

UNVEILING MMPS' CONTRIBUTION TO CARTILAGE DEGRADATION IN KASHIN-BECK DISEASE

Dr. David Lee and Dr. Sophia Khan

Department of Pathology, University of Toronto, Canada

Abstract: Kashin-Beck disease (KBD) is a chronic osteochondrosis endemic to certain regions, notably in Russia and China, characterized by profound articular and epiphyseal cartilage damage. Chondrocyte necrosis, excessive apoptosis, and extracellular matrix degradation are hallmarks of KBD pathology. While various factors have been linked to chondrocyte injury in KBD, comprehensive understanding of underlying mechanisms remains elusive, hindering effective treatments.

Matrix metalloproteinases (MMPs), a family of zinc-dependent proteins associated with extracellular matrix degradation, notably MMP-13, have been implicated in cartilage destruction. However, their precise role in KBD pathogenesis is not yet well-delineated. Recent findings suggest that the regulatory interaction of miR-488 with ZIP-8 can inhibit MMP-13 activity, fostering chondrocyte differentiation and cartilage recovery. MMP-13, a critical enzyme in cartilage degradation, primarily targets type II collagen. Unraveling the interplay between MMP-13 and other factors in KBD progression holds promise for more effective treatments and enhanced clinical management. Moreover, exploration of additional molecular and cellular mechanisms at play in KBD development is vital.

Keywords: Kashin-Beck disease (KBD), Matrix metalloproteinases (MMPs), Chondrocyte injury, Extracellular matrix degradation, Pathogenesis mechanisms.

1. Introduction

Kashin-Beck disease (KBD) is an endemic chronic osteochondrosis with a high prevalence and incidence in the eastern Siberia region of Russia and the northeast to southwest wide diagonal zone of China and North Korea. KBD mainly affects articular cartilage, epiphyseal cartilage, and epiphyseal plate chondrocytes, leading to their irreversible coagulation necrosis and apoptosis. The major pathological changes in the disease include chondrocyte necrosis, excessive apoptosis, and the degradation of the extracellular matrix [1]. Although many processes of metabolism, apoptosis, adaptive immune defense, cytoskeleton, cell movement, and extracellular matrix turnover are identified related with KBD chondrocyte injury process, no clear underlying mechanisms have been involved. This leads to the lack of effective treatment options for the development and development of KBD [2-6]. Studies have shown that the opposite is true for matrix metalloproteinases (MMPs). Overall, these indicators represent the

degradation of the extracellular matrix (ECM) of the articular cartilage in patients with KBD. MMPs are a family of zinc-dependent proteins that accumulate in the synovium during inflammation and play an important role in cartilage destruction in arthritis. Among the MMP involved in cartilage degradation, MMP-13 is the main MMP expressed by chondrocytes and synovial cells in human

osteoarthritis (OA) and rheumatoid arthritis (RA). MMP- 13 plays an important role in cartilage damage by cleaving type II collagen, type IX collagen, collagen X collagen, and other extracellular matrix components (e.g., fibronectin, aggregated proteoglycans, and fibromodulin). It was shown that ZIP-8, through the regulatory interaction of miR-488 (a microRNA found in chondrocytes) in mouse OA chondrocytes, can inhibit MMP- 13 activity, thus promoting chondrocyte differentiation and recovery of cartilage development. MMP- 13 is one of the major enzymes catalyzing cartilage degradation. Compared to other MMPs, MMP- 13 expression is more restricted and mainly targets type II collagen degradation in cartilage [7]. Therefore, further studies investigating the role of MMP- 13 in the process of development and progression of KBD and its interrelationship with other factors may help to discover more effective treatment options and provide better clinical management for patients with KBD. Moreover, further exploration of other molecular and cellular mechanisms potentially involved in the development of KBD is also very important [8].

2. **Extracellular matrix ECM (extracellular matrix, extracellular matrix)**

The extracellular matrix (ECM) is composed of self-assembled macromolecular complexes such as collagen, non-collagen glycoproteins, hyaluronic acid, and proteoglycans. ECM is not only a scaffold but also a major regulator of cell behavior, which can serve as a reservoir of growth factors and cytokines, affecting cell proliferation, differentiation, and migration, thus participating in and affecting the overall function of the cell. Secreted matrix molecules from various cell types have different properties and quantities, and changes in these molecules play important roles during growth and development. In the case of cartilage, its ECM is mainly composed of collagen and proteoglycan. The collagen network is responsible for the tensile strength of cartilage matrix, while proteoglycan is responsible for the elastic properties and permeable swelling of cartilage tissue. The conversion of cartilage into bone requires the involvement of multiple different ECM components, and the renewal and remodeling of the ECM is one of the complex mechanisms that maintain the homeostasis of both cartilage and bone. The ECM also plays a role in regulating cell behavior in bone and cartilage. Cells residing in the ECM produce local factors, inflammatory mediators, and matrix-degrading enzymes, and the renewal and degradation of the ECM depends on the local cellular response to metabolic pathways. Both renewal and degradation of normal and pathological stroma occur in the ECM, thereby affecting bone and cartilage function. In the field of ECM research, the function and regulatory mechanism of ECM are mainly explored through the interaction with ECM, ECM degradation and remodeling. ECM studies helps to explain the mechanisms of cell behavior, providing novel therapeutic targets for disease prevention and treatment [9]. The ECM also plays a crucial role in maintaining tissue mechanical and biochemical properties. Tissue-building cells are responsible for the biosynthesis of ECM components, while the matrix also has a direct effect on cell function. Cell-matrix interactions are the result of interactions between matrix molecules and the specific cellular receptors and epitopes on the binding cell surface. These receptors and epitopes not only play a dominant role in cell connectivity and migration, but also regulate cell differentiation and specific protein expression at the gene level, and are involved in signaling [10]. The extracellular matrix creates a specific physiological microenvironment that protects the cell from harmful physical factors and facilitates signal transmission. Collagen is one of the most important components of the ECM and is characterized by a proline-rich repetitive tripeptide domain. Knowledge of the collagen family and collagen-degrading enzymes has been significantly expanded in recent years. This diverse protein family not only plays a role in forming the structural scaffold of the ECM, but also exerts a broader function through its additional domains.

The collagen family includes many types of collagen: I, V, X, XI, X, etc. Among them, type I collagen is one of the most important components of ECM, which is found in various tissues, such as bones, teeth, muscles, skin, tendons and ligaments. Deficiency of type I collagen causes many diseases, such as osteoporosis, Marfan syndrome, and OI. In addition to forming the structural scaffold of the ECM, type I collagen can also participate in signal transduction and control of cell proliferation and differentiation

by binding to receptors on the cell surface. Collagen degrading enzymes are a class of enzymes capable of grading ECM components, including matrix metalloproteinases (MMPs), cathepsin (tPA), protease K, etc. These enzymes play important roles in normal physiological processes, such as cell migration, tissue repair, and regeneration. However, if the activity of collagen-degrading enzymes is uncontrolled or excessively secreted, it will lead to ECM imbalance, resulting in the occurrence and development of diseases. For example, excessive activation of MMPs leads to the rupture of cardiac muscle fibers and cardiomyopathy, while defects in cathepsin can lead to the occurrence of diseases such as pulmonary fibrosis. There are some other ECM components, such as fibrin, elastin and codoin, which are also important. Fibrin is one of the major adhesion molecules in the ECM, binds to integrin receptors on the cell surface and is involved in intracellular signaling. Elastin is mainly found in elastic tissues, such as the arterial wall and the skin. Its main function is to provide resilience to the tissue, allowing it to withstand changes in external forces. Codoprotein is an ECM molecule associated with blood coagulation, which promotes the generation of thrombin and participates in platelet aggregation and coagulation processes. In conclusion, the extracellular matrix functions not only as a structural scaffold for tissues, it can play a crucial role in cell function, differentiation and signaling by binding to specific receptors on the cell surface. The collagen family and collagen-degrading enzymes are one of the most important components of the ECM, and are crucial for the structural integrity of the maintenance and other functional aspects of the ECM. Research on ECM has important implications in the prevention and treatment of many diseases. Collagen has certain physiological functions in living organisms, including the construction of extracellular matrix, the structural support of organs and tissues, cell adhesion, cell signaling, and so on. Its degradation process plays a very important role in development, morphogenesis, tissue remodeling, and repair. However, when the degradation of collagen is unbalanced, it triggers a variety of diseases, especially cancer, rheumatoid arthritis, nephritis, encephalomyelitis, chronic ulcers and fibrosis. The degradation of ECM involves many types of proteases, the main one of which is matrix metalloproteinases (MMP). MMP belongs to a group of endopeptidases whose enzymatic activity is regulated by both Ca^{2+} and Zn^{2+} ions. The first enzyme identified in this group was collagenase- 1 (now known as MMP- 1). MMP- 1 was found in 1962 by Gross and LaPiere, who used the North American frog species *Rana catesbiana*, and found that the degradation of collagen was caused by collagenase ^[11,12].

3. **MMP s (matrix metalloproteinase)**

MMP- 1 is an enzyme class that targets collagen and that is able to cut the 3- 1 peptide bond in collagen molecules. The expression and activity of MMP- 1 are influenced by many factors, including physiological and pathological factors. The expression and activity levels of MMP- 1 are generally relatively low during normal development and maturation. However, in disease states, such as chronic infection, tumor, arthritis, etc., the expression and activity levels of MMP- 1 will increase significantly, thus triggering tissue destruction and disease occurrence. In addition to MMP-1, there are other types of MMP involved in the degradation process of ECM, such as MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, etc. MMP-2 mainly acts on components such as collagen, prothrombin and vitreous protein in the matrix. MMP-3 mainly acts on components such as proteoglycans, proteoglycans and amyloid substances in the matrix. MMP-7 mainly acts on the components such as macromolecular proteins, adhesion molecules and lipoproteins in the matrix. MMP-8 mainly acts on components such as collagen I, III, and V in the matrix. MMP-9 acts primarily on collagen IV and V in the matrix. In addition to MMP, there are other proteases involved in the ECM degradation process, such as asparag, adhesion molecule proteases, angiogenesis inhibitors, etc. These proteases are expressed and active differently in different tissues and cell types, so their roles in ECM degradation are vary ^[13]. In conclusion, the degradation process of ECM plays important roles in the development, morphogenesis, tissue remodeling, and repair of living organisms. As one of the major proteases for ECM degradation, the dysregulation of MMP triggers the occurrence of many diseases. Therefore, deepening the research on the mechanism of ECM degradation is important for the prevention and treatment of diseases

associated with ECM. The biological activity of MMP is regulated in multiple organisms and plays important roles in many biological processes. Currently, 24 known MMP species have been identified in vertebrates, of which 23 have been identified in humans. These metalloproteinases are extensively classified, starting with MMP-1 and ending with MMP-28, but not including MMP-4, MMP-5, MMP-6, and MMP-22. MMP is involved in trophoblast implantation, embryogenesis, bone growth, angiogenesis, wound healing, and tissue regeneration. Expression of the MMP gene was mainly in fibroblasts but also in neutrophils, monocytes, macrophages and endothelial cells. The biological activity of MMP is regulated at several levels, including gene transcription, regulation of MMP mRNA half-life, regulation of cell-secreted MMP, activation of enzymatic forms (pro-MMP, zymogen), inhibition of catalytically active enzymes, and enzymatic activation. MMPs are maintained at constant low levels in tissues and are under the control of neuroimmune hormones. Transcriptional regulation at the cellular level is influenced by growth factors, cytokines, hormones, cell-cell, and cell-ECM interactions, as well as physical factors (e.g., UV radiation). Metalloproteinase activity is tightly regulated by activation of the inactive zymogen and natural inhibition of endogenous inhibitors. Enzymogen activation occurs through proteolytic cleavage of the prodomain protecting the catalytic site. Several MMP are involved in the activation of other pre-MMP, often leading to a cascade of activations. For example, MMP-3 and MMP-10 activate MMP-1, MMP-7, MMP-8, and MMP-9 to enhance ECM degradation, whereas MMP-14 activates the presence of MMP-2 and MMP-13 TIMP-2. MMP-2 and MMP-13 also contribute to the cleavage of the pro domain in pro-MMP-9 by affecting cell migration in tumor invasion and metastasis. This complex MMP cross-activation network is able to disrupt the ECM if strict regulation of MMP is compromised. Therefore, the study of MMPs has become a hot spot in the field of life sciences [14-16].

4. **Activation and inhibition of MMP are a series of controlled processes**

MMP is a class of proteases that can degrade the extracellular matrix, and they play important roles in the pathological processes of many diseases. The activity of MMP is influenced by various factors, including inflammatory cytokines, hormones, growth factors, metalloproteases, etc. Various factors increase MMP expression, including inflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor A [TNF- α]), hormones and growth factors (transforming growth factor [TGF- β], epidermal growth factor [EGF], platelet-derived growth factor [PDGF], and basic fibroblast growth factor [bFGF]). These factors can increase the expression level of MMP by regulating the gene expression of MMP, thereby promoting the MMP activity. In contrast, the expression of MMP inhibitors (MMPI) includes: corticosteroids, retinoic acid, heparin, and interleukin 4. These inhibitors can inhibit MMP activity by regulating gene expression of MMP or by directly binding to MMP. In addition to being directly regulated by these factors, MMP activity is regulated by other proteases. For example, the action of proteases (plasmin, trypsin, chymotrypsin, elastase) and certain metalloproteinases (MMP-1, MMP-2, MMP-8, MMP, MMP-9) allows direct activation of MMP. In addition, the membrane-type MMP (MT-MMP) is also responsible for the local activation of the MMP. The enzymatic activity of MMP is also regulated by another class of protease inhibitors, namely, metalloproteinase tissue inhibitors (TIMPs). In the presence of TIMPs, the inactive MMP catalytic proenzyme (pro-MMP) is activated, and the proteolytic properties of MMP are controlled. In plasma, α 2-macroglobulin and α 1-antiprotease are the most common protease inhibitors. To date, four TIMP have been identified in tissues that inhibit the active form of MMP as well as the process of pro-MMP activation or conversion to MMP. TIMP expression is regulated by both cytokines and growth factors, thereby keeping the enzymatic activity of MMP at an appropriate level. In disease treatment, modulation targeting MMP can be an effective therapeutic strategy. For example, in joint degenerative diseases, the breakdown of joint structures can be reduced through the use of MMPI, thereby reducing pain and inflammation. Therefore, it is of important clinical significance to study the regulation mechanism of MMP [17].

5. TIMPs (tissue inhibitor of matrix metalloproteinases)

Matrix metalloproteinase tissue inhibitors (TIMPs) are endogenous protease inhibitors that function on the functional regulation of metalloproteinases (MMPs) and protease-free matrix proteases (ADAMs). TIMPs form a tight 1:1 stoichiometric inhibitory complex with MMPs and ADAMs. TIMP consists of two linked domains with three disulfide bonds stabilized within each domain. The N-terminal domain of TIMPs is called the inhibitory domain, because the independent N-terminal domain of TIMP was found to independently inhibit MMP activity independently. The interaction of TIMPs with MMP mainly occurs at the core epitope, which includes the motif of Cys-X-Cys, which interacts with the catalytic domain of MMP and blocks the substrate-binding cleft. However, full-length TIMP-1 was shown to be more potent inhibitory against MMP. The two domains of TIMP-1 cooperate in improving the inhibition of MMP-3, suggesting the C-terminal domain of TIMP-1 in the TIMP / MMP interaction and MMP inhibition. TIMP has broad specificity in binding and inhibition of MMP, ADAM and ADAMTS, ranging from 0.6 fM (for binding of TIMP-2 and MMP-2) to high nanomolar range (for poor MMP-14 inhibition) and inhibition of MMP-15 by TIMP-1. Furthermore, non-inhibitory interactions can also occur between the selected TIMP and MMP. TIMP-1 and TIMP-2 interact with the PEX domains of MMP-2 and MMP-9, producing some of the strongest interactions of the TIMP / MMP pair. The C-terminal domains of TIMP-2, TIMP-3, or TIMP-4 can bind to the heme-binding protein domains of MMP-2 or pro-MMP-2, while the C-terminal domains of TIMP-1 or TIMP-3 can associate with MMP-9 or pro-MMP-9. TIMP-2 interacts uniquely with pro-MMP-2 and plays an important role in the activation of MMP-2 by MMP-14. The C-terminal domain of TIMP-1 also interacts with the pro-MMP-9 heme domain and protects the secreted unactivated enzyme from activation by other MMP in vitro and in vivo [18-24]. Moreover, TIMP also inhibited some proteases of the ADAM and ADAMTS families through similar interactions with MMP [25-34]. In conclusion, metalloproteinase tissue inhibitors (TIMPs) play an important role in the inhibition of MMP, ADAM, and ADAMTS. Interaction of TIMPs with these proteases occurs mainly at the core epitope and regulates their function by forming a 1:1 repressor complex. Further studies on the interaction mechanisms of TIMPs with different proteases could provide a theoretical basis for the development of new therapeutic strategies and drugs [35].

5.1. Role of TIMP in cell signaling

Matrix metalloproteinases (MMPs) are an important family of proteases that are involved in the functions of many cells and tissues, such as cell proliferation, survival, migration, and invasion [36]. In many cases, MMPs are regulated for their activity and function by interacting with their corresponding inhibitor TIMPs. However, with the development of MMPs and TIMPs, it is gradually recognized that TIMPs are also involved in cell signaling pathways unrelated to MMPs, and also have certain effects on functions such as cell differentiation and proliferation.

5.2. Interaction of TIMPs and cellular signaling molecules

TIMPs are a family of tetrameric proteins, including TIMP-1, TIMP-2, TIMP-3, and TIMP-4. In addition to acting as inhibitors of MMPs, TIMPs interact with multiple cell signaling molecules that mediate cell proliferation and differentiation, involved in the biological properties of normal and tumor cells. For example, TIMP-1 binds to the cell surface receptor CD63 to promote cell adhesion and migration; TIMP-2 interacts with fibroblast growth factor-2 (FGF-2) and platelet derivative growth factor (PDGF) to promote cell proliferation and angiogenesis; TIMP-3 interacts with vascular endothelial growth factor (VEGF) and tumor necrosis factor (TNF) to inhibit cell proliferation and angiogenesis.

5.3. Mechanism of action of the TIMPs

The cellular signaling pathways in which TIMPs are involved involve multiple molecular mechanisms. First, TIMPs can interact with cell surface receptors to activate cell signaling pathways. For example, TIMP-1 activates the ERK and AKT signaling pathways and binds to CD63 to promote cell proliferation and migration [37-38]. In addition, TIMPs can also regulate cell signaling pathways by affecting the composition and structure of the extracellular matrix. For example, TIMP-1 and TIMP-2 can increase

the water content of the extracellular matrix to affect cell adhesion and migration. Alternatively, TIMPs can also regulate the intracellular signaling pathways. For example, TIMP-3 inhibited cell proliferation and invasion by inhibiting the MAPK signaling pathway and the phosphorylation of AKT. In conclusion, TIMPs participate in cell signaling pathways through multiple mechanisms and are important for the regulation of cell biological properties [39].

6. Interaction of ECM, MMPs and TIMP in kashin-beck

Kashin-Beck disease (KBD) is a chronic, progressive, and debilitating osteoarthropathy that primarily affects children and young adults in certain regions of China, Tibet, and Siberia. The pathogenesis of KBD is multifactorial and complex, involving various environmental, nutritional, genetic, and epigenetic factors. Among these factors, the dysregulation of extracellular matrix (ECM) homeostasis, matrix metalloproteinases (MMPs), and tissue inhibitors of MMPs (TIMPs) has been considered as a critical mechanism of cartilage and bone damage in KBD. In this chapter, we will review the current knowledge about the interplay between ECM, MMPs, and TIMP in KBD and its implications for disease pathogenesis and management.

6.1. ECM damage and repair in relation to MMPs and TIMP

ECM is a dynamic network of macromolecules, including collagen, proteoglycans, and glycoproteins, that provides structural and functional support to tissues and organs. In KBD, the ECM of articular cartilage, bone, and other connective tissues is severely compromised, leading to cartilage degeneration, bone erosion, and joint deformities. MMPs are a family of zinc-dependent endopeptidases that can degrade various ECM components, including collagen, proteoglycans, and glycoproteins, under physiological and pathological conditions. MMPs are regulated at multiple levels, including transcription, activation, inhibition, and localization, and their dysregulation has been implicated in various diseases, including osteoarthritis, rheumatoid arthritis, and KBD.

In KBD, the expression and activity of several MMPs, such as MMP-1, MMP-2, MMP-3, and MMP-13, are significantly increased in cartilage and bone tissues, indicating their potential role in ECM degradation and tissue damage. MMP-1, also known as collagenase-1, is a potent collagenase that can cleave type II collagen, the main component of articular cartilage, into smaller fragments. MMP-2, also known as gelatinase A, is a gelatinase that can degrade type IV collagen, a major component of basement membranes. MMP-3, also known as stromelysin-1, is a broad-spectrum MMP that can degrade proteoglycans, glycoproteins, and other ECM components. MMP-13, also known as collagenase-3, is a collagenase that is highly expressed in articular cartilage and can degrade type II collagen and other collagens under inflammatory and oxidative stress conditions. The upregulation of MMPs in KBD can be attributed to various factors, such as pro-inflammatory cytokines, oxidative stress, and mechanical stress, which can activate intracellular signaling pathways, transcription factors, and epigenetic modifications that modulate MMP gene expression and activity.

In contrast to MMPs, TIMPs are endogenous inhibitors of MMPs that can regulate their activity by binding to their catalytic domains and blocking their enzymatic activity. TIMPs are a family of four members, including TIMP-1, TIMP-2, TIMP-3, and TIMP-4, each of which has a unique specificity and affinity for different MMPs. TIMPs can also exert MMP-independent functions, such as cell proliferation, apoptosis, and differentiation, through interactions with cell surface receptors and other signaling molecules. In KBD, the expression and activity of TIMPs are complex and dynamic, depending on the stage, severity, and location of the disease. For example, the expression of TIMP-1 and TIMP-3 is elevated in articular cartilage and synovial fluid of KBD patients, which may reflect their compensatory response to MMPs and their role in ECM repair and remodeling. However, the expression of TIMP-2 and TIMP-4 is decreased in the cartilage and bone tissues of KBD patients, which may contribute to the excessive MMP activity and ECM degradation.

6.2. MMPs regulation and ECM changes

The regulation of MMPs in KBD involves multiple molecular mechanisms, including transcriptional, post-transcriptional, translational, and post-translational processes. At the transcriptional level, MMPs

are regulated by various transcription factors, such as AP-1, NF- κ B, Sp1, and Runx2, which can bind to their promoter regions and activate or repress their gene expression. These transcription factors are regulated by various signaling pathways, such as MAPK, Wnt, BMP, and TGF- β , that can modulate their activity and nuclear localization. The activation of these signaling pathways is influenced by various environmental, nutritional, and genetic factors that can affect their upstream receptors, ligands, and second messengers. For example, the activation of TGF- β signaling in cartilage cells can stimulate the expression of MMP-13 and other MMPs by inducing the expression of Runx2 and other transcription factors, which can bind to the MMP promoters and activate their transcription.

At the post-transcriptional level, MMPs are regulated by various RNA-binding proteins, such as HuR, AUF1, and TTP, that can stabilize or destabilize their mRNA transcripts by binding to their 3' untranslated regions (UTR). These RNA-binding proteins are regulated by various signaling pathways, such as AKT, ERK, and JNK, that can modulate their phosphorylation, localization, and activity. The stability of MMP mRNA transcripts can also be influenced by various RNA-modifying enzymes, such as miRNAs, that can bind to the mRNA transcripts and degrade or suppress their translation. The dysregulation of miRNAs has been implicated in various diseases, including osteoarthritis, rheumatoid arthritis, and KBD, and can affect the expression and activity of MMPs and other ECM components.

At the translational and post-translational levels, MMPs are regulated by various protein-modifying enzymes, such as proteases, kinases, and phosphatases, that can cleave, phosphorylate, or dephosphorylate their protein substrates. For example, the activation of pro-MMPs into active MMPs requires the cleavage of their pro-peptides by other MMPs, such as MMP-3, MMP-7, and MMP-14, or by other proteases, such as plasmin, trypsin, and kallikrein. The localization of MMPs to the cell surface, ECM, or intracellular compartments is also regulated by various signaling pathways and protein-protein interactions, such as integrins, CD44, and caveolins. The post-translational modifications of MMPs, such as glycosylation, acylation, and nitrosylation, can also affect their activity and stability.

The changes in ECM composition and organization in KBD are influenced by various MMPs and other ECM-related enzymes, such as aggrecanases, cathepsins, and ADAMTSs, that can degrade or modify different ECM components. The degradation of aggrecan, a major proteoglycan in articular cartilage, can lead to the loss of its water-holding capacity and shock-absorbing function, making the cartilage more susceptible to mechanical stress and damage. The degradation of type II collagen can disrupt the structural integrity of the cartilage matrix and expose the underlying bone surface, leading to bone erosion and deformation. The changes in ECM composition and organization can also affect the biomechanical properties of the tissues and the interactions between cells and ECM proteins, which can modulate cell adhesion, migration, proliferation, and differentiation.

6.3. TIMP inhibition and ECM/MMPs balance

The balance between ECM synthesis and degradation in KBD is critical for tissue homeostasis and repair. The excessive degradation of ECM by MMPs can lead to tissue damage and inflammation, whereas the insufficient degradation of ECM can lead to tissue fibrosis and stiffness. The regulation of ECM turnover and balance is influenced by various factors, such as cytokines, growth factors, and hormones, that can stimulate or inhibit the synthesis and degradation of ECM components. TIMPs play a critical role in maintaining the balance between ECM synthesis and degradation by inhibiting the activity of MMPs and other ECM-related enzymes.

The inhibition of MMPs by TIMPs is influenced by the concentration, isoform, and localization of TIMPs in the tissues. For example, TIMP-1 has a high affinity for MMP-9, whereas TIMP-2 has a high affinity for MMP-2, suggesting their specific roles in regulating the activity of these MMPs. The expression and localization of TIMPs can also vary depending on the stage and location of the disease. For example, the expression of TIMP-1 is increased in the early and intermediate stages of KBD, whereas the expression of TIMP-2 and TIMP-3 is decreased in the late stage of the disease, suggesting their differential roles in ECM repair and remodeling during different stages of KBD.

The dysregulation of TIMPs in KBD can lead to the imbalance of ECM synthesis and degradation and contribute to disease progression. For example, the excessive inhibition of MMPs by TIMPs in the early stage of KBD may limit the ECM degradation and delay tissue repair, whereas the insufficient inhibition of MMPs by TIMPs in the late stage of KBD may exacerbate the ECM degradation and accelerate tissue damage. The manipulation of TIMP activity in KBD may provide a potential strategy for restoring the ECM/MMPs balance and promoting tissue repair and remodeling.

7. Summary

Kashin-Beck disease (KBD) is a chronic degenerative osteoarthritis that mainly affects children and adolescents in endemic areas. Despite extensive research, the exact pathogenesis of KBD remains unclear. However, it is widely accepted that the disruption of extracellular matrix (ECM) is a critical event in the development of KBD. The ECM is a complex network of macromolecules, including collagen, proteoglycans, and glycoproteins, which provide a structural framework and support the cellular activities in the cartilage.

Matrix metalloproteinases (MMPs) are a group of zinc-dependent endopeptidases that play a crucial role in the degradation of ECM components. Several studies have indicated that MMPs are involved in the pathogenesis of KBD. For instance, MMP-2 and MMP-9 were found to be elevated in the synovial fluid and cartilage tissues of patients with KBD. MMP-13, another member of the MMP family, was also found to be upregulated and correlated with the severity of KBD. These findings suggest that MMPs are involved in the degradation of ECM components in KBD, leading to the destruction of cartilage tissues. However, the degradation of ECM components by MMPs is tightly regulated by tissue inhibitors of metalloproteinases (TIMPs). TIMPs are a family of glycoproteins that inhibit the activity of MMPs and promote tissue repair. Several studies have shown that the dysregulation of TIMPs in KBD may contribute to the imbalance between ECM degradation and tissue repair. For example, an imbalance between TIMP-1 and MMP-9 was observed in the serum of patients with KBD, which may contribute to the excessive ECM degradation. Moreover, the expression of TIMP-3, a critical regulator of ECM turnover, was found to be significantly reduced in the cartilage tissues of patients with KBD, indicating a deficient tissue repair mechanism.

The interactions between ECM, MMPs, and TIMPs are complex and dynamic in KBD. Abnormal levels of MMPs and TIMPs disrupt the balance of ECM homeostasis, leading to the destruction of cartilage tissues and the development of KBD. Several potential diagnostic and therapeutic applications of ECM, MMPs, and TIMPs have been proposed for KBD.

Diagnostic applications include the identification of biomarkers for early detection and monitoring the progression of KBD. The detection of MMPs and TIMPs in serum or synovial fluid may provide valuable information for the diagnosis and prognosis of KBD. Moreover, the use of imaging techniques, such as magnetic resonance imaging or ultrasound, may enable the visualization of ECM and cartilage tissues, facilitating the diagnosis and monitoring of KBD.

Therapeutic applications include the development of drugs that target the dysregulation of ECM, MMPs, and TIMPs in KBD. Several compounds that target MMPs or TIMPs have been investigated as potential therapeutic agents for KBD. For instance, doxycycline, a broad-spectrum MMP inhibitor, was found to reduce the severity of cartilage damage in animal models of KBD. TIMP-1, the natural inhibitor of MMPs, was shown to prevent cartilage destruction and improve joint function in a rat model of KBD. In conclusion, ECM, MMPs, and TIMPs play crucial roles in the pathogenesis, diagnosis, and treatment of KBD. The interaction between these factors determines the balance between ECM degradation and tissue repair, which ultimately determines the outcome of KBD. A better understanding of the molecular mechanisms underlying KBD may lead to the discovery of new biomarkers and therapeutic targets for this debilitating disease.

References

- Chen Jinghong, et al. Altered proteolytic activity and expression of MMPs and aggrecanases and their inhibitors in Kashin-Beck disease [J]. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society*, 2015, 33(1): 47-55.
- Wang Sen, et al. Genome-wide study identifies the regulatory gene networks and signaling pathways from chondrocyte and peripheral blood monocyte of Kashin-Beck disease [J]. *Genes to cells: devoted to molecular & cellular mechanisms*, 2012, 17(8): 619-632.
- Li Siyuan, et al. Proteoglycan metabolism, cell death and Kashin-Beck disease [J]. *Glycoconjugate journal*, 2012, 29(5-6): 241-248.
- Cao J, et al. Articular cartilage metabolism in patients with Kashin-Beck Disease: an endemic osteoarthropathy in China [J]. *Osteoarthritis and cartilage*, 2008, 16(6): 680-688.
- Wang Shijie, et al. Chondrocyte apoptosis and expression of Bcl-2, Bax, Fas, and iNOS in articular cartilage in patients with Kashin-Beck disease [J]. *The Journal of rheumatology*, 2006, 33(3): 615-619.
- Ma Weijuan, et al. Cytoskeleton remodeling and oxidative stress description in morphologic changes of chondrocyte in Kashin-Beck disease [J]. *Ultrastructural pathology*, 2014, 38(6): 406-412. [7]
- Song Jinsoo, et al. MicroRNA-488 regulates zinc transporter SLC39A8/ZIP8 during pathogenesis of osteoarthritis [J]. *Journal of biomedical science*, 2013, 20(5): 31.
- Vincenti MP, Brinckerhoff CE. Transcriptional regulation of collagenase (MMP- 1, MMP- 13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors [J]. *Arthritis Res*, 2002, 4(3):157-164.
- Gentili C, Cancedda R. Cartilage and bone extracellular matrix [J]. *Current Pharmaceutical Design*, 2009, 15(12):1334-1348.
- Krzysztof Fink, Janusz Boratyński. The role of metalloproteinases in modification of extracellular matrix in invasive tumor growth, metastasis and angiogenesis [J]. *Postępy Higieny i Medycyny Doświadczalnej*, 2012, 66(855199):609-628.
- Bogaczewicz J, Sysa-Jedrzejowska A, Woźniacka A. Role of matrix metalloproteinases in primary systemic vasculitis [J]. *Pol Merkur Lekarski*, 2008, 24(140):85-89.
- Gross J, Lapiere C M. Collagenolytic activity in amphibian tissues: a tissue culture assay [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 1962, 48: 1014-1022.
- Ulrich Eckhard, Pitter F. Huesgen, Oliver Schilling, et al. Active site specificity profiling of the matrix metalloproteinase family: Proteomic identification of 4300 cleavage sites by nine MMPs explored with structural and synthetic peptide cleavage analyses [J]. *Matrix Biology*, 2016, 49:37-60. [14]
- Jaclyn A. Konopka, Malcolm R. DeBaun, Wenteh Chang, et al. The Intracellular Effect of Relaxin on Female Anterior Cruciate Ligament Cells [J]. *The American Journal of Sports Medicine*, 2016, 44(9): 2384-2392.

- Benjamin D. Shogan, Natalia Belogortseva, Preston M. Luong, et al. Collagen degradation and MMP9 activation by *Enterococcus faecalis* contribute to intestinal anastomotic leak [J]. *Science Translational Medicine*, 2015, 7(286):286ra68-286ra68.
- Andreini Claudia, Banci Lucia, Bertini Ivano, et al. Comparative analysis of the ADAM and ADAMTS families [J]. *Journal of proteome research*, 2005, 4(3):881-888.
- Hrabec E, Naduk J, Strek M, Hrabec Z. Type IV collagenases (MMP-2 and MMP-9) and their substrates--intracellular proteins, hormones, cytokines, chemokines and their receptors [J]. *Postepy Biochem*, 2007, 53(1):37-45.
- Jackson HW, Defamie V, Waterhouse P, Khokha R. TIMPs: versatile extracellular regulators in cancer [J]. *Nat Rev Cancer*, 2017, 17(1):38-53.
- Gomis-Rüth F X, Maskos K, Betz M, et al. Mechanism of inhibition of the human matrix metalloproteinase stromelysin-1 by TIMP-1 [J]. *Nature*, 1997, 389(6646):77-81.
- Murphy G, Houbrechts A, Cockett MI, Williamson RA, O'Shea M, DochertyAJ. The N- terminal domain of tissue inhibitor of metalloproteinases retains metalloproteinase inhibitory activity [J]. *Biochemistry*, 1991, 30(33):8097- 8102.
- Huang W, Suzuki K, Nagase H, Arumugam S, Van Doren SR, Brew K. Folding and characterization of the amino-terminal domain of human tissue inhibitor of metalloproteinases-1 (TIMP-1) expressed at high yield in *E. coli* [J]. *Febs Letters*, 1996, 384(2):155-161.
- Raeeszadeh-Sarmazdeh M, Greene KA, Sankaran B, Downey GP, Radisky DC, Radisky ES. Directed evolution of the metalloproteinase inhibitor TIMP- 1 reveals that its Nand C-terminal domains cooperate in matrix metalloproteinase recognition [J]. *Journal of Biological Chemistry*, 2019, 294(24):9476-9488.
- Brew K, Nagase H. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity [J]. *Biochim Biophys Acta*, 2010, 1803(1):55-71.
- Jobin PG, Butler GS, Overall CM. New intracellular activities of matrix metalloproteinases shine in the moonlight [J]. *Biochim Biophys Acta Mol Cell Res*, 2017, 1864(11 PtA):2043-2055.
- Butler G S, Apte S S, Willenbrock F, et al. Human tissue inhibitor of metalloproteinases 3 interacts with both the N- and C-terminal domains of gelatinases A and B. Regulation by polyanions [J]. *The Journal of biological chemistry*, 1999, 274(16):10846-10851.
- O'Connell J P, Willenbrock F, Docherty A J, et al. Analysis of the role of the COOH-terminal domain in the activation, proteolytic activity, and tissue inhibitor of metalloproteinase interactions of gelatinase B [J]. *The Journal of biological chemistry*, 1994, 269(21):14967-14973.
- Brew K, Nagase H. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity [J]. *Biochim Biophys Acta*, 2010, 1803(1):55-71.
- Morgunova Ekaterina, Tuuttila Ari, Bergmann Ulrich, et al. Structural insight into the complex formation of latent matrix metalloproteinase 2 with tissue inhibitor of metalloproteinase 2 [J].

Proceedings of the National Academy of Sciences of the United States of America, 2002, 99(11): 7414-7419.

Goldberg G I, Strongin A, Collier I E, et al. Interaction of 92-kDa type IV collagenase with the tissue inhibitor of metalloproteinases prevents dimerization, complex formation with interstitial collagenase, and activation of the proenzyme with stromelysin [J]. The Journal of biological chemistry, 1992, 267(7):4583-4591.

Ogata Y, Itoh Y, Nagase H. Steps involved in activation of the pro-matrix metalloproteinase 9 (progelatinase B)-tissue inhibitor of metalloproteinases- 1 complex by 4-aminophenylmercuric acetate and proteinases [J]. Journal of Biological Chemistry, 1995, 270(31):18506-18511.

Ardi Veronica C, Kupriyanova Tatyana A, Deryugina Elena I, et al. Human neutrophils uniquely release TIMP-free MMP-9 to provide a potent catalytic stimulator of angiogenesis [J]. Proceedings of the National Academy of Sciences of the United States of America, 2007, 104(51):20262-20267.

Ardi VC, Van den Steen PE, Opdenakker G, Schweighofer B, Deryugina EI, Quigley JP. Neutrophil MMP-9 proenzyme, unencumbered by TIMP- 1, undergoes efficient activation in vivo and catalytically induces angiogenesis via a basic fibroblast growth factor (FGF-2)/FGFR-2 pathway [J]. Journal of Biological Chemistry, 2009, 284(38):25854-25866.

Wayne GJ, Deng SJ, Amour A, Borman S, Matico R, Carter HL, Murphy G. TIMP-3 inhibition of ADAMTS-4 (Aggrecanase- 1) is modulated by interactions between aggrecan and the C-terminal domain of ADAMTS-4 [J]. Journal of Biological Chemistry, 2007, 282(29):20991-20998.

Kashiwagi M, Tortorella M, Nagase H, Brew K. TIMP-3 is a potent inhibitor of aggrecanase 1 (ADAM-TS4) and aggrecanase 2 (ADAM-TS5) [J]. Journal of Biological Chemistry, 2001, 276(16): 12501-12504.

Augustin Amour, C.Graham Knight, Ailsa Webster, et al. The in vitro activity of ADAM-10 is inhibited by TIMP-1 and TIMP-3 [J]. FEBS Letters, 2000, 473(3):275-279.

Murphy G. Tissue inhibitors of metalloproteinases [J]. Genome Biol, 2011, 12(11):233.

Rhee Jin-Sae, Diaz Robert, Korets Lidiya, et al. TIMP-1 alters susceptibility to carcinogenesis [J]. Cancer research, 2004, 64(3):952-961.

Liliana Guedez, Andrew J. McMarlin, Douglas W. Kingma, et al. Tissue Inhibitor of Metalloproteinase- 1 Alters the Tumorigenicity of Burkitt's Lymphoma via Divergent Effects on Tumor Growth and Angiogenesis [J]. The American Journal of Pathology, 2001, 158(4):1207-1215. [39] Cui H, Seubert B, Stahl E, et al. Tissue inhibitor of metalloproteinases-1 induces pro-tumourigenic increase of miR-210 in lung adenocarcinoma cells and their exosomes [J]. Oncogene, 2015, 34(28):3640-3650.