Evaluation of Human Chondrocyte Cells Viability by Caspase Gene Expression Analysis

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Introduction

Autologous chondrocyte implantation has been proposed as an alternative to classical treatments of the chondral lesions of the human knee, especially in young patients (1). However, and although the variability among studies is very high, systematic reviews have demonstrated that there is a lack of scientific evidence supporting the effectiveness of this treatment in comparison to other conventional techniques for the treatment of chondral lesions of the knee (2). One possibility is that the variability of the results and the failure of the treatment in certain series could be due to the lack of cell viability of the chondrocyte cultures that are implanted in the patient.

In this work, we have performed a cell viability study of primary cultures of human hyaline articular chondrocytes by determining the expression levels of several genes associated to cell death using expression microarrays.

Materials and Methods

Primary cell cultures of human articular hyaline chondrocytes were established from small biopsies of the cartilage of the knee using collagenase II. Primary cultures were subcultured for 8 cell passages using trypsin-EDTA. At each cell passage, total RNA was extracted from each cell passage using a commercial kit and the RNA expression level was quantified by using Affymetrix Human Genome U133 plus 2.0 arrays. Expression of the genes encoding for caspases 1 to 8 was analyzed and compared among all the cell passages analyzed here.

Results

The gene expression analysis revealed the existence of three different patterns among the 8 caspase genes analyzed here (Fig. 1): A- genes that did not significantly modify their expression in regard of the cell passages (caspases 5 and 7); B- genes with high expression at the first two cell passages and at the last ones, with a minimum expression at the third cell passage (caspases 2 and 3); C- genes whose expression was minimum at the fourth cell passage (caspases 1, 4, 6 and 8).

![Fig. 1. Average RNA expression levels for caspases 1 to 8 as determined by RNA microarray for each cell passage of human hyaline chondrocytes cultures.](image)

Discussion and Conclusions

The analysis of gene expression suggests that caspases expression could be the responsible for the variable cell viability that is commonly found among different cell passages of chondrocytes cultures. Our analysis revealed that the minimum expression of these apoptosis-related genes could be found between the third and the fourth cell passage, implying that these should be preferentially used for cell therapy protocols using human cultured chondrocytes.

References


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Disclosures

Authors have nothing to disclose.