Inflammatory Cytokines Can Cause Loss of DNA Methylation in Healthy Articular Chondrocytes together with Induction of Abnormal Gene Expression

INTRODUCTION

Osteoarthritis (OA) is characterised by progressive erosion of articular cartilage, which, once started, cannot be halted. The breakdown of cartilage is mediated by proteases, such as MMPs and ADAMTSs. In normal articular chondrocytes, these proteases are thought to be silenced by epigenetic mechanisms, such as DNA methylation. In OA chondrocytes, on the other hand, expression of the enzymes is induced, especially after exposure to inflammatory cytokines. This aberrant gene expression has been shown to be associated with an epigenetic “un-silencing” via DNA de-methylation of specific CpG sites within the promoter regions (Arthritis & Rheumatism, 2005; 52:3110-24). The mechanisms that trigger or cause this loss of DNA methylation in OA are not known. Since inflammatory cytokines are known to induce abnormal gene expression in cultured chondrocytes, we asked whether this induction involved loss of DNA methylation. If so, the aberrant gene expression would be permanent and transmitted to daughter cells rather than a temporary up-regulation. To test this hypothesis, we selected IL-1β as the abnormally expressed gene, after preliminary experiments showed a strong inverse correlation between expression and CpG methylation of the IL-1β promoter.

METHODS

Healthy chondrocytes, harvested from human femoral head cartilage following a fracture (with ethical permission from the Southampton & South West Hampshire Research Ethics Committee and patients’ consent), were divided into five experimental groups: (1) non-culture; (2) control culture; (3) culture with the de-methylating agent 5-aza-deoxycytidine (5-aza-dC); (4) culture with the inflammatory cytokine IL-1β; or (5) with TNF-α and oncostatin M (OSM). The factors were added to groups (3), (4) and (5) at every medium change. At confluence, total RNA and genomic DNA were extracted simultaneously so as to correlate mRNA expression with DNA methylation.

To determine which CpG sites in the IL-1β promoter were most crucial for gene expression, the methylation status of 16 CpG sites in the 1200 bp 5'-upstream region was determined by bisulfite modification in control and experimental samples. Relative mRNA expression of IL-1β was quantified by SYBRGreen-based real-time PCR. In addition a method, based on methylation-sensitive restriction enzymes and PCR, was developed to quantify the fraction of cells with DNA methylation at the most crucial CpG sites (Epigenetics, 2007; 2: 86-95).

To determine whether increased expression of IL-1β was translated into protein, the release of IL-1β into the medium was measured by Elisa.

RESULTS

Bisulfite modification identified the CpG sites at –247 and -290bp in the IL-1β promoter as the most important ones for the epigenetic regulation of IL-1β, since these sites were methylated in control cultures, but de-methylated in cultures with the inflammatory cytokines. The CpG site at -290 bp was selected for quantification of DNA methylation. 5-aza-dC reduced DNA methylation, as expected, and this resulted in a 5-fold increase in IL-1β expression (see Fig. 1), showing that loss of DNA methylation per se increases gene expression.

Far greater effects were seen with the inflammatory cytokines (Fig. 2). Before culture, IL-1β was not expressed (Fig. 2A). Culture alone induced a low level of expression, which was set to =1. Addition of IL-1β increased its own expression ~100-fold, whereas the combination of TNF-α and OSM induced a 1000-fold increase in expression of IL-1β. DNA methylation varied inversely (Fig. 2B). Before culture, the CpG site at -290 bp was methylated in 60% of the cells and culture alone reduced CpG methylation to 40%. IL-1β addition reduced DNA methylation to ~15% and TNF-α/OSM abolishing DNA methylation almost completely.

DISCUSSION

This is the first demonstration that inflammatory cytokines can cause loss of DNA methylation along with permanent induction of gene expression. At present, the mechanisms can only be speculated upon. De-methylation can either occur by passive mechanisms whereby DNMT1, the maintenance DNA methyl transferase, is inhibited at specific sites during cell division, or by active mechanisms that do not require cell division. Signal transduction pathways for the cytokines include the three classical MAPK-signaling pathways (ERK, p38 and JNK) as well as NF-κB. Conceivably, binding of specific DNA binding proteins/ transcription factors may prevent DNMT1 from methylating the newly-synthesized strand during cell division (Thomassin et al, ENMO, J, 2001; 20:1974-83). In other systems, de-methylation via passive mechanisms depended on the rel/ NF-κB family of transcription factors (Kirkillov et al, Nat Genet, 1996; 13:435-41), but it is not yet known whether this also applies to OA chondrocytes.

We also confirmed previous work that IL-1β induces its own expression in healthy chondrocytes, thus setting up a dangerous positive feed-back mechanism. Since IL-1β was released into the medium, it is possible that IL-1β, initially produced by OA chondrocytes in the superficial zone, diffuses to adjacent chondrocytes in vivo to initiate loss of DNA methylation and induction of abnormal gene expression. Auto-induction and the heritable expression of IL-1β by a growing number of chondrocytes could explain the unrelenting progression of osteoarthritis.