Methylation Status Of The Promoter Region Of Matrix Genes Collagen I, Collagen II And Aggrecan In NP And AF Cells Of Degenerated And Non-degenerated IVD

Vivian Tam¹, Victor YL Leung, Ph.D.², Kenneth Cheung³, Danny Chan¹.
¹The University of Hong Kong, Hong Kong, Hong Kong, ²The University of Hong Kong, Hong Kong, China.

Disclosures:
V. Tam: None. V.Y. Leung: None. K. Cheung: None. D. Chan: None.

Introduction: During intervertebral disc (IVD) degeneration, changes in the nucleus pulposus (NP) results in the loss of water, proteoglycan and cells, which impacts on disc function and can lead to back pain¹,². Whilst several studies have demonstrated changes in the matrix gene and protein expression in the diseased state and in vivo animal models¹,³⁻⁷, we still do not fully understand the processes that are involved in IVD degeneration. The regulation of genes may be controlled by numerous factors and can occur at different stages including transcription, during translation, post-translational modification, or due to the structural or chemical modification of DNA.

DNA methylation is a type of chemical modification in the DNA⁸ on the cytosine nucleotides in a CpG dinucleotide sequence, which can alter the expression of specific genes. The methylation of the promoter region of a gene (which usually contains densely clustered CpG ‘islands’) can regulate the expression or switching off of a gene. Various studies have reported on the regulation of matrix genes such as collagen I by specific regions in the promoter⁹,¹⁰⁻¹². However, few studies have examined the methylation patterns of the promoter regions of the matrix genes with regards to cells of the IVD, where differences in the methylation status of the promoter may indicate different types of regulation in the diseased vs non-diseased state. We hypothesised that DNA methylation in the promoter region of matrix may be different in degenerated and non degenerated IVD, and thus the aim of this study was to examine the promoter region of the matrix genes collagen I, collagen II and aggrecan to determine the methylation status.

Methods: Genomic DNA was isolated from degenerated (n=4) and non-degenerated (n=3) clinical IVD samples and bisulfite converted using the Epitect Bisulfite Kit (Qiagen). The converted DNA was then subject to PCR to amplify the region of interest within the promoter region with primers for collagen I, collagen II, and aggrecan. The amplicons were then subject to pyrosequencing (PSQ 96MA, Biotage-Qiagen) for analysis of the percentage methylation of the CpG sites.

Results: For collagen I, the CpG sites for the non-degenerated NP cells had an increased percentage of methylation of the CpG sites in comparison to the degenerated NP cells (Fig 1) but this was not significant. A similar trend was observed for the AF cells (Fig 2), however, there was a significant difference in the percentage methylation at CpG site 7. For collagen II, there was no significant difference in the percentage methylation for NP and AF cells, with the exception of CpG site 7 for the AF cells (Fig 3). There was no significant difference in the percentage methylation in the CpG sites of degenerated/non degenerated NP and AF cells for aggrecan.

Discussion: Differences in the methylation pattern within the promoter region of degenerated or non-degenerated cells may give light to how the matrix genes are regulated in the cells of the IVD. Although there was an increased trend in percentage methylation of the CpG sites of collagen I of the non-degenerated NP cells in comparison to degenerated NP cells, this was not significant and may have been associated with the low number of clinical samples that were examined. Studying other regulatory elements such as enhancers in degenerated and non-degenerated samples may further our understanding in the regulation of matrix genes and subsequent process that occur during IVD degeneration.

Significance: Few studies have investigated the methylation pattern of promoters of matrix genes specifically in IVD cells. Significant differences in the percentage methylation of Cpg sites of collagen I and II in the AF between degenerated and non-degenerated IVD indicate that these sites could play a role in the regulation of these genes in the IVD. Understanding the regulation of the matrix genes could shed light on the control of the processes that occur during IVD degeneration.

Acknowledgments: This project is supported by the AOSpine East Asia Research Grant.

5. Kozaci LD, Guner A, Oktay G, Guner G. Alterations in biochemical components of extracellular matrix in intervertebral disc...
8. Jones Pa Fau - Takai D, Takai D. The role of DNA methylation in mammalian epigenetics. 20010810 DCOM- 20010906(0036-8075 (Print)).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reported promoter region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen I</td>
<td>-2000bp to -1501bp (distal promoter), -452 bp to +116bp (proximal promoter)¹³</td>
</tr>
<tr>
<td>Collagen II</td>
<td>-6368 bp to +125 bp¹⁴</td>
</tr>
<tr>
<td>Aggrecan</td>
<td>-784bp to +485bp¹⁵</td>
</tr>
</tbody>
</table>
Fig. 1: Methylation in the promoter region of collagen I in cells from degenerated or non-degenerated AF tissue. Comparison between degenerated and non-degenerated AF shows that there was a slight increase in non-degenerated AF methylation at analyzed CpG sites except for site 9 and 10, although this was not significant.

Fig. 2: Methylation in the promoter region of collagen II in cells from degenerated or non-degenerated AF tissue. Comparison between degenerated and non-degenerated AF showed that there was a slight increase in the amount of methylation at sites 1, 3, 4, 5, 7, 9, and 10.