Role of matrix metalloproteinases in skeletal muscle

Migration, differentiation, regeneration and fibrosis

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Matrix metalloproteinases (MMPs) are key regulatory molecules in the formation, remodeling and degradation of extracellular matrix (ECM) components in both physiological and pathological processes in many tissues. In skeletal muscle, MMPs play an important role in the homeostasis and maintenance of myofiber functional integrity by breaking down ECM and regulating skeletal muscle cell migration, differentiation and regeneration. Skeletal muscle satellite cells, a group of quiescent stem cells located between the basement membrane and the plasma membrane of myofibers, are responsible for lifelong maintenance and repairing, which can be activated and as a result migrate underneath the basement membrane to promote regeneration at the injured site. MMPs are able to degrade ECM components, thereby facilitating satellite cell migration and differentiation. This current review will focus on the critical roles of MMPs in skeletal muscle injury and repair, which include satellite cell activation with migration and differentiation. The effect of MMPs on muscle regeneration and fibrous scar tissue formation, as well as therapeutic insights for the future will be explored.

Introduction

Matrix metalloproteinases (MMPs) are a family of enzymes that can selectively digest individual components of the extracellular matrix (ECM)1,2 in the processes of both normal physiological and pathological states.3-6 Twenty-five members of the MMP family have been identified, of which six are membrane-type (MT) MMPs. Each MMP interacts in specific ways with certain elements of the ECM. MMP collagenases (MMP-1, -8, -13 and -18) have the ability to cleave interstitial collagen types I, II and III, and MMP gelatinases (MMP-2 and -9) degrade denatured collagen types IV, VII and X in many tissues.1-3 MMPs are generally secreted as zymogens and are able to extracellularly activate by several proteinases. In vitro studies have indicated that plasmin could directly activate a group of pro-domains of MMPs, such as proMMP-1, proMMP-3, proMMP-9, proMMP-10 and proMMP-13.4 However, the activation of proMMP-2 involves hydrolysis by MT1-MMP during plasmin stimulation. Several other active MMPs also can further activate the proMMPs to constitute the positive feedback mechanisms in many tissues.4,6 On the other hand, MMPs are also adjusted by specific tissue inhibitors of metalloproteinases (TIMPs). Four members of the TIMP family have been identified including TIMP-1, which is synthesized by most types of connective tissue cells as well as by macrophages. TIMP-1 is also able to act against all types of collagenase, stromelysin and gelatinase.2 MMPs, alone or in conjunction with the plasminogen/plasmin system, have the ability to degrade extracellular matrix components. This ability is a requirement for cell migration and tissue remodeling, both of which play essential roles in many physiological and pathological processes.2,6

Skeletal muscle healing after injury and the formation of skeletal muscle during development are similar processes because they both involve progressive specifications, such as proliferation, migration and differentiation of muscle precursors. The fusion of skeletal muscle satellite cells to form terminally differentiated, contractile, highly patterned myofibers is a key step of the process that relates metalloproteinases.7,8 A role for MMPs in myogenesis has long been proposed, and some of the responsible proteases have been identified.5,6,9 Recent studies have demonstrated the important roles of MMP-1,3 MMP-2,6,10,11 MT1-MMP10,12 and MMP-9,5,6,13 in skeletal muscle satellite cell migration and differentiation both in vitro cultured muscle cells and in vivo animal models including mdx mice, a X-link dystrophic skeletal muscle mouse model. The interaction of MMPs and TIMPs in the skeletal muscle system with various conditions has also been well studied.9,14

Muscle Cell Migration and Differentiation In Vitro

It has been reported that MMPs and TIMPs play important roles in skeletal muscle-derived myoblast migration and differentiation...
in vitro.\(^3\)\(^,\)\(^6\)\(^,\)\(^12\)\(^,\)\(^15\) MMP-1 is able to enhance myoblast migration and differentiation in an in vitro wound-healing assay.\(^3\) There is a significant difference in migration distances for C2C12 myoblasts treated with MMP-1 after being wounded when compared with controls at various conditions (e.g., on the flanks coated with collagen, fibronectin or blanket control, Fig. 1) without chemotactic affect.\(^3\) Indeed, MMP-1 treatment directly stimulates the migration of target cell by increasing the expression of migration-related marker proteins N-cadherin and β-catenin, as well as inducing the expression of pre-MMP-2 and TIMP significantly in vitro. The activation of either N-cadherin and β-catenin or pre-MMP-2 and TIMP are able to explain why the MMP-1 treated myoblasts had an increased migration capacity. Additionally, these C2C12 cells displayed a dose-dependent increase in differentiation/fusion capacity to produce significantly more myotubes as well as upregulated myogenin expression when cultured in differentiation media with MMP-1 treating.\(^3\) Besides, numerous studies have indicated the important role of MMP-2, -7, -9 and MT1-MMP in myotube formation.\(^11\),\(^13\),\(^16\) The mechanism behind MMPs in myoblast differentiation/fusion process may be due to the fact that these enzymes can serve to eliminate ECM and/or cell surface components that intercalate and thereby hinder the fusion between two cell membranes. Besides the fusion itself, the preceding steps, such as migration and alignment of myoblasts, may also be affected directly by these MMPs. Cultured embryonic cells failed to form myotubes efficiently in the absence of MMP-2 and MT1-MMP, indicating that MMP-2 and MT1-MMP are the major factors that alter myotube formation in vitro.\(^11\) In fact, the correlation between the expression of MMPs and TIMPs in myogenic differentiation has long been investigated.\(^11\),\(^13\) Masster myotubes express high levels of TIMP-1 mRNA without MMP-9 activity in vitro. This situation is highly analogous to that observed in masseter muscle in vivo, where TIMP-1 protein is abundantly localized to the basal lamina and endomysium of muscle myofibres while MMP-9 protein is detected in a very low level. The regulation of matrix turnover via MMP-9 activation is involved in the events leading to muscle cell migration and myotube formation. This suggests that the ratio between MMPs and TIMPs is an important factor for determining myoblast migration and differentiation. Of the identified 25 members of MMPs and 4 members of TIMPs, the trio TIMP-2, MT1-MMP (MMP-14) and MMP-2 were thought to be more essential in myoblast differentiation in vitro, because these molecules are components of a ternary complex known to act in concert.\(^17\) Biological TIMP-2 plays a role not only in MMP inhibition in some MMPs, but also in MMP activation in others. For example, the relative levels of TIMP-2 and MT1-MMP are critical, since low TIMP-2 levels are associated with MT1-MMP mediated activation of pro-MMP-2; but at higher levels of TIMP-2, MT1-MMP function is blocked, and pro-MMP-2 activation is prevented.\(^3\),\(^6\),\(^11\) The expression of networks among TIMP-2, MT1-MMP and MMP-2 has been detected during myoblast C2C12 differentiation. Among these networks, TIMP-2 expression is upregulated at the same time as in C2C12 differentiation process, but MMP-2 expression is only increased at later stages of this process. In contrast, MT1-MMP expression is downregulated with increased time in a growth medium but is upregulated with differentiation medium. Thus, these three molecules play distinct roles in myoblasts differentiation in vitro.\(^5\),\(^11\),\(^17\) These continuing investigations have repercussions in understanding the in vitro phenomenon of muscle cell migration and differentiation and may therefore give insight into the process of muscle development, adaptation and repair.

**Muscle Cell Migration and Myogenesis During Muscle Injury and Repair**

Skeletal muscle fibers are surrounded by a basement membrane composed predominantly of type IV collagen, laminin, fibronectin and heparan sulfate proteoglycan.\(^4\),\(^8\) Skeletal muscle satellite cells are considered to be a group of quiescent stem cells that are localized between the plasmalemma and the basement membrane of...
muscle fiber. Satellite cells play an important role in the repair of injured or inflamed muscle.\textsuperscript{7,8} Once satellite cells are activated following traumatic injury, they migrate to the injured site and differentiate to form new myofibers that fuse to each other or fuse to local surviving muscle fibers, contributing to muscle regeneration. It is believed that a key step for muscle regeneration is when satellite cells migrate across the basement membrane to access the injured site.\textsuperscript{7,8,18} Therefore, the degradation of ECM must be a central component for satellite cell migration and regeneration in vivo. MMPs have been shown to play an essential role in the remodeling and maintenance of the ECM in a wide range of normal and pathological tissues in many organs.\textsuperscript{1,3,6,9,18} There is evidence of extensive remodeling of the ECM of muscle satellite cells during regeneration. It seems that MMPs have a different pattern of expression during the course of muscle injury and repair. MMP-2 and MMP-9 are mainly involved in this ECM remodeling of skeletal muscle.\textsuperscript{18-21} MMP-2 is extensively upregulated during the first three days following cryolesion injury and the amount of mRNA for this enzyme was reduced at day 7 post-lesion, whereas MMP-2 mRNA returned to baseline levels in the skeletal muscle regeneration process at 10 days post-lesion.\textsuperscript{19} MMP-9 activity is extensively upregulated during the first three days following cardiotoxin injury in tibialis anterior (TA) muscle, whereas after three days following the injury, the amount of MMP-9 mRNA and protein begins to decrease.\textsuperscript{20} Moreover, MMP-9 is localized in inflammatory cells identified as polymorphonuclear leucocytes and macrophages.\textsuperscript{18,20} The MMP-2 activity is concurrent with the regeneration of new myofibers probably due to degradation of type IV collagen of the basement membrane during myoblast proliferation, migration and fusion.\textsuperscript{19} Conversely, MMP-9 might be associated with not only ECM degradation during inflammation, but also activating satellite cells during the initiation of muscle regeneration.\textsuperscript{20,21} Studies also indicated that MMP-1 can effectively reduce muscle scarring and that its activity is related to the ability of the enzyme to digest collagen, thereby facilitating remodeling and regeneration of the injured muscle.\textsuperscript{22} In a mouse model with muscle injury, C2C12 cells were transplanted with exogenous MMP-1 at the site of injury, resulting in the MMP-1 treated limbs contained greater regenerating myofibers and less fibrous scar tissue, which indicates that MMP-1 could enhance muscle regeneration by increasing the number of myofibers and decreasing the amount of fibrous tissue.\textsuperscript{23} Other members such as MMP-13 expression also related to the muscle regeneration process. Researchers found that only the migrating muscle satellite cells with activation, but also all skeletal muscle cells, selectively expressed MMP-13 during skeletal muscle regeneration. MMP-13 activity in vivo may also be correlated with the extent of tissue damage.\textsuperscript{24} One experiment in a transgenic mouse strain showed that MMP-13 expression was biphasic, with peak activities only at days 15 and 37 after injury. These results revealed that MMP-13 was involved in a series of coordinated events during wound healing, which includes not only the long-term remodeling of wound connective tissue, but also the process of skeletal muscle repair. MT1-MMP-deficient mice have smaller and heterogeneous myofibers compared with those in the wild-type mice. In addition, some centrally nucleated myofibers exist in the mutant mice. These fibers represent those that occur during muscle regeneration following tissue damage and are typical in patients with muscular dystrophy. This observation suggests that apart from its role in myogenesis, MT1-MMP also maintains myofiber integrity.\textsuperscript{25}

### Inflammation and Myofiber Regeneration in mdx Mice

Duchenne muscular dystrophy (DMD) is a lethal, X-linked disorder associated with dystrophin deficiency that results in chronic inflammation, sarcolemma damage, and severe skeletal muscle degeneration. Skeletal muscle of patients with DMD follows a general pathological cascade of damage, necrosis and fibrosis leading to muscle weakness and increased stiffness.\textsuperscript{26,27} The biological mechanisms responsible for the dysfunction have not been completely elucidated, but are associated with the absence of the subsarcolemmal protein dystrophin. The mdx mouse is routinely used to study mechanisms of dysfunction in DMD because it too lacks the dystrophin protein.\textsuperscript{26} Numerous studies have indicated that MMPs are key regulatory molecules in the ECM remodeling of DMD patients and mdx mice.\textsuperscript{1,6,26,27} In adult mdx mice, both pro- and active forms of MMP-2 and MMP-9 have been detected in the skeletal muscle. MMP-9 mRNA was localized in inflammatory cells and putative activated satellite cells in injured muscles by detecting with in situ hybridization. The data allowed the correlation of the differential expression of pro- and/or active forms of MMP-2 and MMP-9 with different stages of the degeneration-regeneration process: MMP-9 expression is related to the early stages of inflammatory response and probably to the activation of satellite cells, whereas MMP-2 activation is concomitant with the regeneration of new myofibers at late stages.\textsuperscript{28} The marked inflammation and myo-necrosis were associated with the increased MMP-9 activity as well as TNF\textsubscript{α} production, whereas muscle regeneration evidenced by MMP-2 activity varied at different stages of this disease. Soleus muscles showed a high percentage of NCAM-positive myofibers and MMP-2 activity in the early stages (two weeks) of the disease, but they appeared in the gastrocnemius muscles at 12 weeks and in the diaphragm at 24 weeks.\textsuperscript{29} Increased MMP-2 activity in the diaphragm throughout all stages of the disease suggests important tissue remodeling, which is probably associated with persistent inflammation.\textsuperscript{29} This data indicates that the microenvironment of distinct skeletal muscle may influence a particular kinetic pattern of MMP activity, which ultimately favors persistent inflammation and myofiber regeneration at different stages of the myopathy in mdx mice.\textsuperscript{29} Skeletal muscles in mdx mice also exhibit differing degrees of pathological changes, such as fibrosis formation. Fibrous scar tissue is a potential factor that impedes muscle regeneration by posing as a mechanical barrier to cell migration and fusion, providing inappropriate signals for cell differentiation, and limiting vascular perfusion of the injury site, subsequently leading to incomplete functional recovery.

A persistent imbalance between collagen biosynthesis and degradation has been suggested to contribute to hypertrophic scars and fibrosis in different tissues. Similarly, high levels of collagens deposition have been found in the diseased and injured area.
Matrix metalloproteinases in skeletal muscle

of skeletal muscle.\(^{22,23}\) MMPs are naturally responsible for the degradation of collagen as well as a great number of other ECM compounds (such as fibronectin). In the injured and diseased skeletal muscle, the predominant components of the ECM and the resulting fibrous scar tissue include collagen types I and III. MMP-1, a class of naturally occurring collagen-digesting enzyme, is able to digest fibrous scar tissue as well as to aid regeneration by removing this blockade, and improve muscle healing after injury.\(^{22}\) The introduction of MMP1 into previously formed scar tissue, which specifically denatures collagen I and III, could result in degradation of the principal component, collagen, and thus facilitate scar-free muscle healing. MMP-1 also enhances myoblast migration and differentiation, which is a critical step in the sequence of muscle regeneration.\(^{3}\) After MMP-1 treatment within the dystrophic skeletal muscles of \(mdx\) mice, myoblast transplantation was greatly improved through the promotion of migration and differentiation, leading to more myofibers formation with greater size in transplanted myoblast coverage (Fig. 2).\(^{3}\) Combining with differentiation, leading to more myofibers formation with greater size in transplanted myoblast coverage (Fig. 2).\(^{3}\) The introduction of MMP1 into previously formed scar tissue, which specifically denatures collagen I and III, could result in degradation of the principal component, collagen, and thus facilitate scar-free muscle healing. MMP-1 also enhances myoblast migration and differentiation, which is a critical step in the sequence of muscle regeneration.\(^{3}\) After MMP-1 treatment within the dystrophic skeletal muscles of \(mdx\) mice, myoblast transplantation was greatly improved through the promotion of migration and differentiation, leading to more myofibers formation with greater size in transplanted myoblast coverage (Fig. 2).\(^{3}\) Combining with differentiation, leading to more myofibers formation with greater size in transplanted myoblast coverage (Fig. 2).\(^{3}\) The introduction of MMP1 into previously formed scar tissue, which specifically denatures collagen I and III, could result in degradation of the principal component, collagen, and thus facilitate scar-free muscle healing. MMP-1 also enhances myoblast migration and differentiation, which is a critical step in the sequence of muscle regeneration.\(^{3}\) After MMP-1 treatment within the dystrophic skeletal muscles of \(mdx\) mice, myoblast transplantation was greatly improved through the promotion of migration and differentiation, leading to more myofibers formation with greater size in transplanted myoblast coverage (Fig. 2).

Figure 2. MMP enhances myoblast transplantation in the skeletal muscle of \(mdx\) mice. \(1 \times 10^5\) LacZ positive C2C12 cells and fluorescent beads were injected into the gastrocnemius muscles (GMs) of \(mdx/SCID\) mice along with 200 ng of MMP-1 (Sigma) in the left GMs and PBS in the right GMs. GMs were harvested at different time points. LacZ staining with Eosin, along with immunohistochemistry for dystrophin was performed. Results showed that MMP-1 treated cells fused into muscle grafts to a greater degree (A) compared to control cells (C) at two weeks post transplantation. Using immunohistochemistry, higher numbers of dystrophin positive myofibers with larger size were detected within MMP-1 treated muscle (B) compared to control (D) at two weeks. It shows that MMP-1 treated C2C12 cells migrated at increased distances from the original injection site and showed a greater degree of engraftment. Red, dystrophin; green, beads; blue, nuclei; white asterisks, dystrophin positive myofibers.

Therapeutic Insights for the Future

With the increased research data that concerns the pathogenesis of skeletal muscle injury and repair as well as dystrophic muscles, new cellular and molecular therapies aimed at altering these processes are being investigated. As reviewed above, in both cell culture in vitro and in vivo animal models, it has been uncovered that MMPs play an essential role in ECM remodeling compounds and skeletal muscle satellite cell migration. In the development of fibrosis, the balance between collagen deposition and degradation is a key to settle scar tissue formation in injured skeletal muscle as well as other different organs and tissues. Degradation of collagen, as well as a great number of other extracellular matrix compounds, is initiated by MMPs, of which also can promote muscle cell in migration, proliferation and differentiation in injured and diseased skeletal muscles. The application of MMPs also faces the aspects of determining the adequate dosing and sustained delivery. Therefore, prolonging the life of MMPs has been suggested including continuous intravenous injection, gene therapy or combining degradable polymers. On the other hand, the ratio between MMPs’ or TIMPs’ expression also can positively or negatively alter the stability of the tissue state, balancing both of their expression is necessary during MMP application in muscle healing. In fact, an increased mRNA expression of TIMP-1 and -2 had been detected in the skeletal muscle of Duchenne muscular dystrophy, which is a cause of MMPs’ inhibition and fibrous scar tissue formation in the diseased...
mature. Additionally, using other growth factors or non-toxic chemical agents to promote MMP expression may prove beneficial for tissue remodeling. To support the applications, the signaling mechanisms involved in regulating the effects of MMPs on skeletal muscle pathogenesis need to be further clarified. On the basic research, the mechanism behind MMP in muscle cells, including genetic networks and signaling pathway activation within satellite cell and stem cell proliferation, migration and differentiation should be addressed in future studies. Thus, clinical trials and applications of nontoxic MMPs or their inhibitors based on their signaling mechanisms for dystrophic muscles are expected to be carried out in the near future.

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