Regulation of Periostin for Osteoarthritic Cartilage Repair Applications

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INTRODUCTION: Osteoarthritis (OA) is one of the leading causes of disability in the adult population characterized by disruption in the structural and functional properties of articular joints. Although the etiology of the disease is unclear, it is widely accepted that these degenerative changes arise from an imbalance of synthetic and degradative pathways that control cartilage extracellular matrix (ECM) metabolism. Although several factors have been implicated in the development of OA; Periostin (Postn) has recently been described to play a contributing role. Increased expression of Postn after an injury appears to be detrimental to articular cartilage, as it stimulates a catabolic cascade in chondrocytes and may contribute to the development of OA. The matriline Link N (DHLSDNYTLDHDRAIH, LN) is a 16 residue peptide fragment derived from proteolytically processed extracellular matrix molecule Link protein. We and others have shown that LN can behave as an anabolic-like factor by stimulating matrix protein synthesis in cartilage and the intervertebral disc. In an in vivo rabbit model of osteoarthritis, intraarticular injection of LN significantly reduced disease burden. Despite the importance of Postn in the development of OA, to date, there are no known targeted peptide inhibitors. The goal of this study is to test the effectiveness of LN as a therapeutic target against Postn in cartilage degeneration and post-traumatic OA.

METHODS: In vitro: Articular cartilage was isolated from four donors undergoing total knee replacement (50 – 70 yrs). Chondrocytes were recovered from the cartilage and osteochondral explants were prepared from each knee. Freshly isolated cells were treated with LN (1 or 10 µg/ml) for 72 hrs to measure periostin synthesis by RT-qPCR or 6 days to quantify for protein synthesis by Western blotting of periostin or IL-1β (1ng/ml) to mimic an OA environment for 72h. Osteochondral explants were cultured for 21 days in growth medium containing IL-1β (1ng/ml) or IL-1β and LN [1 µg/ml] to mimic an inflammatory milieu. Immunohistochemistry was performed to analyze for periostin expression and matrix protein content. To determine if LN can inhibit periostin signaling in OA chondrocytes we measured changes in β-catenin signaling. Peptide docking of LN to Postn (crystal structure, 5yjg) was determined using CABS-dock web server (www. http://biocomp.chem.uw.edu.pl/CABSdock/). The best prediction generated by CABS-dock was added to PyMOL (Schrodinger, LLC) to create the model. Docking of LN and periostin was verified by immunoprecipitation.

In vivo: Skeletally mature New Zealand white rabbits underwent unilateral anterior cruciate ligament transection (ACLT) of their left femorotibial joint operatively, and every three weeks thereafter for 12 weeks, either saline (1 mL) or sLN (100 µg in 1 mL saline) was injected intra-articularly in the operated knee. Additional rabbits underwent Sham surgery but without ACLT.

RESULTS: Western blotting of OA chondrocytes indicate the periostin is upregulated when compared to normal cells. Treatment with LN decreased the synthesis and expression of periostin in chondrocytes (p < 0.01; n = 3; Figure 1). Immunohistochemical analysis of osteochondral explants indicated increased expression of periostin that was subverted following incubation with LN. Although periostin was upregulated in the joints of OA-induced rabbits, intra-articular injection of LN retained proteoglycan and collagen content whilst suppressing periostin expression. Immunohistochemical analysis of Postn expression in the rabbit model of OA, where the stains were divided into 3 categories: cells unstained with Postn, cells with minimal Postn staining, and cells with saturated Postn. chi-square test; ****p < 0.0001. Periostin-induced increases in β-catenin (β-Cat) accumulation in chondrocytes however, this was inhibited when LN was co-incubated in a dose-dependent manner (ANOVA, posthoc Dunnett’s; ***, p < 0.0001; n = 3). Peptide docking of LN to Postn (crystal structure, 5yjg) was determined using CABS-dock web server. Model was created using PyMOL (Schrodinger, LLC). Immunoprecipitation (IP) of LN with Postn demonstrated a direct interaction that was abrogated when a scrambled LN peptide was used (Figure 2).

DISCUSSION: Periostin is increasingly characterized as a contributing factor in the development of OA. LN has been previously described as an anabolic-like agent for in the treatment of OA. Although the mechanism of LN in cartilage repair remains unclear, we provide evidence that regulation of periostin may be one of its targets.

SIGNIFICANCE: Targeting periostin during the early stages of OA has been suggested to improve disease outcome.

![Figure 1](Image 339x206 to 458x288)  
**Figure 1.** Effect of LN on periostin expression in human OA chondrocytes. Cells were treated for 6 days with the indicated concentrations of LN and Western blotting was performed to determine changes in periostin expression.

![Figure 2](Image 59x235 to 218x288)  
**Figure 2.** LN interacts with Postn and induces dissociation. Immunoprecipitation demonstrating LN interaction with periostin.