Collagen type IX is present on the surface of type II collagen and binds covalently to two collagen II molecules and to COMP. Although only 1% to the total collagen of mature cartilage consists of collagen type IX, it is important for the integrity and stability of articular cartilage. Mice which lack COL9A1 develop normally, but display osteoarthritic-like cartilage degradation in older animals (1). In human OA, reduction in COL9A1 expression over decades would ultimately lead to a gradual loss of collagen IX in the matrix, which would result in loss of cartilage matrix integrity. This reduced stability might, in turn, render the cartilage more susceptible to damage by mechanical forces. Reduced collagen IX expression could thus contribute to the patho-etiologic of osteoarthritis (OA).

Gene expression is regulated by both epigenetic and non-epigenetic mechanisms. The latter involve up- or down-regulation of those genes that are part of the expression repertoire of a specific somatic cell type. Regulation by transcription factors and other DNA binding proteins, non-epigenetic gene regulation is short-term and expression reverts upon withdrawal of the relevant factors. By contrast, epigenetic regulation of gene expression involves long-term silencing of all those genes that are not normally expressed by a specific cell type. This silencing is important for genomic stability and changes in epigenetic status are associated with disease. The main heritable component of epigenetics is DNA methylation. Pathological loss of DNA methylation may result in induction of aberrant genes, while increased methylation may explain silencing of normally expressed genes. We have previously shown that loss of DNA methylation underlies the aberrant expression of MMPs, ADAMTS-4 and IL-1beta in OA chondrocytes (2,3). Here we provide evidence that increased DNA methylation is associated with loss of COL9A1 expression in OA chondrocytes.

METHODS

Human chondrocytes were isolated from the articular cartilage of femoral heads, obtained with ethical permission, after operations following neck of femur (NOF) fractures (controls, 10 patients) or total hip replacements (OA, 12 patients). For controls, only deep zone cartilage was used, because this contained healthy, albeit aged, chondrocytes, as shown in previous studies. For OA samples, only the surface zones were dissected, as only these contained the typical OA chondrocytes with the altered ‘degradative’ phenotype. Total RNA and genomic DNA were extracted simultaneously from each sample. Gene expression of COL9A1 was determined by SybrGreen-based qRT-PCR. The % DNA methylation in the COL9A1 promoter was quantified after bisulfite modification with a pyrosequencer (Biotage).

RESULTS

Although there was considerable variation between patients, high expression of COL9A1 was found in most control samples. In OA patients, on the other hand, expression was considerbly down-regulated. On average, COL9A1 expression in OA patients was 500-fold lower than in control patients, see Figure 1.

To determine whether the loss of COL9A1 expression in OA cartilage was associated with epigenetic silencing by DNA hypermethylation, the DNA methylation status was quantified. The COL9A1 promoter (AF036110) contains just 8 CpG sites within the 890 bp region upstream from the transcription start site. Three nested primer pairs were designed to cover the CpG sites located at (-632, 614, 599bp), (-400, -382bp), and (-95,-49bp). The CpG site at -8bp was not analyzed.

At the CpG sites closest to the transcription start site (-95 and -49bp) DNA methylation was virtually absent (<4%) in control samples, but increased to 8 and 14% in OA samples (p<0.01). At the -400 and -382bp sites, the overall % of methylation was higher than at the previous two sites, 5% and 20% respectively in NOF patients and 13% and 24% in OA samples. The difference between NOF and OA at -400bp, but not at -382bp, was statistically significant. Considerably more cells contained methylated cytosines at the CpG sites -632, 614 and -599bp. On average ~40% of control chondrocytes were methylated and ~60% in OA chondrocytes – a 20% increase in the number of cells with methylated cytosines in OA patients. Overall, the % DNA methylation correlated inversely with mRNA expression. The magnitude of the hypermethylation in OA samples was greatest in the region between -632 and -599bp.

DISCUSSION

This is the first demonstration that, in osteoarthritis, hypermethylation is associated with down-regulated expression of COL9A1, a typical chondrocytic gene. Other studies have examined the DNA methylation status of the promoters of aggrecan (4) and p21WAF1/CIP1 (5), which are also typical chondrocytic genes that are down-regulated in OA. However, none of these studies could demonstrate hypermethylation. We believe that the crucial difference lies in the promoter structure. Both the aggrecan and the p21 promoter contain a Cpg island, whereas the COL9A1 promoter does not. As the Human Epigenome Project has shown (6,7), genes with CpG islands are generally not methylated, irrespective of expression. (Exceptions are tumor suppressor genes in cancer).

In summary, the present study has shown that epigenetic changes in OA not only involve hypomethylation and consequent activation of aberrant genes, but also hypermethylation leading to silencing of at least one of those chondrocytic genes that do not contain CpG islands. No doubt other such genes will be identified in the future.

REFERENCES