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Extracellular Matrix, Proteases and Physical Exercise

Extracellular-Matrix, Proteasen und körperliche Belastung

REVIEW

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Summary

- **Physical exercise** evokes mechanical loading conditions and thereby controls a diversity of physiological and biological reactions ranging from improved endurance capacity to increased muscle strength.
- **This holds true for the endothelial cell system,** which is controlled by exercise-dependent shear forces and for the skeletal muscle system subjected to mechanical loading. Shear forces/mechanical loading result in the activation of proteolytic enzymes, called proteases, which are capable of remodeling the central mechanosensitive cell scaffold, the extracellular matrix (ECM). Remodeling of ECM components evokes the release of cleavage fragments. These cleavage fragments in turn exert defined biological functions, such as angiogenesis modulation, nitric oxide generation or muscle regulation.
- **This mini-review** (i) describes the main players involved in this processing cascade and discusses the influence of physical exercise on mechanisms of ECM remodeling in the endothelial cell and muscle systems and (ii) discusses influences of societal challenges, such as aging or chronic diseases on ECM remodeling.

KEY WORDS:
ECM, Exercise,
Endothelial Cells, Skeletal Muscle

Zusammenfassung

- **Körperliche Belastung** beschreibt einen physiologischen Reiz, welcher eine Vielzahl physiologischer und biologischer Reaktionen hervorruft, die von der Steigerung der Ausdauerleistungsfähigkeit bis zur Erhöhung der Muskelkraft reichen. Im Detail kann argumentiert werden, dass Bewegung die mechanische Belastung des Gewebes erhöht, um gezielt biologische Prozesse zu kontrollieren.
- **Dieser Zusammenhang ist belegt für das Endothelzellsystem,** welches durch (belastungsabhängige) Scherkräfte beeinflusst wird. Scherkräfte resultieren in einer Aktivierung proteolytischer Enzyme, die als Proteasen bekannt sind. Diese Proteasen sind in der Lage, die zentrale mechanosensitive Einheit, die Extracellular Matrix (ECM), umzubauen. Der Umbau bedingt die Freisetzung definierter Spaltfragmente, welche wiederum biologische Prozesse vermitteln, z. B. Angiogenese-Inhibition.
- **Dieses Mini-Review** beschreibt (i) die Hauptkomponenten dieser Umbaukaskade und diskutiert den Einfluss körperlicher Belastung auf Mechanismen dieses Umbaus. Weiterhin ist neben dem Endothelzellsystem auch das Skelettmuskelgewebe konstant einer mechanischen Belastung ausgesetzt. Aus diesem Grunde will dieses Mini-Review weiterhin die Effekte (belastungsinduzierten) ECM-Umbaus und die Konsequenzen auf das Skelettmuskelgewebe diskutieren.

SCHLÜSSELWÖRTER:
ECM, Bewegung,
Endothelzellen, Skelettmuskulatur

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Introduction

Tissue functions rely on defined cell types. One example is the vascular system composed of endothelial and vascular smooth muscle cells (ECs, VMSCs, respectively) and additional supportive cell types, such as pericytes, fibroblasts etc. (8). Among defined cell types, the extracellular matrix (ECM), a cell-specific and highly specified environment, contributes to the function, capability and structure of a tissue (16). The ECM is constantly remodeled in a physiological (as opposed to pathophysiological) manner resulting in ECM component breakdown and synthesis. The ECM remodeling is mainly controlled by a protein superfamily known as proteases, which are key to conserve the tissue-specific functions of the ECM (5).

Physical exercise has been identified to exert beneficial and health-promoting effects in a variety of disease entities and pathophysiological conditions (15). To this respect, it was demonstrated that regular physical exercise improves insulin sensitivity and hence to improve metabolic functions of diabetes type 2 patients (6, 41), to reduce cardiovascular ›

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malfunctions (3, 61) or improve systemic functions of patients suffering from different cancer types (14, 17, 64). A central tissue stressed by physical exercise is the skeletal muscle tissue. The skeletal muscle tissue represents about 40-50% of the entire body weight and hence characterizes the largest 'organ' of the human body (18). Skeletal muscles consist of myofibers, which exert different, but highly defined capabilities (48). Type 1 myofibers show oxidative capacities, contain a high proportion of mitochondria and myoglobin, are fatigue-resistant, but are slow-twitching generating low forces (48). On the other hand, type 2 myofibers represent glycolytic fiber, fatigable, fast-twitch fibers that generate high forces and which can be classified into type 2A (intermediate fiber type), type 2X and type 2B (in rodents) (48).

Myofibers are assembled into complex entities to generate controlled and directed contractions for force production (42). The functional assembly is governed and maintained by components of the ECM (20). Also skeletal muscle tissues harbor specialized ECM structures (20). It is important to mention here that the ECM is not just a 'passive' structure providing support to tissues as a scaffold, but that the ECM is rather 'active' in the way that it controls shapes, functions and capabilities of tissues, including skeletal muscle fibers, by a variety of mechanisms (26).

This mini-review mainly focusses on the ECM, its processing by specific proteases and its biological functions. A second part of the review will concentrate on linking protease activity and subsequent ECM remodeling to physical exercise. The last part describes the remodeling of the ECM and protease activity under disease settings and which effects physical exercise can exert. Where appropriate, a link to striated muscle, specifically skeletal muscle tissue will be provided.

Extracellular Matrix

The extracellular matrix is a non-cellular component forming an essential microenvironment within all tissues and organs (16, 50). The ECM provides thereby a physical scaffold for cells and skeletal muscle fibers and its constituents. Further, the ECM initiates and controls critical biochemical as well biomechanical cues essential for physiological capacities of embedded cells and skeletal muscle fibers (26). The impact and significance of the ECM is evidently highlighted by the wide range of diseases directly caused by or associated with ECM disorganizations, such as cancers, cardiovascular diseases, neurological diseases or myopathies (4, 5, 28). The stiffness of the ECM is a critical regulator in this respect, because it differs between tissues and actively adapts to tissues' properties as well as to extrinsic and intrinsic signals.

The ECM is fundamentally composed of water, proteins and polysaccharides and unique combinations of ECM components can be found in different tissues. It is important to mention that each tissue has an unique ECM composition and topology (16). The two main classes of macromolecules composing the ECM are proteoglycans (PGs) and fibrous proteins (16). Fibrous proteins are mainly represented by collagens, elastins, laminins and fibronectin. Collagens represent a large superfamily of triple helical proteins that have important roles in tissue scaffolding, cell adhesion, cell migration or tissue repair (29, 31). Out of so far 28 identified collagens, collagen I (Col1) is the archetypical collagen characterized by a triple helical structure without imperfections and fibril assemblies (29, 31). However, many collagens differ from Col1 in that they show triple helix interruptions, fail to assemble into fibrils or are transmembraneous (30). The most important and relevant groups of collagens are the following: fibril-forming collagens, fibril-associated collagens with interrupted triple helices (FACITs), network-forming collagens (e.g. Col4), transmembrane collagens, anchoring fibrils and beaded-filament-forming collagens (e.g. Col6) (29–31). Different reports have highlighted the importance of collagens for skeletal muscle function and integrity. It was demonstrated that Col15/18, an integral membrane collagen, is critical for skeletal muscle biology. In detail, Col15/18 deletion in mice resulted in a myopathy-like phenotype with severe pathological signs of muscle weakness (10). Another example of skeletal muscle-controlling collagens is Col6. Col6 is a satellite cell (the natural skeletal muscle stem cell, (57)) niche component with essential functions in satellite cell activations, as the deletion of Col6 in mice resulted in reduced skeletal muscle regeneration upon injury (55). These data demonstrate that the ECM controls skeletal muscle physiology through collagens.

Proteoglycans are heavily glycosylated proteins consisting of a core protein with at last one or more covalently attached glycosaminoglycan (GAG) chains (44). The glycosaminoglycan chain binds to the core protein through a tetrasaccharide bridge via a serine residue in the sequence -Ser-Gly-X-Gly- (where X can be any animo acid except for proline). However, this sequence does not automatically provoke a glycosaminoglycan chain attachment. Due to the occurrence of sulfate in the chain sequences, PGs are usually negatively charged under physiological conditions (30). PGs are classified by their relative size (small PGs vs. large PGs) and the nature of their glycosaminoglycan chains (chondroitin sulfate/dermatan sulfate vs. heparan/chondroitin sulfate vs. chondroitin sulfate vs. keratan sulfate) (27). To exemplify this nomenclature in more detail, the syndecans represent small heparan/chondroitin sulfate PGs, whereas perlecan represents a large heparan/chondroitin sulfate PG (27). Syndecans play critical roles in skeletal muscle physiology as they control satellite cell activities (54) and perlecans manages cardiac muscle properties by providing a scaffolding for proper basement membrane organization (47). Furthermore, perlecan is a critical basement membrane constituent to maintain skeletal muscle properties during development. It was demonstrated in zebrafish that deletion of perlecan resulted in abnormal actin filament orientation and disorganized sarcomeres, suggesting a role of perlecan in the development of myopathies (65). These data underline the significance of the ECM PGs in tissue, and specifically in skeletal muscle integrity and physiology.

Among these properties, ECM components fulfil another, yet still underestimated, function. ECM components bind a variety of growth factors like a sponge and thereby enhance the direct and acute bioavailability of these growth factors (26, 50). This principle is also important in skeletal muscle tissues, as the heparan/chondroitin sulfate PGs bind to heparin-binding sequences harboring growth factors, such as IGF-1 (26). IGF-1 in turn plays a critical role in skeletal muscle metabolism control by initiating signals that result in the translocation of the glucose transporter 4 (Glut-4) to and an incorporation of Glut-4 into the sarcolemma to foster glucose uptake (53). Hence, ECM components indirectly contribute to skeletal muscle energy supply by providing the opportunity to stimulate glucose uptake by ECM-bound and –released IGF-1.

Among these factors, the influence of aging, a major societal factor, which influences skeletal muscle and vascular system properties, on ECM regulation and function is critical. It has been widely recognized that collagen deposition and crosslinking increases with aging in both striated muscle tissue (62) and vascular systems (37). Further, ECM degradation capacity also

increases due to elevated protease activities. In cardiac tissue, total collagen represents about 5-6% of left ventricular protein content in young rats of one month of age (9). This increases to about 10-12% in 26 months-old rats. This goes along with elevated collagen fibril diameter in aged tissues (9). In humans, autopsy studies of cardiac tissue from an 80 years-old individual demonstrated that Col1 increased, whereas Col3 decreased compared to young subjects (36). This collagen shift evokes increased ECM stiffness, resulting in impaired tissue biomechanics. In skeletal muscle tissue, the ECM accounts for up to 10% of total muscle weight and is dominated by Col1 (31). Collagen fibrils are more loosely oriented near individual fibers to permit muscle contractions. Contrary, collagen fibrils are densely layered and highly oriented in the perimysium to transmit force to tendons in an optimally manner (19). Comparable to cardiac tissue, collagen deposition also increases in skeletal muscle with aging with similar ratio shifts between Col1 increases and Col3 decreases. This results in increased Young's modulus of skeletal muscle tissue with aging, reflecting increased tissue stiffness (32, 62). A recent example of negative effects of aged skeletal muscle ECM describes the loss of myogenicity of satellite cells, whereas fibrogenic markers were increased, which terminates reduced skeletal muscle regeneration capacity (49). These data highlight that aging has a tremendous effect on ECM remodeling, mainly by increasing ECM stiffness causing negative adaptations of tissues, such as cardiac and skeletal muscles.

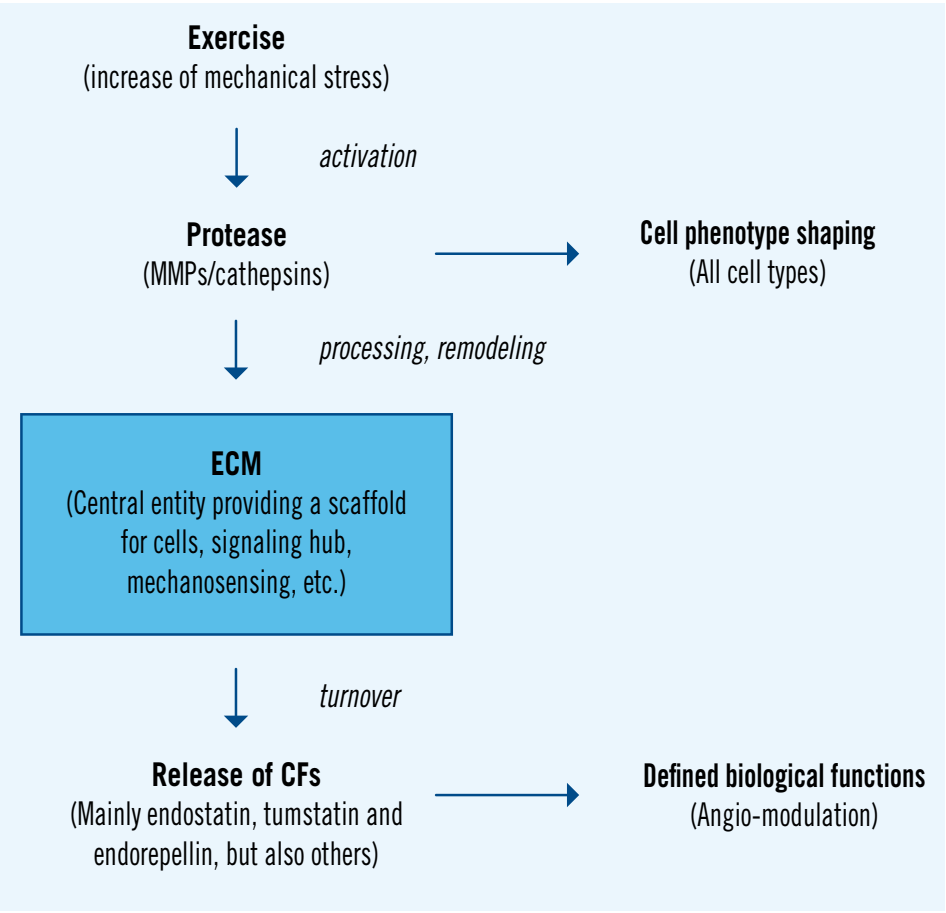

Figure 1

Overview of sequence of actions evoked by exercise. Exercise increases mechanical forces in tissues, such as skeletal muscles or endothelial cells. This mechano-loading is a potent stimulus for the activation of proteases, which further are able to remodel and process nearly all kinds of ECM components. The result of ECM processing and remodeling is the release of ECM cleavage fragments (CFs), in this context here (but not limited to) mainly endostatin, tumstatin and endorepellin. CFs exert defined biological functions, mainly studied in the context of angiogenesis (details see text).

Role of Proteases in the ECM

Cleavage of ECM components is a central process during ECM and tissue remodeling to promote structural and functional changes as well as releasing biologically active molecules (5), such as growth factors (see above). This ECM remodeling is governed and controlled by protein families of proteases. Proteases classify enzymes that initiate and control proteolysis. Proteolysis describes the process of protein catabolism or breakdown by hydrolysis reactions. A wide range of different proteases has been identified and is nowadays classified into seven broad families: serine proteases, cysteine proteases, threonine proteases, aspartic proteases, glutamic proteases, metalloproteases and asparagine peptide lysases (5). In this mini-review, the focus will be on cysteine and metalloproteases.

Metalloproteases (MMPs) are the main enzymes involved in ECM degradation. Under basal physiological condition, the activity of MMPs is low. However, stress (e.g. inflammation, cancer environments or exercise (21)) or repair mechanisms (e.g. angiogenesis (46)) increase MMPs activity several fold. MMPs are produced either as membrane-anchored or as soluble isoforms and cleave ECM components with wide substrate specificities (5). MMPs are secreted as zymogens, meaning that they are released as inactive enzymes and biochemical processes controlled by serine proteases or other MMPs activate them in the extracellular space. The family of MMPs is the central family of proteases, because collectively they degrade all known ECM components (5). Out of the family of MMPs, the gelatinases A and B (MMP-2 and MMP-9, respectively) should be highlighted briefly. These two MMP isoforms control the degradation of a variety of ECM molecules. Specifically, MMP-2 and MMP-9 are involved in angiogenic processes (46, 51). These (exercise-induced) angiogenic processes are key for the remodeling the capillary bed to support skeletal muscles with sufficient amounts of oxygen (23).

Cysteine proteases summarize ubiquitously expressed proteolytic enzymes that are found at high concentrations in the endolysosomal system (56). Cathepsins represent a major subclass of cysteine proteases involved in numerous physiological processes. In humans, eleven cathepsins have been identified (56). To reach their final destination of action, cathepsins are either transported to the cell membrane or are directly secreted into the extracellular space. Similar to MMPs, ›

cathepsins are usually secreted as zymogens, which are then turned into active enzymes in the extracellular space (56). Cathepsins are stable under unfavorable oxidative and pH conditions and degrade a number of ECM components, such as fibronectin, elastin, laminin or perlecan (56). Of interest in the context of this mini-review is cathepsin L. Cathepsin L is a ubiquitously expressed lysosomal endopeptidase involved in the initiation of protein degradation. It is capable of degrading skeletal muscle ECM components, including Col15/18 or perlecan (13). By this, cathepsin L might be involved in shaping skeletal muscle structure and functional characteristics under stress conditions.

Cleavage Fragments of Extracellular Matrix Components

As mentioned above, the ECM underlies a constant turnover. One result of ECM degradation by proteases is a newly organized ECM network. A second result of ECM degradation is the liberation of cleavage fragments from ECM components, such as collagens or PGs. These cleavage fragments possess biological activities and hence are biological determinants rather than waste products (50).

Several biologically active cleavage fragments have been identified. Herein, the following cleavage fragments will be briefly highlighted: endostatin, tumstatin, and endorepellin.

Endostatin was identified as an angiostatic peptide able to suppress endothelial sprouting in cancer environments (39). The endostatin peptide is harbored within the non-collagenous NC1 domain of Col15/18. The molecular size of endostatin varies from 38 kDa to 20/22 kDa (24). Of note, endostatin exerts its proposed biological functions in a size-independent manner. Cathepsin L (but also B) and elastases are able to liberate the 20/22 kDa endostatin peptide, whereas MMP-2 and MMP-9 liberate the 38 kDa endostatin fragment. Beside the angiostatic properties of endostatin, it was demonstrated that endostatin evokes also angiogenic-modulatory functions exerted by its ability to activate the endothelial nitric oxide synthase (eNOS) in endothelial cells in a concentration-dependent manner (59). This finding was important, because it demonstrated that endostatin is not solely angiostatic, but also possesses angiogenic functions. The receptor network of endostatin signaling is not fully elaborated, but it was found that endostatin amplifies its signals via vascular endothelial growth factor receptor 2 or integrins (11).

Tumstatin is the 28 kDa cleavage fragment of the α3 chain of Col4. Its structure is similar to that of endostatin and both peptides share about 14% amino acid sequence homology. Similar to endostatin, tumstatin also serves as an angiostatic peptide and is pro-apoptotic. Tumstatin binds to integrin αVβ3 and inhibits the activation of the focal adhesion kinase, PI3K, protein kinase B and the mechanistic target of rapamycin (mTOR). In contrast to endostatin, tumstatin has yet been solely identified as an angiostatic agent. Further research might uncover novel yet unknown capabilities of tumstatin in ECs and skeletal muscle tissues.

Endorepellin represents the 85 kDa large C-terminal cleavage fragment of the heparan/chondroitin sulfate PG perlecan (38). Endorepellin has been shown to function as an angiogenesis inhibitor (38). Furthermore, endorepellin potentially binds endostatin to counteract its angiostatic effects (38). Consequently and similar to endostatin, endorepellin is an angiostatic peptide on the one hand with angiogenesis-modulating functions on the other hand. Mechanistically, endorepellin binds to α2β1 integrins of ECs to trigger a pathway that increases the second messenger cAMP, activation of protein kinase B/Akt and focal adhesion kinase and a transient activation of p38 MAP kinase (2). This pathway ultimately results in the disassembly of actin stress fibers and focal adhesions.

Conclusively, ECM cleavage fragments possess highly specific biological properties and control ECs as well as skeletal muscle properties through defined signaling pathways.

How Physical Exercise Activates Proteases and Changes the Extracellular Matrix

The onset of physical exercise provokes increased blood flow and causes increased shear forces at the endothelial cell layer (63). Mechanical forces, such as shear forces, are sensed by protein complexes known as focal adhesions ((34), striated muscle equivalents are known as costameres, (40)). Central components of focal adhesions are integrins representing transmembrane proteins consisting of an α- and a β-subunit (25). Intracellularly, integrins connect to focal adhesion components, such as integrin-linked kinase, talin or vinculin. From these structural proteins, signaling molecules, including kinases, are regulated to govern downstream events, such as protein synthesis, cell proliferation/differentiation etc. (34). Endothelial cells sense increased shear forces and by this activate programs to actively remodel their surrounding ECM microenvironment to liberate ECM cleavage fragments and consequently to release them into the interstitial space or into the circulation.

Only a few studies have been performed to investigate the potential of exercise to increase ECM cleavage fragements into the circulation. Gu et al. (22) demonstrated that acute exercise caused significantly increased circulating endostatin levels, which further correlated with increased VO2peak values during an incremental running test (22). The group of Wilhelm Bloch also focused on effects of physical exercise on endostatin regulations. They demonstrated that acute intensive exercise increased circulating endostatin, independent from additional mechanical input and additional metabolic stress, such as hypoxia (51). Furthermore, they found that regular training decreased circulating endostatin concentrations indicating a training-dependent stabilization of endostatin-related ECM turnover in physiologically stressed tissues (52). In a third project, the Bloch group demonstrated that resistive vibration exercise lasting six weeks provokes initial endostatin elevations acutely upon exercise. In addition to these finding, it was demonstrated that endostatin-enriched serum collected from the subjects increased ECs proliferation. Rullman et al. (45) reported similar results, namely acutely increased endostatin levels in humans upon exercise. No data are available for tumstatin and endorepellin regulations during physical exercise.

Beside endostatin, also MMPs and their activities have been determined by exercise. Rullman et al. (45) demonstrated by in situ zymography that the activity MMP-9 increased significantly upon acute exercise in human muscle tissues, whereas MMP-2 remained unchanged. In line with these observations, MMP-2 and MMP-9 have been demonstrated to be elevated by acute exercise under different acute (1, 51) and chronic training regimes (52). The active isoform of the cysteine protease cathepsin L is also triggered by acute exercise (52).

Collectively, these data highlight the potential of physical exercise to activate different classes of proteases, which activity levels correlate with liberated ECM cleavage fragments, such as endostatin. The benefit of endostatin liberation could be an endostatin-dependent increase of nitric oxide generation by endothelial cells (59) to actively regulate the vascular tone and vessel diameter during acute exercise.

ECM Remodeling and Protease Activity under Disease Settings

A wide spectrum of diseases is either directly caused by or associated with remodeling of the ECM and hence with increased activities of proteases. Without going into detail, common and well-established examples for these postulates are cancers (5), cardiovascular diseases (33), myopathies (4) or metabolic disorders, such as type 2 diabetes mellitus (7).

However, the effect of exercise on ECM cleavage fragments and proteases in disease-associated circumstances is more or less unknown. One study measured endostatin levels in a six months lasting training intervention on elderly overweight men. The authors found that both running and cycling exercises caused a significantly decrease in circulating endostatin levels (7), which is in contrast to other studies (1, 22, 45, 51, 52). The reason is unclear, but it is reasonable that the overweight conditions of the subjects might be a causative factor for this discrepancy. Brixius et al. (7) and Makey et al. (35) both reported that obese women show significantly lower circulating endostatin levels. In this regard, it was postulated that ECM remodeling could be associated with diet-induced insulin resistance in different metabolic tissues and circumstances (60). Here, the focus has been on integrin receptors, which influence the regulation of insulin action. This is of particular interest, because ECM cleavage fragments bind to a variety of different integrin receptors (12) and could hence influence insulin sensitivity in different tissue. In this regard, inflammation and inflammatory conditions are also regulated by the ECM and its cleavage fragments. Chronic inflammation evoked by malnutrition or as a secondary effect of chronic diseases results in ECM synthesis reflected by increased collagen production, whereas at the same time ECM degradation is reduced reflected by decreased MMP activities. Related to this finding, a selective cleavage of Col1 has been observed, which results in chemotactic Col1 fragments that contribute to inflammation. The acetylated tripeptide, Pro-Gly-Pro (acetyl-PGP), generated by MMP8- or MMP9-mediated Col1 cleavage mimics the chemotactic effect of CXC-chemokine ligand 8 (CXCL8) neutrophils (58). In skeletal muscles, inflammation controls ECM production and hence functionality of this tissue. To exemplify this, acute injury of healthy skeletal muscle results in rapid and controlled inflammation that supports removing necrotic and damaged muscle fibers. On the contrary, chronic inflammation (e.g. under diabetic conditions, muscle dystrophies, etc.) triggers excessive accumulation of ECM components. The functional consequence is i.a. reduced muscle repair upon injury due to reduced satellite cell activity and muscle tissues being replaced by fibrotic tissue (43).

Collectively, ECM remodeling controls a variety of disease conditions. Only a few studies analyzed the potential of ECM cleavage fragments in this regards; however, future studies should elaborate on the role of cleavage fragments in more detail, because they might beneficially be involved in the control of e.g. insulin sensitivity. In this regard, the effect of physical exercise is important to study, because (i) ECM cleavage fragments have been shown to be liberated upon exercise (51) and (ii) exercise is beneficial in many disease conditions – it is time to analyze this connection in detail.

Concluding Remarks

The ECM represents a highly dynamic entity responsible for the control of several biological and (patho)physiological processes. The ECM turnover of mainly mediated by protease families, with MMPs as the major class. The ECM turnover and hence protease activation results in the liberation of ECM cleavage fragments, which possess biological properties ranging from angiostatic capabilities in cancer environments to rather angiogenic processes, such as nitric oxide generation. ECM and its cleavage fragments contribute to the control of disease regulation, such as impaired muscle repair or insulin sensitivity. However, the knowledge about precise mechanisms is still lacking. Aging is another societal challenge that alters ECM composition in the vascular and muscle systems, hence, contributing to malfunctions. Physical exercise has been shown to influence the ECM turnover and cleavage fragment liberations by enhancing protease activities potentially to regulate signaling pathways supporting exercise-demanding requirements (Figure 1). Therefore, effects of acute exercise and chronic training on ECM remodeling and cleavage fragment liberation under diseased conditions should be studied to open novel avenues for the understanding, by which pathways and mechanisms exercise/training positively influence disease outcomes or aging-related impairments.

Conflict of Interest

The authors have no conflict of interest.

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