Review

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The anti-catabolic role of bovine lactoferricin in cartilage

Abstract: Bovine lactoferricin (LfcinB) is a multifunctional peptide derived from bovine lactoferrin that demonstrates antibacterial, antifungal, antiviral, antitumor, and immunomodulatory activities. Recently, studies have focused on the anti-catabolic and anti-inflammatory potential of LfcinB. LfcinB is able to modulate the effects cytokines such as IL-1 and fibroblast growth factor 2 as well as promote specific cartilage anabolic factors. These properties are particularly important in maintaining cartilage homeostasis and preventing a catabolic state, which leads to clinical pathology. This review focuses on the recent literature elucidating the role of LfcinB in preventing cartilage degradation.

Keywords: cartilage; homeostasis; lactoferricin.

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Introduction

Cartilage degenerative diseases such as osteoarthritis (OA) in joints and degenerative disc disease (DDD) in the spine are prevalent diseases that impart a significant impact on today's society. OA is the leading cause of disability among the elderly population (1), and DDD contributes to the debilitating nature of chronic back pain (2–4). At present, the pathogenesis of these two conditions is largely unknown, but both involve the progressive deterioration and degradation of cartilage tissue. Recent literature has focused on understanding many of the pathophysiological processes involved in cartilage degradation, with the intention of developing novel therapies aimed at slowing and/or reversing these diseases.

Under normal conditions, both articular chondrocytes and intervertebral disc (IVD) cells maintain a dynamic equilibrium between synthesis and breakdown of extracellular matrix (ECM) components (5, 6). In degenerative states, there is a disruption of matrix equilibrium, leading to progressive loss of cartilage tissue, suppression of proteoglycan (PG) production, and clonal expansion of cells in the depleted regions. Chondrocyte metabolism is unbalanced due to the excessive production of catabolic mediators, including matrix metalloproteases (MMPs), aggrecanases (ADAMTS), and other cytokines and growth factors released by chondrocytes that aid in the destruction of the ECM (7–9). Therefore, identifying novel growth factors or mediators that biochemically suppress the catabolic and/or anti-anabolic pathways involved in cartilage degradation may shift homeostasis toward a pro-anabolic state, potentially serving as one therapeutic strategy to slow both OA and DDD.

Interestingly, several recent studies have revealed the potential for the glycoprotein bovine lactoferricin (LfcinB), and its precursor bovine lactoferrin (bLf), to induce anti-catabolic, pro-anabolic, and anti-inflammatory effects in cartilage. First identified in bovine milk in 1939 (10), bLf is an 80-kDa glycoprotein found in mucosal secretions, synovial fluids, plasma, and neutrophil granules (11) and plays a key role in various antibacterial, antiviral, anticarcinogenic, and anti-inflammatory pathways (12). Recently, bLF has been found to provide therapeutic value in musculoskeletal etiologies. For example, oral administration of bLF in a rat adjuvant arthritis model suppressed the development of arthritis and hyperalgesia (13), suggesting its anti-catabolic and anti-inflammatory properties in synovial joints. These results are corroborated by other findings in which periarticular injections of human LF

suppressed local inflammation in a murine septic arthritis model (14).

LfcinB is formed by pepsin-mediated cleavage of the glycoprotein bLf, a key member of the transferrin family. LfcinB has been retrieved from the acidic gastric contents of humans following digestion of bLf (15). Similar to bLF, LfcinB exerts a variety of effects in several different tissues and organisms. The antibacterial effects of LfcinB have already been well documented. When released from its parent protein, LfcinB transforms from an α-helical structure to an amphipathic β-hairpin structure, allowing the peptide to bind to bacterial membranes with high affinity (16). Once bound, LfcinB increases the permeability of the cell membrane, thus eliciting a bactericidal effect (17). Studies in other biological systems also reveal LfcinB-mediated antifungal, antiparasitic, antiviral, antitumor, and immunomodulatory effects (17–19), much like bLf. Given their structural similarities, these findings suggest that it is most likely the basic hydrophobic N-terminus of bLf (which contains the LfcinB residues) that accounts for these shared effects (20).

This review will focus on the powerful anti-catabolic and anti-inflammatory effects of LfcinB in both articular cartilage and the IVD, which highlight its potential as a therapeutic agent for OA and DDD in the future.

Lactoferricin in articular cartilage

Cartilage homeostasis is maintained by a delicate balance between catabolic and anabolic pathways. When this balance is disrupted, catabolic pathways often predominate and induce degeneration of cartilage, manifested clinically as OA. The effect of LfcinB on the catabolic pathways associated with cartilage degradation was first investigated by Yan et al. (21). In their study, human articular cartilage and synovium were cultured with IL-1β and fibroblast growth factor 2 (FGF-2) (two molecules known to drive catabolism in articular cartilage), with or without LfcinB. They demonstrated that LfcinB potently inhibits the catabolic effects of both IL-1β and FGF-2. In particular, in human articular chondrocytes and synovial fibroblasts treated with IL-1β and FGF-2, the expression of matrix-degrading enzymes (i.e., MMP-1, MMP-3, and MMP-13) and ADAMTSs (i.e., ADAMTS4 and ADAMTS5) were downregulated at both the mRNA and protein levels in the presence of LfcinB. This finding is significant, as MMPs and ADAMTSs have been shown to contribute to cartilage degradation (22), and finding ways to decrease their activities has been the focus of multiple studies (23, 24).

Yan and colleagues also elucidated that LfcinB may interfere with the catabolic activities of FGF-2 (also known as basic FGF) by potentially binding to heparan sulfate proteoglycans (HSPGs) such as syndecan 4 (21). Syndecan 4 facilitated the binding of FGF-2 to the FGF receptor on the cell surface. The ability of LfcinB to bind to syndecan has been previously described. Mader et al. (25) found that LfcinB binds with syndecan, preventing FGF-2 and vascular endothelial growth factor (VGEF) binding in human and mice umbilical vein endothelial cells. It is proposed that a similar mechanism is present in articular cartilage cells (21). LfcinB competitively binds syndecan and prevents FGF-2 from binding to its articular cell receptor, hence preventing its downstream catabolic and/or anti-anabolic signaling cascades (21).

Along with the suppression of catabolic mediators, LfcinB also inhibits inflammatory mediators in articular cartilage (21). In articular cartilage, LfcinB decreased the expression of several pro-inflammatory genes, such as IL-6 and toll-like receptor 2 (TLR-2). IL-6 can incite cartilage degeneration through autocrine and paracrine mechanisms, and TLR-2 incites an innate immune response and inflammation in OA joints (26, 27). Moreover, LfcinB also upregulated anti-inflammatory cytokines, including IL-4 and IL-10 (21). By simultaneously modulating pro-inflammatory and anti-inflammatory activities, LfcinB caused an overall reduction of inflammation in OA.

In a follow-up study, Yan et al. (28) further characterized the signaling pathways used by LfcinB and discovered yet another anti-catabolic mediator upregulated in the presence of LfcinB (28), tissue inhibitor of metalloproteinase 3 (TIMP-3). This finding is significant, in that TIMP-3, unlike the other members of the TIMP family (TIMP-1–4), is a potent inhibitor with a relevant substrate spectrum in cartilage biology, including MT-MMPs, ADAMTS4, ADAMTS5, and tumor necrosis factor convertase (29, 30). Given that TIMP-3 is the only endogenous inhibitor of ADAMTSs (31), this may explain why LfcinB was able to block PG depletion by IL-1β and FGF-2 *ex vivo* (19).

Although many of the downstream signaling pathways mediated by LfcinB remain largely unknown, recent studies have begun to uncover specific cascades stimulated by LfcinB in human articular cartilage. By pharmacologically inhibiting ERK, Akt, and p38 pathways, Yan et al. (28) determined that ERK-1/2 pathway mediated most aspects of LfcinB effects in articular chondrocytes. Further, mechanistic studies on LfcinB-induced TIMP-3 transcription uncovered an essential role of the transcription factor Sp1 (32, 33). Sp1 belongs to the core

transcriptional machinery during TIMP-3 induction because knocking down Sp1 abrogates LfcinB-induced TIMP-3 expression (32). Interestingly, Sp1 was also critical for TGF-β-mediated TIMP-3 production (33), suggesting this transcriptional program can be activated by different signaling inputs.

In a yet unpublished study, Yan et al. further demonstrate that the anti-inflammatory cytokine IL-11 was dramatically upregulated by LfcinB. This increase of IL-11 was a result of activation of the ERK-1/2 pathway, which subsequently activated the c-Fos/JunD heterodimer to initiate transcription. Induced IL-11 then stimulated anti-inflammatory as well as anti-catabolic cascades *via* the induction of TIMP-1 in a Stat3-dependent manner.

Based on these studies, we hypothesize that LfcinB promotes anti-inflammatory and anti-catabolic activities *via* three mechanisms (Figure 1): (i) competitive binding of LfcinB to HSPGs, thus preventing efficient IL-1 and FGF-2 signaling; (ii) induction of TIMP-3 to limit endogenous proteolytic activities; and (iii) induction of protective cytokines, particularly IL-11, which promote anti-inflammation and anti-catabolism. Although these studies have provided the foundation for understanding the role of LfcinB in articular cartilage, further studies are needed to dissect the exact pathways and target genes regulated by LfcinB.

Lactoferricin in IVD cells

Another area where LfcinB has shown great promise as a potential therapeutic option has been in the degeneration of the nucleus pulposus (NP) of the IVD. Much like articular cartilage, the maintenance of the NP tissue homeostasis relies on a balance between anabolic and catabolic processes. When this balance is altered, degeneration of the IVD is initiated, potentially culminating in chronic back pain for the patient.

Kim et al. (34) examined the effects of LfcinB on the IVD of bovine as well as human tissue. They demonstrated that the addition of LfcinB to a culture of NP cells led to an increase in PG synthesis. One mechanism by which LfcinB increased PG synthesis was through the upregulation of SOX-9. SOX-9 is a key transcription factor that has been shown to increase aggrecan and collagen type II expression in bovine NP cells (35, 36). Cells cultured with LfcinB have nearly 2.5–3.0 times greater concentrations of SOX-9 depending on the LfcinB dose compared with those without LfcinB, demonstrating its ability to promote anabolic pathways in bovine NP tissue (34). In addition to promotion of SOX-9, there is evidence of suppression of key cartilage-degrading enzymes including MMP-1, MMP-13, and ADAMTS5. This suppression was complimented with promotion of multiple TIMPs including TIMP-1, TIMP-2,

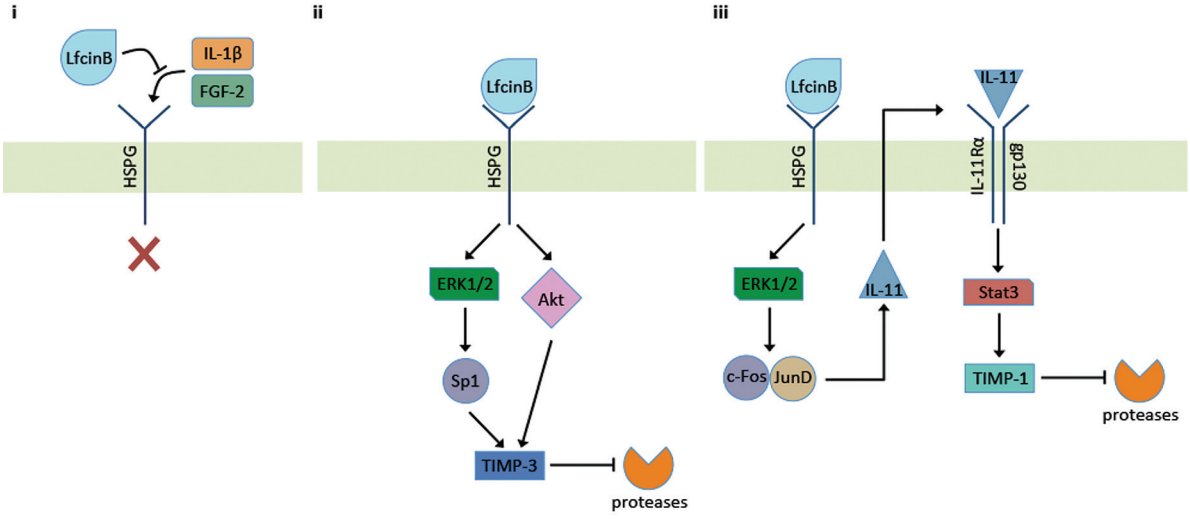

Figure 1 LfcinB uses multiple mechanisms to promote anti-catabolic and anti-inflammatory cellular processes. (i) LfcinB competes with IL-1β and FGF-2 for HSPG binding. Failure of binding to their co-receptors results in inability of IL-1β and FGF-2 to trigger downstream signaling. (ii) LfcinB-bound HSPG activates ERK-1/2 MAPK and Akt pathways. Active ERK-1/2 subsequently activates transcription factor Sp1 and coordinates with active Akt in TIMP-3 upregulation. TIMP-3 then targets proteases to curtail collagenolytic and aggrecanolytic activities. (iii) In parallel, active ERK-1/2 promotes c-Fos and JunD dimerization, and the resultant heterodimer transactivates IL-11. Secreted IL-11 protein can bind to its cognate receptor complex (IL-11Rα and gp130) and activate the Stat3 pathway. After translocalization into the nucleus, Stat3 upregulates TIMP-1 expression, which further limits extracellular proteolytic activities.

and TIMP-3, further solidifying the anti-catabolic role of LfcinB (34). Finally, the signaling pathways by which LfcinB exerts its anabolic effects were elucidated. The activation of mitogen-activated protein kinase (MAPK) subgroups (ERK, p38, and JNK) were evaluated, and only the ERK and p38 pathways are activated by the presence of LfcinB, whereas the JNK pathway was not activated at any time point (34). The extent of involvement of each activated pathway was assessed using specific pharmacologic inhibitors. Inhibition of the p38 pathway leads to a significant decrease in aggrecan gene induction to below the level of the control group. ERK pathway inhibition also led to a decrease in aggrecan induction but not to the extent of p38 inhibition (34).

Ellman et al. (37) further investigated the signaling pathway through which LfcinB acts and identified a synergistic relationship between LfcinB and another anabolic mediator: bone morphogenetic protein 7 (BMP-7). In their study, treatment of bovine IVD cells with the combination of LfcinB and BMP-7 led to a greater increase in PG accumulation and synthesis, as well as *aggrecan* gene induction. They proposed that the synergistic mechanism was the result of shared activation of SMAD-1/5/8 by both BMP-7 and LfcinB (38, 39). Also, LfcinB downregulated SMAD-6 (a potent inhibitor of SMAD-1/5/8) as well as Noggin (an inhibitor of BMP-7) (37). The decrease of these inhibitory factors allows for LfcinB to eliminate the negative feedback on BMP-7 and allow for maximal anabolic contribution from BMP-7. The authors concluded that this combination could potentially be used clinically in the prevention and treatment of IVD degeneration (37).

Further insight into the anti-inflammatory nature of LfcinB in the IVD is provided by a follow-up study by Kim et al. (40). This study focused primarily on the interaction of LfcinB with the inflammatory mediators IL-1 and endotoxin lipopolysaccharide (LPS). Both IL-1 and LPS have been shown to be potent inflammatory mediators leading to degeneration of IVD cells (41, 42). Through their experiments, Kim et al. (40) demonstrated that LfcinB induces an increase in pericellular matrix formation when added to bovine IVD cultures. This anabolic effect was so potent that the addition LfcinB rescued the suppressed pericellular matrix formation in the presence of IL-1 and LPS. The addition of LfcinB also suppressed the IL-1- and LPS-mediated production of MMP-1, MMP-3, MMP-13, and ADAMTS-5, which have been shown be involved in disc degeneration. Finally, the addition of LfcinB to IVD cultures containing IL-1 and LPS led to the attenuation of TLR-2 and TLR-4 upregulation. TLR-2 and TLR-4 have important regulatory functions in the inflammatory and oxidative pathways leading to degeneration of the IVD (27, 43). The ability to suppress the expression of the TLRs as well as the other catabolic mediators, demonstrates potent anti-catabolic potential of LfcinB in the IVD.

Kim et al. (40) also investigated the therapeutic potential of LfcinB by examining *ex vivo* organ cultures of IVD of New Zealand white rabbits and mice. Prior to culture, the discs were injected *en bloc* with LfcinB (200 μg per rabbit disc and 100 μg per mouse disc). The LfcinB and the control discs without LfcinB injections were cultured in medium containing IL-1, LPS, or neither. The discs without the LfcinB injection had significant PG depletion in the presence of IL-1 and LPS as well as decreased cellularity in the pericellular matrix. The LfcinB-injected discs demonstrated an attenuation of the effect of IL-1 and LPS. This finding is significant in demonstrating LfcinB potential to be a therapeutic agent against the catabolic activates of IL-1 and LPS in the IVD.

Conclusion

Lactoferricin is a molecule that has been shown to have multiple functions in various biological systems. Its role in the musculoskeletal system is just starting to be elucidated. In particular, the anti-inflammatory and anti-catabolic properties of LfcinB make it an attractive option in the treatment of DDD and IVD degeneration. Both of these pathologic processes are the result of potent inflammatory and catabolic mediators that shift the homeostatic balance toward catabolism. Further studies are needed to elucidate the exact mechanisms and pathways activated by LfcinB; however, the potential of LfcinB as a therapeutic option in OA and DDD shows great promise.

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