INTRODUCTION:
Osteoarthritis (OA) is a chronic degenerative joint disorder of high prevalence that remains the leading cause of disability in aged people. A balance between anabolic and catabolic mechanisms maintains extracellular matrix homeostasis in articular cartilage. Among mechanisms to control gene expression, genomic DNA methylation, modification of nucleosome histone tails, and chromosome remodeling are essential contributors to the mechanisms of epigenetic control. Specially, genomic DNA methylation is known as the most fundamental process. A methyl group is added to the 5’ cytosine in a CpG dinucleotide, leading to gene silencing. However, it is not known whether DNA methylation contribute to OA progression. In this study, we examined the methylation status of the promoter region for chondrogenesis- or degradation-related genes using MS-PCR and bisulfite sequencing from osteoarthritic and normal cartilages.

METHODS:
Cell culture and Procurement of samples Articular cartilage was obtained from normal human femoral heads from femoral neck fractures without OA (age range 64-83, n=9, four males and five females), and from OA patients (age range 58-69, n=9, all females) undergoing total hip arthroplasty.

Methylation Specific Polymerase Chain Reaction (MS-PCR) Genomic DNA was extracted from the cells and cartilage using a DNeasy tissue system (Qiagen, USA) according to the manufacturer’s instructions. Bisulfite-modified genomic DNA was prepared using an EZ DNA Methylation-Gold kit (Zymo Research, USA), according to the manufacturer’s instructions. The PCR reaction was carried out in a volume of 20μl with converted genomic DNA using AccuPowerHotStart PCR PreMix (Bioneer, Korea). CpG rich regions within upstream sequences 5 kb from the transcription start site were analyzed. Putative CpG-rich islands and respective primers for MS-PCR were derived from the CpG Island Searcher (http://cpgislands.usc.edu/) and the MethPrimer program (http://www.urogene.org/methprimer/), respectively. The PCR reaction was confirmed by electrophoresis in a 1.8% agarose gel, visualized by SYBR® Safe DNA gel stain (Invitrogen, USA) staining and the images were taken using LAS3000 (FujiFilm, Japan). Intensity of each band was analyzed using the Image J program (National Institutes for Health, USA).

Bisulfite Sequencing Bisulfite sequencing primers were designed based on MethPrimer program and. The PCR reaction was confirmed by electrophoresis in a 1.8% agarose gel, visualized by SYBR® Safe DNA gel stain (Invitrogen, USA) and specific bands were eluted using GeneAll®Expip® Gel SV kit (GeneAll, Korea). Elute of specific PCR product was ligated with TA vector using TOP cloner™ TA kit (Enzymomics, Korea) according to the manufacturer’s instructions. After transformation of ligated products into DH5α chemically competent E. coli (Enzymomics, Korea), clones were screened by PCR. Positive clones were cultured and plasmid DNA was isolated using GeneAll® Exprep™ Plasmid SV according to its manual. Sequences of each plasmid were analyzed using M13R or M13F primer (Macrogen, Korea). Sequences were analyzed using 2BLAST (http://blast.ncbi.nlm.nih.gov/).

RESULTS:
We first checked methylation status for the promoter regions covering -5kb from transcription start site (TSS) of chondrogenesis-related genes (SOX-9, COL2A1, IGF-1) and cartilage degradation-related genes (IL-1α/β and TNF-α) via MS-PCR in normal and OA cartilage. We found that methylation status of SOX-9 for R3 (from -3653 to -3496) and R4-1 (from -3111 to -2983) significantly increased in OA cartilage compared to normal cartilage (Figure 1B and C). We performed analysis of bisulfite sequencing for region from -4548 to -2846 in the promoter of SOX-9. Methylated CpG sites significantly increased in all the examined regions (BSQ1-5) and total methylated CpG sites increased about eight-fold in OA cartilage (14.04%) than in normal (1.66%).

Figure 1. MS-PCR analysis of SOX-9 promoter in normal and osteoarthritic (OA) cartilages. (A) Scheme of MS-PCR analysis. R1-R6; regions for MS-PCR. (B) Representative electrophoresis results after MS-PCR. M; methylated DNA; U; unmethylated DNA. (C) Dot plot and statistic analysis of MS-PCR. p; p-value, n=9

DISCUSSION:
Our study suggests that the increased methylation status in the SOX-9 promoter region may have a close relation to the progression of OA.

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