Reduced Expression of Collagen type IX in Human Osteoarthritic Chondrocytes is Associated with Epigenetic Silencing by DNA Hypermethylation

INTRODUCTION:
Collagen type IX comprises only 1% of the total collagen of mature cartilage yet is critical for the integrity and stability of articular cartilage. Gene expression is regulated by both epigenetic and non-epigenetic mechanisms. The main heritable component of epigenetics is DNA methylation. The aim of this study was to investigate whether increased DNA methylation is associated with loss of COL9A1 expression in OA chondrocytes.

METHODS:
Human chondrocytes were isolated from articular cartilage of femoral heads obtained from fracture neck of femur (controls, 10 patients) or total hip replacements (OA, 12 patients). Gene expression for COL9A1 and COL2A1 were determined using SybrGreen-based qRT-PCR. The % DNA methylation in the COL9A1 promoter was quantified after bisulphite modification with a pyrosequencer and the effect of methylation on COL9A1 promoter was analyzed using a luciferase assay. After constructing the pCpGfree-Luc-COL9A1 vector (C9-WT), vectors with mutations in the CpG sites were also created to determine which CpG sites are the most important for COL9A1 promoter activity (Figure 2B) to give pCpGfree-Luc-COL9A1-M1, M2 and M3 vectors and vector mutations in the CpG sites; M1: -632, -614 and -599; M2: -400 and -382; M3: -95, -49 and -8). Co transfection with transcription factors were undertaken to determine effects on COL9A1 promoter activity.

RESULT:
COL2A1 expression was increased in OA chondrocytes compared to control (Figure 1A), however, there was no difference in methylation status of COL2A1 promoter CpGs between control and OA chondrocytes (Figure 1B). COL9A1 expression of OA chondrocytes was 6000-fold lower than control (Figure 1C). 6 CpG sites of COL9A1 promoter were significantly hypermethylated in OA compared to control (Figure 1D).

DISCUSSION:
The present study demonstrates for the first time that hypermethylation is associated with the down-regulation of the expression of COL9A1, a typical chondrogenic gene. Thus epigenetic changes in OA not only involve hypomethylation and consequent activation of aberrant genes, but also hypermethylation leading to gene silencing. This novel finding that increased DNA methylation is associated with loss of COL9A1 expression in OA chondrocytes offers new insight into the pathoetiology of osteoarthritis and the potential therein for innovative therapeutic strategies for OA.