Telomerase-transduced Osteoarthritis Fibroblast-like Synoviocytes Display Distinct Gene Expression Patterns

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Introduction: Although the most striking pathological changes in osteoarthritis (OA) are usually found in articular cartilage, OA is not merely a disease of articular cartilage. There is increasing evidence suggesting that other tissues are involved (1). Abnormal levels of certain molecules in the joint fluid, mainly secreted from OA synovium, may contribute to the degeneration of articular cartilage. There has been little effort to investigate the alterations in OA synovium, especially to identify the abnormal gene expressions in OA fibroblast-like synoviocytes (FLS). Using microarrays and RT-PCR, we found that human telomerase transduced OA FLS (hTERT-OA 13A FLS) compared to telomerase transduced rheumatoid arthritis (RA) FLS (hTERT-RA 516 FLS) (4).

Materials and Methods: Microarray analysis: Total RNA was extracted from passage 8 hTERT-OA 13A FLS and hTERT-RA 516 FLS using Trizol reagent, and prepared for microarray analysis according to Agilent protocols. Data was analyzed using Genespring software (Silicon Genetics). Total RNA was extracted from long-term cultures of hTERT-OA 13A FLS (passage 25) and hTERT-RA 516 FLS (passage 25), and prepared for microarray analysis according to Affymetrix protocols. Data was analyzed using Genesifter software (VizX Labs).

RT-PCR: Following RT with TaqMan® Reverse Transcription (Applied Biosystems, Inc.), real time PCR was carried out using TaqMan® Gene Expression reagents and ABI7000 system (Applied Biosystems, Inc.). Semi-quantitative RT-PCR was performed as described (2).

Cell culture: Cells were plated in 100 mm plates at 70% confluence. On the second day, Medium containing 10% serum was added. Twenty-four hours later, total RNA was extracted.

Results: Microarrays were carried using Agilent human cDNA array B chips. Among more than 20,000 genes examined, 89 up-regulated genes and 145 down-regulated genes (more than 2 fold) were detected in hTERT-RA 516 FLS. Select differential expressions were further confirmed by real time RT-PCR.

Discussion: Our findings suggest potential applications of telomerase transduced OA FLS in the study of OA disease biomarkers and in the search for novel new therapeutic targets because of the phenotypic stability as well as the distinct, presumably disease-specific gene expression patterns displayed in telomerase immortalized OA FLS.


Figure 1. hTERT-OA 13A FLS and hTERT-RA 516 FLS were plated in 100 mm plates at 70% confluence. On the second day, medium containing 10% serum was added and the cells were cultured for another 24 hours. RNA was then extracted and examined for gene expressions by semi-quantitative RT-PCR.

This study; ** Kato et al. 2007 (3)

* Among the other five group I candidate biomarkers, three had closely related genes being detected in this study Table 1. Differential gene expression of several group I candidate biomarkers of OA.

Microarray analysis: Total RNA was extracted from long-term cultures of hTERT-OA 13A FLS and hTERT-RA 516 FLS was carried out. Among more than 50,000 transcripts examined, 2,420 up-regulated transcripts and 2,795 down-regulated transcripts (more than 1.6 fold) in hTERT-OA 13A FLS compared to hTERT-RA 516 FLS were detected. Among the 89 up-regulated genes that were detected by Agilent microarray, 62 genes (70%) were again detected by the Affymetrix microarray, which confirmed the phenotypic stability of hTERT-OA 13A FLS. Significantly, among the twelve group I candidate biomarkers of OA (3), seven were detected in this study and five had differential expression similar to that previously reported Table 1. Differential gene expression of several group I candidate biomarkers of OA.

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Furthermore, among the nine group II candidate biomarkers of OA (3), four were detected in this study. They were CXCL2, GSTT1, RPL15 and P2PR5C.

The distribution of these genes in biological processes was examined. Again, the most notable differential gene expressions were the up-regulated expressions of several MHC class II molecules suggesting that hTERT-OA 13A FLS might possess certain trait of macrophage-like synoviocytes. Other notable differential expressions included the up-regulated expressions of many genes whose products functioned in lipid and phosphate transport. In contrast, several putative autoantigens, many angiogenesis-related and anti-apoptosis genes had up-regulated expressions in hTERT-RA 516 FLS. Select differential expressions were further confirmed by real time RT-PCR.

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