**INTRODUCTION:**

Osteoarthritic chondrocytes (OC) suffer dedifferentiation, terminal differentiation and redifferentiation towards fibroblast, which contributes to its inability to self-repair in osteoarthritic cartilage. However, little is known about the underlying molecular mechanism. Two-dimensional gel electrophoresis (2-DE) technique provides an ideal tool for delineating the molecular mechanism topography underlying chondrocyte mal-differentiation. This study sought to optimize a feasible scheme to use the 2-DE technique to understand more about the molecular mechanism of osteoarthritic chondrocyte mal-differentiation.

**METHODS:**

2-DE Design: 2-DE was performed using protein samples obtained from freshly isolated chondrocytes from normal and osteoarthritic cartilage graded with Outerbridge Classification. Differentially expressed spots were isolated and identified by mass spectrometry. This study was approved by the Medical Ethics Committee of the hospital and informed consent was given by all patients.

**CoREST Differential Expression Validation:** The differentially expression pattern of a novel protein, the REST corepressor (CoREST), was validated with the Real-time RT-PCR.

**CoREST RNA Interference:** Different siRNAs and time-points were explored to achieve the most effective CoREST knock-down.

**Chondrocyte Phenotype changes after CoREST Knock-down:**

mRNA levels of the normal chondrocyte phenotypic genes, the type II collagen and aggrecan, the terminal differentiation marker gene, the type X and I collagen, and the phenotypic gene of fibroblast, the type I collagen, were compared before and after CoREST knock-down.

**RNA in situ Hybridization:** Digoxin labeled probe was used to bind and visualize CoREST mRNA distribution in cartilage tissues of different Outerbridge Grade.

**Statistical Analysis:** CoREST mRNA levels among Outerbridge classifications were compared with Mann-Whitney U-test. Phenotypic gene expression levels before and after CoREST knock-down were compared with Real-time RT-PCR. P<0.05 was considered to be significant.

**RESULTS:**

A perfect 2-D gel focusing was achieved and the gel images were generated in high-quality (Figure 1A). 135 protein spots were screened to have been differentially expressed and 31 protein spots were identified by MS. Numerous molecules functionally correlated with chondrocyte phenotype modulation were detected. Among them, human protein disulfide isomerase (PDII), procollagen-proline, 2-oxoglutarate 4-dioxygenase (P4H2), TGF-J2 were upregulated in OAI, while mitogen-activated protein kinase kinase 7 (MAPKK7) and CoREST were downregulated.

mRNA level of CoREST was confirmed to be downregulated by 22.9% (Z=2.623, P=0.009) and 64.4% (Z=-4.510, P<0.001) respectively in Outerbridge II (OAI) and II (OAI) cartilage compared to that of normal cartilage (NC) with real-time RT-PCR, which showed significant difference between normal and OA chondrocytes (Figure 1B).

**DISCUSSION:**

With freshly isolated chondrocytes and the soluble protein fraction used for analysis, problems concerning disturbed 2D gel focusing and cell culture induced phenotype loss were avoided to investigate the etiology of OC mal-differentiation with 2-DE. CoREST, known as an important factor involved in neuron phenotype modulation, was firstly detected to be downregulated in osteoarthritis. The downexpression of CoREST suppressed type II collagen and aggrecan expression while increased type X collagen expression, coincided with phenotype changes in osteoarthritis. We thus conclude CoREST also a factor responsible for chondrocyte dedifferentiation and terminal differentiation in osteoarthritis.

However, type I collagen was documented to have been downregulated in response to CoREST knock-down, indicating phenotype modulation effect driven by CoREST is not accompanied by a redifferentiation towards fibroblast phenotype.

Upregulation of TGF-beta[2], PDI and P4H2 in OAI were interpreted to be positive factors benefiting chondrocyte phenotype stability, however, downregulation of CoREST and MAPKKK7 were triggering factors for chondrocyte mal-differentiation. The contradictory biological effect of these differentially expressed phenotype regulating factors demonstrated a disordered mechanism in OC phenotype modulation. CoREST downexpression mainly existed in the middle layer of Outerbridge I deep layer of Outerbridge II cartilage, indicating that chondrocyte mal-differentiation was inhomogeneous in different cartilage regions.

**REFERENCES:**