quiescent undifferentiated state. Satellite cells can be activated after muscle injury and Pax7 expression is crucial for their specification/expansion.

Muscle regeneration includes the degenerative phase (necrosis and inflammatory response) and the regenerative phase (proliferation, differentiation and fusion of myogenic cells).

Satellite cell pool’s renewal depends on the satellite cell compartment and on adult stem cells derived from bone marrow, adult muscles, neuronal compartment and mesenchymal tissues that are able to undergo myogenic differentiation.

This review article exposed some of the existing results and hypothesis concerned to muscle regeneration: facts that can be mentioned, some of which were analyzed and described in this review: FGF-6 is critical for the mammalian muscle regeneration, possibly because it stimulates or activates satellite cells and the expression of MyoD; MDSC is a population of muscle stem cells with great potential for muscle regeneration and enhancement of muscle cell-mediated therapies; mIGF-1 is a powerful regenerative agent that mediates the recruitment of bone marrow cells to sites of muscle damage; increases bone marrow and local stem cells’ pools, enhances local repair mechanisms and provides an explanation for the effects of supplemental MLC/mIGF-1 transgene expression on muscle mass and integrity, in vitro and in vivo, both tis7 and SKMc15 are present in TIS7(-/-) mice during muscle regeneration, which may enhance muscle stem cells differentiation in vitro and tis7 also plays a regulatory role in adult muscle regeneration; it has been defined a MyoD-Tead2-Fgfr4 pathway that is important for an effective muscle regeneration; the resting interval between two periods of eccentric exercises affects the histological properties of muscle regeneration and is essential to avoid the accumulation and further muscle damage in mice; F-MDSCs regenerate skeletal muscle more efficiently; supplemental mIGF-1 creates a qualitatively different environment capable of sustaining a more efficient muscle regeneration and repair, by modulating the inflammatory response and limiting fibrosis; cdk9-55 has a critical role in muscle regeneration and differentiation in vitro, as it is specifically induced in injured myofibers and its activity is critical to achieve muscle regeneration.

Reversine, may become a future powerful tool to generate and enhance muscle regeneration in many situations, such as musculoskeletal pathologies. It is necessary further investigation, however scientific evidence shows that primary murine and human fibroblasts treated with reversine could be induced to differentiate into skeletal muscle at high frequency both in vitro and in vivo.

The evolution of muscle regeneration knowledge will bring new multiple applications and therapeutic approaches in order to provide a better long-term prognosis for diverse pathologies, such as musculoskeletal disorders.
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