P21 Regulates Mmp-13 Expression Through Stat3 Signaling In Chondrocytes

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Disclosures:

Introduction:
Osteoarthritis (OA) is a multifactorial disease, and biomechanical stress is a major contributor to OA pathology. However, the underlying mechanisms remain unclear. Recent data have suggested that cell cycle-related proteins play a role in OA pathology. The cyclin-dependent kinase inhibitor p21 was initially identified as a potent inhibitor of cell cycle progression. Recently, however, it has been proposed that p21 is a regulator of transcription factor activity. In this study, we evaluated the role of p21 in the control of gene expression in response to cyclic tensile strain by using an experimental mouse model of biomechanical stress.

Methods:
Normal human knee chondrocytes (Cambrex, Charles City, IA) were cultured in a humidified atmosphere of 5% CO₂ and 95% air at 37°C in a Bullet kit (Cambrex). OA chondrocytes were isolated and cultured from cartilage tissues. Cartilage tissues were obtained during total hip joint replacement surgery from 11 patients with OA. Human chondrocytes were treated with p21-specific siRNA, and cyclic tensile strain was introduced in the presence or absence of a STAT3-specific inhibitor. In order to investigate p21 function in response to mechanical stress in vivo, we developed a destabilization of the medial meniscus (DMM) model in a p21-knockout background at 10 week age. p21+/− littermates were used as wild type controls. The mice were sacrificed at 8 weeks after surgery and subjected to Histological evaluation. Statistical analysis was performed using one-way analysis of variance, with Tukey’s post-hoc test for multiple comparisons of paired samples. Mann-Whitney U test was used for comparisons between the 2 groups. P-values less than 0.05 were considered significant.

Results:
In order to analyze the function of p21 in chondrocytes in response to mechanical stress, we compared COL2A1, ACAN, MMP, and ADAMTS mRNA levels in p21-knockdown chondrocytes subjected to cyclic tensile strain. Real-time PCR analysis showed that p21 expression was inhibited by ~80% by p21-specific siRNA transfection in all cells subjected to tensile strain (Figure 1). In normal chondrocytes subjected to 5% strain, downregulation of p21 led to a reduction in ACAN expression and increased MMP-13 expression (Figure 1b). Furthermore, the expression of ACAN was decreased, but MMP-13 levels were unchanged compared with control cells after 10% stress was applied (Figure 1c).
In OA chondrocytes, there was no change in response to mechanical loading after downregulation of p21 (Figure 1d, e, f). In order to analyze the mechanism by which p21 regulates ACAN and MMP-13 expression, we pretreated control and p21-knockdown cells with a STAT3 inhibitor and measured gene expression after application of 5% cyclic tensile strain. Real-time PCR analysis showed that MMP-13 expression decreased upon STAT3 inhibition, but ACAN levels were not changed in normal chondrocytes (Figure 2a). In OA chondrocytes, the expression levels of MMP-13 and ACAN were not changed after application of 5% cyclic tensile strain (Figure 2b). Western blot analysis of normal chondrocytes confirmed that phosphorylation of STAT3 increased after p21 knockdown, but the effect was abolished with a STAT3 inhibitor (Figure 2c).

In order to realize the effect of p21 function in vivo, we made DMM model for p21 knockout mice and compared with wild type mice at 8 weeks after surgery. Safranin O and Fast Green staining showed that Wild type mice had matrix staining depletion within the upper one-third of cartilage (figure 3a) and p21 deficiency mice had midzone excavation of cartilage tissue and loss of hyaline cartilage proteoglycan staining (Figure 3b). Immunohistochemistry showed that the phosphorylation of STAT3 was elevated (Figure 3c, d), and the expression levels of CXCR4 in cartilage (Figure 3e, f) and SDF-1 in synovial tissues (Figure 3g, h) were increased in p21 deficiency mice compared with wild type mice. Therefore, p21 deficiency mouse is susceptible to OA and was associated with increased STAT3 phosphorylation, and elevated CXCR4 and SDF-1 expression.

**Discussion:**
Our results suggest that p21 in chondrocytes functions to maintain matrix synthesis by regulation of aggrecan and MMP-13 expression via STAT3 phosphorylation. Our data imply that stabilization of p21 would be useful as a therapeutic strategy for OA treatment.

**Significance:**
Our results suggest that p21 in chondrocytes functions to maintain matrix synthesis by regulation of aggrecan and MMP-13 expression via STAT3 phosphorylation.

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**References:**