A High-throughput Gene Expression-based Screen For Factors That Modulate In-vitro Chondrogenesis Of Stem Cells Identifies Optimal Conditions And Novel Factors

INTRODUCTION

The in vitro process of chondrogenic differentiation of mesenchymal stem cells for tissue engineering has been shown to require three-dimensional culture along with the addition of differentiating factors to the culture medium. This, however, in general leads to a phenotype lacking some of the cardinal features of native articular chondrocytes and extracellular matrix. The factors used vary but regularly include members from the TGF beta super family and dexamethasone (DEX), sometimes in conjunction with FGF-2 and IGF-1. The use of soluble factors to induce chondrogenesis has largely been studied on a single factor basis or with combinations of a few factors. We have combined a factorial design experiment with high-throughput digital mRNA profiling as a powerful tool to study in vitro chondrogenesis.

AIM OF STUDY

- to evaluate the suitability of studying in vitro chondrogenesis by factorial design and high throughput digital gene expression profiling
- to identify the optimal chondrogenic conditions
- to identify novel inhibitors of unwanted molecules in tissue engineered hyaline cartilage for further testing

METHODS

Quality control of surface markers and multipotentiality were performed on bone marrow derived MSC before embedding into alginate. A 2^5-factorkal design of all combinations of TGF-b1, BMP-2, IGF-1, FGF-2 and DEX was then performed. Adapted designs for isoforms of TGF- (r, 2, 3) and BMP-2, -4, -6 was also tested, giving a total of 48 different conditions. Furthermore, 38 different potential inhibitors of collagen type-I and -X synthesis were tested for their effect on chondrogenesis in a non-combinatorial design. Lysate or RNA was profiled using a custom probe set of 364 chondrogenesis related genes on the Nanostring nCounter platform.

Experimental setup

- 3D-phase: Different cocktails

Consortial analysis of standard differentiation factors

<table>
<thead>
<tr>
<th>High</th>
<th>Samples (day 5)</th>
<th>Low</th>
<th>Samples (day 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Articular cartilage</td>
<td>COLA1, COLA2, COMP</td>
<td>Bone, adipose lineages</td>
<td>COLA1, COLA2</td>
</tr>
<tr>
<td>Undesirable molecules</td>
<td></td>
<td>COLA1, COLA2, COLA3, COLA4</td>
<td></td>
</tr>
</tbody>
</table>

Geneset enrichment analysis

- Analysis of differentially expressed genes between conditions of interest allows for biological interpretation

Novel factors favorable to in vitro chondrogenesis

A cocktail of TGF-b1, BMP-2 and DEX is the most effective from a gene expression perspective, but all factors/beneficial to wanted genes do also increase unwanted genes. Eliminating one or the other factors severely decreases expression of wanted genes, indicating that perfect differentiation to articular cartilage may not be achievable with the currently used factors.

CONCLUSION

Factorial design with high throughput gene expression profiling can be used to investigate differentiation cocktails directly on lysates of cells in alginate.

A cocktail of TGF-b1, BMP-2 and DEX was selected from the screen and is being investigated further.