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Identification of a novel chondrocyte-specific protein

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Keywords

cDNA representational difference analysis (RDA), stem-cell-based chondrogenic tissue engineering

Context

Cell-based therapies based upon regeneration of cartilage involve either harvesting autologous chondrocytes or using mesenchymal stem cells which can be driven into a chondrocytic phenotype *in vitro*. For the latter technique cell-specific markers are essential to differentiate mesenchymal stem cell lineages, which include chondrocytes, osteoblasts and fibroblasts. The aim of this paper was to identify chondrocyte-specific genes by cDNA representational difference analysis (RDA) to use as markers in stem-cell-based chondrogenic tissue engineering. The project resulted in the identification and early characterisation of a novel chondrocyte-specific gene.

Significant findings

RDA of chondrocyte cDNA compared to osteoblast cDNA yielded eight products. Six of these belonged to the gene for cartilage GP39 and one to the related protein YKL-39. The last fragment was derived from an unknown gene designated CEP-68 (chondrocyte expressed protein-68). Using gene-specific primers, expression of CEP-68 was confirmed in cartilage and isolated chondrocytes, but was not present in osteoblasts or mesenchymal stem cells. GP39 and YKL39 were not chondrocyte-specific. Full coding sequence was predicted by the extension of the identified cDNA sequence using a 408 amino acid human cDNA sequence and three expressed sequence tags. The open reading frame was amplified by PCR and sequenced. No homology was found in the protein or DNA databases. The authors conclude that they have identified a novel protein that is a useful marker gene for cultured chondrocytes.

Comments

This is an increasingly used method for identification of cell-specific genes and uncovering novel proteins, particularly where the mRNA is of low abundance. Notable weaknesses of the technique in this paper include the failure to identify known chondrocyte-specific genes such as collagen type II, aggrecan and link protein, and the identification of the gene encoding YKL39 as a chondrocyte-specific gene when it was clearly expressed in one of the osteoblast isolates.

Methods

cDNA representational difference analysis (RDA) - a PCR-based subtractive hybridisation technique

Additional information

The sequence described appears to be identical to that of the cartilage protein deposited in the Translated European Molecular Biology Laboratory (TEMBL) database (accession number Q9NQ78) in July 2000. The authors do not acknowledge this.

References

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