COUNTERACTION OF IL-1-REGULATED GENES BY TGF BETA IN CHONDROCYTES

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Introduction

Osteoarthritis (OA) is the joint disease with the highest prevalence in the human population and is characterized by destruction of articular cartilage eventually leading to loss of joint function. Metalloproteinases are thought to play a crucial role in the process of cartilage breakdown. Although the exact trigger for the production of metalloproteinases in the OA joint is not known yet, interleukin-1 (IL-1) is a likely candidate. IL-1 is one of the most potent catabolic cytokines of articular cartilage and involved in cartilage destruction. On the other hand, TGF beta is a factor that has shown to be protective for articular cartilage. The effect of IL-1 on articular cartilage can be counteracted by TGF beta. However, little is known of the gene regulation by TGF beta of IL-1-controlled genes. The aim of this study was to investigate the counteracting effects of TGF beta on IL-1-induced changes in gene expression in chondrocytes. Therefore the regulation of gene expression by IL-1, TGF beta or a combination of both factors was studied in a murine chondrocyte cell line.

Methods:

A murine chondrocyte cell line derived from adult murine articular cartilage was selected based on similar responses to IL-1 and TGF beta as intact murine articular cartilage in vivo. Therefore, changes in mRNA expression of MMP-3, -9, -13 and -14 in the murine cell line H4 was compared with changes in expression in murine patellar cartilage after IL-1 exposure. The chondrocyte cell line was incubated with IL-1 in vitro while the intact patellar cartilage was exposed to IL-1 in vivo by intra-articular injection of this cytokine. The level of mRNA expression of the MMPs was estimated by RT-PCR. Based on these results the H4 cell line was used as a model system to study IL-1 and TGF beta regulated gene expression. H4 cells were incubated for 9 hours with IL-1 alpha, TGF beta1 or a combination of both factors (10 ng/ml). After incubation, RNA was isolated and gene expression was analyzed using the murine genome array of Affymetrix which represents ~6,000 functionally characterized murine genes and ~6,000 EST clusters.

Results:

Validation of H4 cell line

The effect of IL-1 on MMP expression in the H4 cell line was compared with MMP expression in patellar cartilage after IL-1 exposure in vivo. Both articular cartilage in vivo and the H4 cell line showed up-regulation of MMP expression after exposure to IL-1 (table 1).

Table 1. Relative MMP expression in the murine H4 cell line and patellar cartilage after IL-1 exposure. MMP mRNA expression after IL-1 exposure was compared to expression in control knee joints or in the H4 cell line without IL-1 exposure. Expressed is the change in cycle number, after correction for the expression of GAPDH, at first visual detection. A negative integer means relative increase in mRNA.

<table>
<thead>
<tr>
<th>Relative mRNA expression</th>
<th>MMP-3</th>
<th>MMP-9</th>
<th>MMP-13</th>
<th>MMP-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>H4 cell line</td>
<td>-4</td>
<td>-2</td>
<td>-4</td>
<td>-4</td>
</tr>
<tr>
<td>In vivo cartilage</td>
<td>-14</td>
<td>-2</td>
<td>-2</td>
<td>-2</td>
</tr>
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</table>

Although the up-regulation of the MMPs was not of the same magnitude in the chondrocyte cell line and in vivo cartilage, the H4 cell line shows a similar reaction pattern to IL-1 as intact articular cartilage in vivo. Moreover, TGF beta inhibited expression of all studied MMP genes in this cell line significantly (3 to 10 fold).

Conclusions

The chondrocyte cell line H4 responded with regard to mRNA expression of a number of MMP genes similar as intact murine articular cartilage. The upregulation of MMP expression was not of the same magnitude in both systems but one has to keep in mind that the H4 cell line is an isolated clonal chondrocyte cell line while intact cartilage is a collection of chondrocytes with variable characteristics. However, since the response of the H4 cell line and intact cartilage was comparable this cell line was regarded as a valid model system. It appeared that counterregulation of IL-1-induced gene expression by TGF beta is more pronounced than vise versa. So, IL-1 regulated genes are more under control of TGF beta than TGF beta regulated genes are influenced by IL-1. A large number of expected, new and still uncharacterized genes were regulated by IL-1 and subsequently counteracted by TGF beta in this murine articular cartilage chondrocyte cell line. IL-1 appeared to stimulate matrix catabolism, chondrocyte apoptosis and cellular stress responses. TGF beta counteracted gene induction in all these systems, showing that TGF beta is an important factor in protection of articular cartilage against IL-1-induced cartilage breakdown and chondrocyte death.