Bovine lactoferricin induces TIMP-3 via the ERK1/2-Sp1 axis in human articular chondrocytes

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INTRODUCTION: Bovine lactoferricin (LfcinB), a 25-amino acid peptide derived from lactoferrin, promotes anabolic and anti-catabolic processes in bovine nucleus pulposus cells [1]. Similarly, in human articular cartilage, LfcinB effectively counteracts FGF-2/IL-1β-induced proteoglycan loss and catabolic mediator expression. Furthermore, LfcinB increases anti-inflammatory cytokines, particularly IL-11. LfcinB triggers ERK1/2, p38, and PI3K/Akt signaling to critically control target gene expression. A fraction of LfcinB is also readily internalized into chondrocytes to regulate MMP transcription. However, whether LfcinB is capable of inducing anti-catabolic mediators, such as the TIMP (tissue inhibitor of metalloproteinase) family members, remains unclear. Therefore, the goals of this study were (i) to determine the effect of LfcinB on TIMP expression, (ii) if such an effect exists, to define the molecular mechanism of TIMP regulation.

METHODS: Healthy and OA chondrocytes were maintained in DMEM/Ham’s F-12 media (1:1) supplemented with 10% FBS. For confluent monolayer culture, cells were serum-starved in DMEM/Ham F-12 (1:1) prior to any stimulation. When pharmacological inhibitors were applied, cells were incubated with the inhibitors for 1 hour before LfcinB stimulation. Cell lysates were collected for immunoblotting. Total RNA was used for reverse transcription and real-time qPCR. GAPDH and 18S rRNA were used for normalization, and the ΔΔCt method was adopted to calculate fold induction.

RESULTS: In contrast to the other TIMP family members, LfcinB dramatically upregulated TIMP-3 expression at both mRNA and protein levels. The glycosylated form (27 kDa) and unmodified form (24 kDa) of TIMP-3 were induced, while the putative dimer (50 kDa) level was not changed notably (Figure 1, * p<0.05, ** p<0.01). TIMP-3 induction by LfcinB appears particularly beneficial to OA chondrocytes, because we observed that TIMP-3 expression was significantly decreased in OA cells compared with healthy ones. In OA, all three forms of TIMP-3 protein in our experimental system (24 kDa, 27 kDa, 50 kDa) were downregulated (Figure 2).

DISCUSSION: In this study, we demonstrate that TIMP-3, a potent inhibitor of multiple MMPs and aggrecanases, is strongly induced by LfcinB. ERK and Akt mediate TIMP-3 expression, whereas LfcinB internalization appears not directly involved. We identified Sp1 as a critical transcription factor for TIMP-3 induction. We also observed that Sp1 binding affinity to its consensus sequence was markedly reduced upon ERK inhibition (data not shown), suggesting the ERK-Sp1 axis is mainly responsible for LfcinB-mediated TIMP-3 upregulation. LfcinB effect on TIMP-3 expression is beneficial to OA cartilage, in which TIMP-3 is significantly downregulated.

SIGNIFICANCE: Our findings uncovered the regulatory mechanism of LfcinB on TIMP-3, which furthers understanding on its chondro-protective effect.


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