Quantitation of the Protein MIA as a Marker for Chondrocytes in Research Samples

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The protein MIA is expressed in a cell-type specific manner by melanoma cells and differentiated chondrocytes. Here we show that measuring MIA by a quantitative ELISA can be used as a marker for differentiated chondrocytes in cell culture.

Introduction

MIA (Melanoma Inhibitory Activity) was cloned as a secreted protein from human melanoma cell lines [1]. MIA is expressed in and secreted from malignant melanomas and chondrocytes [2, 3]. As MIA expression in chondrocytes is dependent on the differentiation status of the cells it was also designated CD-RAP (cartilage-derived retinoic acid sensitive protein). Retinoic acid is known as a regulator of chondrocytic cell differentiation *in vitro*.

Recent results indicate an important role in tumor progression and metastasis as MIA mediates detachment of melanoma cells from extracellular matrix molecules such as fibronectin [4]. MIA expression levels closely parallel the capability of melanoma cells to form metastases in syngeneic animals.

Recently, MIA-deficient mice were shown to have structural abnormalities in cartilage [5].

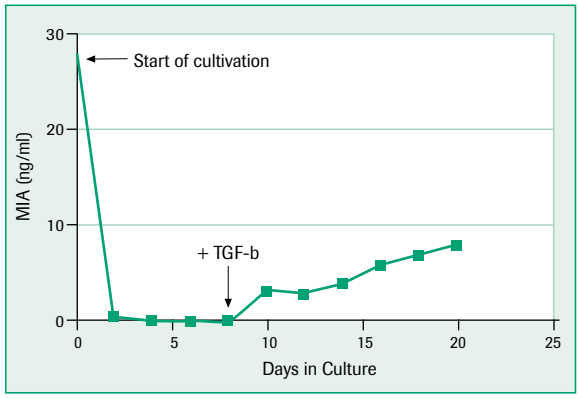

Figure 1: MIA expression using the MIA ELISA was measured in cell-culture supernatant of primary human chondrocytes. Due to cell culturing, a loss of MIA expression is seen during dedifferentiation of the chondrocytes. Re-expression of MIA is detected after induction of re-dedifferentiation by TGF-b.

Materials and Methods

Cell culture

Human primary chondrocytes derived from articular cartilage were cultivated in DMEM supplemented with penicillin (100 U/ml), streptomycin (10 µg/ml) (both Sigma), and 10 % fetal calf serum (Gibco) under a humidified atmosphere of 5 % CO_2 at 37 °C, then split 1:2 at 80 % confluency. As chondrocytes spontaneously dedifferentiate in cell culture, redifferentiation of the cells was induced by treatment with transforming growth factor-b (TGF-b) for 4 days. Differentiation was controlled by determining collagen type II with RT-PCR. Human mesenchymal stem cells were obtained from BioWhittaker Europe (Poietics) and cultured as described by the manufacturer.

MIA-ELISA

MIA was measured by a commercially available ELISA (Roche Applied Science), the manufacturer's instructions were precisely followed and the internal standard reagents provided were used.

Results and Application

MIA as a potential marker for chondrocyte differentiation and chondrocytic disorders

High levels of MIA expression were observed in differentiated chondrocytes. As a result, MIA was also inves-

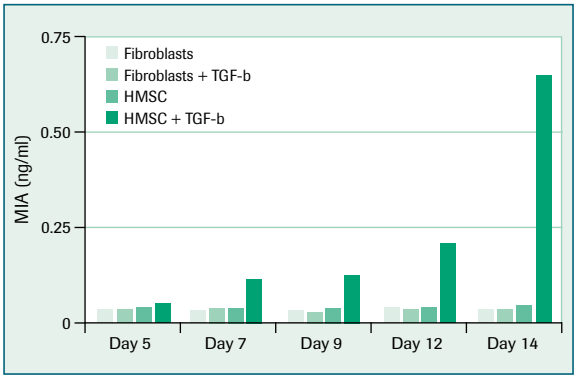

Figure 2: Expression of MIA in chondrocytic differentiation of mesenchymal stem cells. Differentiation of human mesenchymal stem cells (HSMC) using TGF-b results in a chondrocytic phenotype and expression of MIA. In the controls (untreated HMSCs, spheroids of fibroblasts either treated or not treated with TGF-b) no expression was detected.

tigated as a potential marker for cartilage tissue engineering and cartilage damage.

In cell culture of chondrocytes and tissue engineering of cartilage cell differentiation must be carefully monitored. The detection of collagen type II as a marker for chondrocytic differentiation is commonly used. However, using this marker does create problems as ELISAs are not feasible, and for RNA analysis, the cells are lost during extraction procedures. Therefore we established MIA as a chondrocytic marker.

MIA is a secreted molecule, making it easy to measure its levels in the cell culture supernatant. Detection by the MIA-ELISA is highly specific, sensitive, and quantitative. In cell-culture systems using human chondrocytes (Figure 1) and human mesenchymal stem cells (Figure 2), we were able to monitor the process of cell differentiation.

MIA serum concentrations in individuals with different rheumatic diseases were measured, correlated with inflammatory parameters and/or with the degree of joint destruction, and compared with healthy individuals and subjects suffering from melanoma [6]. Increased MIA serum concentrations were only found in individuals with rheumatic diseases associated with joint destruction, such as rheumatoid arthritis, osteoarthritis, HLA B27-associated oligoarthritis, and psoriatic arthritis. Within these rheumatic diseases, the most significant increase in MIA serum concentrations was measured in subjects with rheumatoid arthritis, associated with rheumatoid factor positivity and joint destruction. Therefore, MIA may be useful to discriminate rheumatoid arthritis from nondestructive rheumatic diseases. Further studies are necessary to confirm these preliminary findings.

Summary

Our studies indicate that MIA may be a useful marker for cartilage diseases and the monitoring of chondrocytic differentiation in cell culture. We conclude that MIA has the potential to replace other markers due to its improved applicability.

References

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Order INFO

Product	Pack Size	Cat. No.
MIA ELISA	1 kit (96 reactions)	1 976 826
Also available		
p53 pan ELISA	1 kit (96 reactions)	1 828 789
TeloTAGGG Telomerase PCR ELISA	1 kit (96 reactions)	1 854 666
TeloTAGGG Telomerase PCR ELISA^{PLUS}	1 kit (96 reactions)	2 013 789
TeloTAGGG Telomere Length Assay	1 kit (96 reactions)	2 209 136