Telomerase Transduced Osteoarthritis Fibroblast-Like Synoviocytes Display a Distinct Gene Expression Profile

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Abstract

Objective—To examine the differential gene expression in telomerase transduced osteoarthritis fibroblast-like synoviocytes (hTERT-OA 13A FLS) and telomerase transduced rheumatoid arthritis FLS (hTERT-RA 516 FLS) and test the hypothesis that longterm culture of hTERT-OA 13A FLS display a disease-specific gene expression profile.

Methods—Gene expression in passage 8 hTERT-OA 13A FLS and passage 8 hTERT-RA 516 FLS were compared using microarray assays. Differential expression of selected genes was further examined by reverse transcription-polymerase chain reaction (RT-PCR). After continuous expansion in culture for an additional 4 months, gene expression in the longterm cultures of hTERT-OA 13A FLS and hTERT-RA 516 FLS was again examined with microarray and real-time RT-PCR.

Results—hTERT-OA 13A FLS displayed a distinct gene expression profile. While hTERT-RA 516 FLS expressed ADAMTS1, ADAMTS3, ADAMTS5, and several carboxypeptidases, hTERT-OA 13A FLS expressed matrix metalloproteinase (MMP)1, MMP3, and several cathepsins at higher levels. Numerous genes classified in the immune response, lipid transport/catabolism, and phosphate transport biological processes were also expressed at higher levels in hTERT-OA 13A FLS. In contrast, numerous genes classified in the positive regulation of cell proliferation, anti-apoptosis, and angio-genesis biological processes were expressed at higher levels in hTERT-RA 516 FLS. Further, of the recently proposed 21 candidate synovial biomarkers of OA, 12 (57%) were detected in our study.

Conclusion—The findings indicate that OA FLS may not be a passive bystander in OA and that telomerase transduced OA FLS offer an alternative tool for the study of synovial disease markers and for the identification of new therapeutic targets for OA therapy.

Keywords

OSTEOARTHRITIS; SYNOVIOCYTES; DISEASE MARKERS; ADAMTS; MATRIX METALLOPROTEINASE; RHEUMATOID ARTHRITIS; TELOMERASE

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Osteoarthritis (OA) is a progressive disorder characterized by the degeneration of articular cartilage, formation of osteophytes, and mild synovial inflammation. Because the most striking pathological changes are found in articular cartilage, OA is mainly considered a degenerative disease of articular cartilage. OA synovium has traditionally been considered to be "normal" and was used as controls in many studies to investigate the pathological changes in the synovium of patients with rheumatoid arthritis (RA)[1-4]. OA fibroblast-like synoviocytes (FLS) were used as controls in studies investigating the cellular alterations in RA FLS[5-8]. However, there is increasing evidence that the OA synovium may not be a passive bystander in the initiation or progression of OA[9,10]. A low degree of synovium inflammation exists in OA[11-15]. OA synovium and OA FLS display differential gene expressions compared to RA synovium and RA FLS or compared to normal synovium and normal FLS[16,17]. These observations support the hypothesis that OA FLS, similar to RA FLS, is abnormal.

Analyses of differential gene expressions between OA synovium and RA synovium, or between OA synovium and normal synovium are important for understanding the *in vivo* state of OA synovium; however, such work provides no information regarding cell type specific differential gene expressions. Analyses of primary FLS provide information that is more cell type specific, but they also have drawbacks. Differential gene expressions detected using primary OA FLS and RA FLS may vary due to the variations in the methods of cell preparation or the different passage numbers. Non-FLS types of cells coexist in primary FLS preparations that secrete inflammatory cytokines and express cell type specific proteins[18], which, in turn, may affect the gene expressions in FLS. Primary FLS of early passages (passages 2−3) used in many reported studies may contain up to 5% non-FLS cell types calculated according to the doubling time of synovial cells and initial cell populations[18]. It has been reported that the expressions of numerous genes in arthritis-derived FLS change considerably with passages[17]. The passage-dependent gene expressions in primary FLS not only make it difficult to get consistent and repeatable results using primary FLS derived from a specific patient, but also make it difficult to compare the results from different laboratories. Moreover, the differential gene expression detected using primary FLS (passages 2−3) may not reflect the "true" disease characteristics of the FLS examined but reflect the characteristics of the FLS mixed with other types of cells. These drawbacks can be partially overcome by the examination of telomerase-transduced FLS, since the coexistence of other cell types in telomerase-transduced FLS is highly unlikely and the differential expression of a majority of genes is largely maintained with time.

Currently, diagnosis of OA depends on clinical and radiographic findings such as joint pain and radiographic changes in articular cartilage. These approaches are only effective in detecting intermediate or late-stage OA. New diagnostic methods, especially for early diagnosis of OA, are needed. A recent study reported that OA synovial tissues displayed distinct differential expression patterns compared to RA synovial tissues, and suggested that the differential expression of selected genes might be useful as disease markers[16]. Another study reported that OA FLS displayed distinct differential expression patterns compared to RA FLS and also suggested that the differential expression of selected genes might be useful as disease markers[17]. In our laboratory, we recently successfully established human telomerase-transduced OA FLS and telomerase-transduced RA FLS[19,20]. In the work reported here we examine the differential gene expression between hTERT-OA 13A FLS and hTERT-RA 156 FLS to investigate whether hTERT-OA 13A FLS display a stable and distinctive gene expression profile compared to hTERT-RA 516 FLS, and whether certain distinct gene expression patterns are disease-specific.

MATERIALS AND METHODS

Materials and cells

Dulbecco's modified eagle medium, fetal bovine serum, stock antibiotic and antimycotic mixture were products of Invitrogen (Carlsbad, CA, USA). hTERT-OA 13A FLS and hTERT-RA 516 FLS have been described. They were established from passage 5 primary OA FLS and passage 5 primary RA FLS, respectively, with the approval of the authors' Institutional Review Board[19,20].

Cell culture and RNA extraction

hTERT-OA 13A FLS (passage 8) and hTERT-RA 516 FLS (passage 8) were plated in 100 mm plates at 60% confluence. On the second day, medium containing 10% serum was added and cells were cultured for 24 h. Total RNA was extracted using Trizol reagent (Invitrogen), and purified using Oligotex kit (Qiagen, Valencia, CA). Agilent microarray and semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) were performed using these RNA samples. The passage 8 hTERT-OA 13A FLS and passage 8 hTERT-RA 516 FLS were continuously expanded for 4 months. The longterm cultured hTERT-OA 13A FLS (passage 25) and hTERT-RA 516 FLS (passage 25) were plated in 100 mm plates at 60% confluence and cultured for 24 h. RNA was extracted using Trizol reagent and purified using Oligotex kit (Qiagen, Valencia, CA). Affymetrix microarray and quantitative real time RT-PCR were performed using these RNA samples.

Agilent microarrays

Microarray experiments were carried out to examine the differential gene expression between passage 8 hTERT-OA 13A FLS and passage 8 hTERT-RA 516 FLS using Agilent human cDNA array B chips (Agilent, Palo Alto, CA) as described[20]. Microarray was scanned at 10 µm resolution using a GenePix 4000A scanner (Axon Instruments, Union City, CA) and image was analyzed using the GenePix Pro 5.1 software (Axon Instruments, Union City, CA) for normalization and statistical analysis. Data was filtered for unreliable data points using Acuity software (Axon Instruments, Union City, CA). Filtered data was imported into GeneSpring software for further statistical analysis (Silicon Genetics, Redwood City, CA). Differentially expressed genes were classified according to gene ontology category biological process using Onto-Express software (http://vortex.cs.wayne.edu/Projects.html).

Affymetrix microarray

Double-stranded DNA was synthesized using SuperScript double-stranded cDNA synthesis kit (Invitrogen, San Diego, CA). The DNA product was purified using GeneChip sample cleanup module (Affymetrix, Santa Clara, CA). cRNA was synthesized and biotin labeled using BioArray high yield RNA transcript labeling kit (Enzo Life Sciences, Farmingdale, NY). The cRNA product was purified using GeneChip sample cleanup module and subsequently chemically fragmented. The fragmented, biotinylated cRNA was hybridized to HGU133_Plus_2 gene chip using Affymetrix Fluidics Station 400. Fluorescent signal was quantified during 2 scans by Agilent Gene Array Scanner G2500A (Agilent Technologies), and GeneChip operating Software (Affymetrix). For further analysis of fold changes, false discovery rate, and gene ontology, Genesifter software (VizX Labs, Seattle, WA) was used.

Semiquantitative RT-PCR

Briefly, 1 µg of RNA sample was reverse transcribed at 60°C for 60 min, followed by enzyme inactivation at 85°C for 5 min. PCR experiments were carried out using ThermoScript RT-PCR machine (Invitrogen, Carlsbad, CA). Amplifications were carried out for 30−40 cycles by denaturing at 95°C for 30 s, annealing at 55°C for 30 s, and extending at 72°C for 45 s, with

a final extension at 72°C for 10 min. The products were electrophoresed on 2% agarose gels, stained with ethidium bromide, and photographed using a low light image system (ChemiImager 4000, Alpha Innotech Corporation, San Leandro, CA). Each RT-PCR experiment was repeated at least 3 times using 2 or 3 different RNA batches.

Real-time RT-PCR

cDNA was synthesized using TaqMan® Reverse Transcription Reagents (Applied Biosystems, Inc., University Park, IL). Quantitative real time RT-PCR was then performed. Briefly, quantification of relative transcript levels for selected genes and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed using the ABI7000 Real Time PCR system (Applied Biosystems). TaqMan® Gene Expression assays (Applied Biosystems) were used, which contains a FAM-MGB probe for fluorescent detection. The probe information can be obtained on request from the manufacturer. cDNA samples were amplified with an initial Taq DNA polymerase activation step at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 1 min. For each gene, Ct values were obtained in triplicate. Fold change was calculated[21] and the expression level of the genes of interest was normalized to the expression level of GAPDH. Each real time RT-PCR experiment was repeated twice in triplicate using 2 different RNA batches.

RESULTS

Differential gene expression in passage 8 FLS

Agilent microarray analyses of the differential gene expression in passage 8 hTERT-OA 13A FLS and passage 8 hTERT-RA 516 FLS were carried out as described in Materials and Methods. We found that the dye bias was substantial, and caused many false positive results. Therefore, 2 microarrays were performed by reverse labeling with Cy3 and Cy5. Only the genes that showed similar differential gene expression in both microarrays were selected for further study and/or discussion. Among the more than 22,605 transcripts examined, a total of 178 differentially-expressed genes (more than 2.5-fold) were detected; 62 genes were expressed at higher levels and 116 genes were expressed at lower levels in hTERT-OA 13A FLS compared to hTERT-RA 516 FLS (data not shown). These differentially expressed genes were classified according to gene ontology category biological process. Selected genes, which are involved in a specific biological process that has been implicated in OA or is suspected to play a role in OA pathological calcification such as ectonucleotide pyrophosphatase-phosphodiesterase 2 (*ENPP2*) and adenylate cyclase 3 (*ADCY3*), are listed in Table 1.

A distinct and notable differential gene expression pattern was the elevated expression of many genes classified into the biological process immune response in the hTERT-OA 13A FLS (passage 8) compared to hTERT-RA 516 FLS (passage 8). Of the 12 genes classified into the biological process immune response, 8 genes displayed elevated expressions and only 4 genes displayed decreased expression in hTERT-OA 13A FLS compared to hTERT-RA 516 FLS. Another notable differential gene expression pattern was the elevated expression of several genes associated with lipid transport, catabolism, and metabolism biological processes in the hTERT-OA 13A FLS compared to hTERT-RA 516 FLS.

Many genes implicated in calcification process in OA have been recently identified, including ectonucleotide pyrophosphatase-phosphodiesterase 1 (*ENPP1/PC-1*), *ADCY3*, progressive ankylosis homolog (*ANKH*), and tissue nonspecific alkaline phosphatase (*TNSAP*)[22-25]. These proteins share a common feature in that they are all in some way related to pyrophosphate. Interestingly, *ADCY3*, *ENPP2*, and ectonucleoside triphosphate diphosphohydrolase 1 (*ENTPD1*), which are related to pyrophosphate, were found to be expressed at elevated levels in the passage 8 hTERT-OA 13A FLS compared to passage 8

hTERT-RA 516 FLS (Table 1). The elevated expression of these 3 genes was also detected in passage 25 hTERT-OA 13A FLS (Table 1, column 3; the numbers inside the parentheses indicate the differential expression of these genes between passage 25 hTERT-OA 13A FLS and 25 passage hTERT-RA 516 FLS).

Validation of differential expression of selected genes

We carried out semiquantitative 6RT-PCR to validate the differential expression of 3 genes classified into the biological process immune response: major histocompatibility complex, class II, DR beta 4 (*HLA-DRB4*); MHC, class II, DR beta 3 (*HLA-DRB3*); and MHC, class II, DP alpha 1 (*HLA-DPA*). As shown in Figure 1, elevated expression of these 3 genes in passage 8 hTERT-OA 13A FLS compared to passage 8 hTERT-RA 516 FLS was confirmed. We also carried out semiquantitative RT-PCR to validate the differential expression of 3 genes involved in lipid transport and catabolism: macrophage scavenger receptor type 1 (*MSR1*), phospholipase A2 group IIA (*PLA2G2A*), and CD36 antigen (*CD36*). The differential expression of these 3 genes between hTERT-OA 13A FLS and hTERT-RA 516 FLS was also confirmed by semiquantitative RT-PCR (Figure 1).

Differential gene expression in longterm cultures of FLS

To determine the genotypic and phenotypic stability of hTERT-OA 13A FLS, and further investigate the differential gene expressions, a microarray experiment using Affymetrix HGU133_Plus_2 gene chips and RNA samples extracted from the longterm culture of hTERT-OA 13A FLS (passage 25) and hTERT-RA 516 FLS (passage 25) were performed. We decided to use Affymetrix microarray this time instead of Agilent microarray because we found that Agilent microarray was less sensitive than Affymetrix microarray in detecting differential gene expressions and Affymetrix HGU133_Plus_2 gene chip covers more transcripts (more than 50,000 transcripts) than Agilent human cDNA array B chip (22,605 transcripts). Of more than 50,000 transcripts examined, 2,033 genes that displayed differential gene expression (more than 2-fold) were identified; 895 genes were expressed at higher levels and 1,138 genes at lower levels in passage 25 hTERT-OA 13A FLS compared to passage 25 hTERT-RA 516 FLS (data not shown).

Differentially-expressed genes were classified according to gene ontology category biological process and selected results are listed in Table 2. We were particularly interested in identifying distinct differential gene expression patterns. As shown in Table 2, distinct differential gene expression patterns were apparent. For example, of the 62 differentially-expressed genes classified into the immune response biological process, 39 genes had elevated expressions and 23 genes had decreased expression in hTERT-OA 13A FLS compared to hTERT-RA 516 FLS. In other words, 39 genes had elevated expressions in hTERT-OA 13A FLS compared to hTERT-RA 516 FLS, and 23 genes had elevated expressions in hTERT-RA 516 FLS compared to hTERT-OA 13A FLS. Of the 15 differentially expressed genes classified into the phosphate transport biological process, 10 genes had an elevated expression and only 5 genes had decreased expression in hTERT-OA 13A FLS compared to hTERT-RA 516 FLS. Of the 14 genes classified into the lipid catabolic process/lipid transport biological processes, 11 genes had an elevated expression and only 4 genes had decreased expression in hTERT-OA 13A FLS compared to hTERT-RA 516 FLS. Of the 6 differentially expressed genes classified into the fatty acid metabolic process, 4 genes had an elevated expression and 2 genes had decreased expressions in hTERT-OA 13A FLS.

In contrast, the genes that had elevated expressions in hTERT-RA 516 FLS fell into a different category of biological processes. For example, of the 34 differentially expressed genes classified into the positive regulation of cell proliferation biological process, 24 genes had an elevated expression and only 10 genes had decreased expression in hTERT-RA 516 FLS

compared to hTERT-OA 13A FLS. Of the 22 differentially expressed genes classified into the anti-apoptosis biological process, 17 genes had an elevated expression and only 5 genes had a decreased expression in hTERT-RA 516 FLS compared to hTERT-OA 13A FLS. Of the 20 differentially expressed genes classified into the angiogenesis biological process, 15 genes had an elevated expression in hTERT-RA 516 FLS and only 5 genes had decreased expression. These distinct distributions of the differentially-expressed genes in different sets of biological processes suggest that OA 13A FLS and hTERT-RA 516 FLS have definitely different cellular properties and activities.

Validation of differential expression of selected genes by real-time RT-PCR

The genes selected for validation included MHC, class II, DR alpha (HLA-DRA), microsomal glutathione S-transferase 1 (MGST1), matrix metalloproteinase (MMP)1, 3 rab genes and 2 collagen genes. A disintegrin and metalloproteinase with thrombospondin type 1 motif (ADAMTS1), which was expressed at a lower level in both the passage 8 and passage 25 hTERT-OA 13A FLS compared to hTERT-RA 516 FLS, was also selected for validation. The results of quantitative real-time RT-PCR are listed in Table 3. As shown, the differential expression of these genes in the longterm culture of hTERT-OA 13A FLS and hTERT-RA 516 FLS were confirmed by quantitative real-time RT-PCR.

DISCUSSION

In our study, we demonstrate that hTERT-OA 13A FLS display a stable differential gene expression profile compared to hTERT-RA 516 FLS. Of the 62 genes that were detected with elevated expressions in passage 8 hTERT-OA 13A FLS compared to passage 8 hTERT-RA 516 FLS, 43 genes (69%) showed elevated expression in passage 25 hTERT-OA 13A FLS compared to passage 25 hTERT-RA 516 FLS. All the genes listed in Table 1 which displayed elevated expression in passage 8 hTERT-OA 13A FLS were found to display elevated expression in passage 25 hTERT-OA 13A FLS with the exception of HLA-DRB3, indoleamine-pyrrole 2−3 dioxygenase (INDO) and CCL26/eotaxin-3 (Table 1). There was no elevated expression of HLA-DRB3, INDO, and CCL26/eotaxin-3 detected in passage 25 hTERT-OA 13A FLS (Table 1).

As hTERT-OA 13A FLS and hTERT-RA 516 FLS contain no other types of cells, the differential expressions between hTERT-OA 13A FLS and hTERT-RA 516 FLS may reflect the FLS-specific "true" disease characteristics of OA 13A FLS and RA 516 FLS. In addition, because the differential expression of a majority of genes remains largely unchanged with time, telomerase transduced FLS offer a valuable tool for the study of arthritis-derived FLS and for the identification of disease candidate genes.

MMP, cathepsins, and ADAMS are all implicated in arthritis[3,8,26-30]. MMP-1 and MMP13 degrade type II collagen. MMP2, MMP3, MMP9, and ADAMTS degrade proteoglycans. Our microarray data revealed that a similar number of genes encoding proteolytic proteins was expressed at higher levels in hTERT-OA 13A FLS (49 genes) (Table 2, column 3, the positive numbers) and in hTERT-RA 516 FLS (44 genes) (Table 2, column 3, the negative numbers), indicating that proteolytic proteins play an important role in both OA and RA. However, the functional family classifications of the upregulated genes in hTERT-OA 13A FLS were different from the functional family classifications of the upregulated genes in hTERT-RA 516 FLS. MMP3, MMP1, and several cathepsins were expressed at higher levels in hTERT-OA 13A FLS compared to hTERT-RA 516 FLS (Table 2, column 3, the positive numbers). In contrast, ADAMTS5, ADAMTS3, ADAMTS1, and several carboxypeptidases were expressed at higher levels in hTERT-RA 516 FLS compared to hTERT-OA 13A FLS (Table 2, column 3, the negative numbers). The findings that ADAMTS5 and ADAMTS1 are expressed at decreased levels in hTERT-OA 13A FLS compared to hTERT-RA 516 FLS are consistent with

a previous finding that ADAMTS5 and ADAMTS1 are expressed at decreased levels in OA synovium and OA articular cartilage[31]. These findings together suggest that OA FLS may be involved in the degradation of both proteoglycans and type II collagen, and that RA FLS may be mainly involved in the degradation of proteoglycans, consistent with the clinical observations that degradation of type II collagen in articular cartilage is much more prominent in OA than in RA except at the pannus-cartilage junction. At the pannus-cartilage junction, RA FLS may be involved in the degradation of collagen by secreting inflammatory cytokines and stimulating the expression of collagen II degrading enzymes by articular chondrocytes.

In addition to MMP and ADAMS, many other genes previously found to be aberrantly expressed in OA were detected in our study. They include PLA2[32], collagen, type XI, alpha 1 (COL11A1)[33,34], ANKH[35,36], interleukin (IL)-7[37], IL1 receptor alpha 1 (IL1R1)[16], fibronectin 1 (FN1)[16], human complement subcomponent C1s (C1S)[38], and v-ets erythroblastosis virus E26 oncogene homolog 1 (ETS1)[39]. PLA2 is expressed at higher or similar levels in OA joint tissues compared with RA joint tissues, and may play a role in inflammation[32]. Decreased expression of COL11A1 due to a mutation has been shown to cause OA-like changes including reduced mechanical function of articular cartilage and increased degradation of type II collagen[33,34]. IL-7 is expressed in OA chondrocytes and the expression of collagen-degrading enzymes such as MMP13 is induced by IL-7[37]. Lost-function mutations of ANKH cause calcification and degeneration of articular cartilage[35,36]. Aberrant expression of IL1R1 and FN1 are potential disease markers of OA[16]. C1S and ETS1 have been implicated in RA[40,41]. The consistency between our findings and previous findings provides further support for the hypothesis that OA FLS is not a bystander in OA. In addition, it suggests the validity of our approach to the study of OA FLS, and our approach to the identification of potential disease candidate genes using telomerase transduced OA FLS.

OA is recognized as having a genetic component. Many genes harboring susceptibility for primary OA have been recently identified through genome-wide linkage scan of genetic polymorphisms and association analysis. We recently did a literature search for these genes. The genes we found from published articles are listed in Table 6. Incredibly, of the 28 genes harboring susceptibility for primary OA, 11 genes (39%) are detected in our study and are differentially expressed between hTERT-OA 13A FLS and hTERT-RA 516 FLS (Table 4). This match not only demonstrates that telomerase transduced FLS is a valuable tool for the study of arthritis derived FLS but also provides further support for the previous findings that ADAM metallopeptidase domain 12 (ADAM12), ADAMTS-5, asporin (ASPN), cartilage oligomeric matrix protein (COMP), deiodinase type II (DIO2), ENPP1, estrogen receptor 1 (ESR1), growth differentiation factor 5 (GDF5), isocitrate dehydrogenase (IDH1), neuropilin 2 (NRP2), and matrilin 3 (MATN3) harbor susceptibility for primary OA.

Phosphate-containing crystals are detected in synovial fluid of up to 70% of patients with OA. These crystals may accelerate the progression of OA[42-45]. It has been reported that phosphate plays a role in IL-8 induced hypertrophy of articular chondrocytes[46], and that phosphate affects growth plate chondrocyte maturation, apoptosis, and mineralization[47,48]. In our study, we found that many genes involved in phosphate transport were highly expressed in hTERT-OA 13A FLS (Tables 1 and 2). This distinct differential gene expression pattern suggests that the regulation of phosphate in OA FLS is different from that in RA FLS. Interestingly, several genes implicated in promoting or inhibiting pathological calcification are also expressed at higher levels in passage 25 hTERT-OA 13A FLS compared to passage 25 hTERT-RA 516 FLS including ENPP1 (1.5-fold), ENPP2 (5.2-fold), ENTPD1 (18.3-fold), and ADCY3 (2.6-fold). Although the implication of these differential gene expression patterns is unclear at present, it warrants further study because the deposition of phosphate-containing crystals in the knee joints of patients with OA is a prominent clinical phenomenon.

Increasing evidence suggests that oxidized low density lipoproteins (ox-LDL) may play a role in the pathogenesis of OA[49,50]. The percentage of ox-LDL-positive chondrocytes is much higher in OA chondrocytes and correlates with OA grade[51,52]. Because phospholipid is one of the components of ox-LDL, and has been implicated in calcification[53,54], elevated transport and catabolism of ox-LDL may disturb local homeostasis of phosphate and play a role in pathological calcification. Interestingly, our microarray data revealed that many genes involved in the transport and catabolic process of lipids and ox-LDL were expressed at higher levels in hTERT-OA 13A FLS (Table 2), suggesting a possible involvement of synovial lipids and ox-LDL in the disease process of OA.

Our microarray data also revealed distinct distributions of the genes classified into the positive regulation of cell proliferation biological process including fibroblast growth factor (FGF)2, FGF9, and FGF10, the anti-apoptosis biological process including secreted frizzled-related protein 1 (SFRP1), bcl-2-related protein A1 (BCL2A1), and tumor necrosis factor receptor-related receptor for TRAIL (TNFRSF10D/TRAIL receptor 4) and the angiogenesis biological process including angiopoietin 1 (ANGPT1), vascular endothelial growth factor C (VEGFC), FGF1, and connective tissue growth factor (CTGF) (Table 2). These distinct distributions appear consistent with the clinical observations that severe synovial hyperplasia occurs in RA but not in OA, suggesting an abnormal regulation of proliferation, apoptosis, and angiogenesis in RA FLS. On the other hand, apoptosis of articular chondrocytes has been implicated in OA. The decreased release by OA FLS of certain soluble factors involved in above 3 biological processes may have certain effects on the survival of articular chondrocytes in OA. This subject may warrant further study.

Kato, *et al*[16] reported that the differential expression of selected genes between OA synovium and RA synovium might be used as a synovial disease marker of OA. Significantly, of the proposed 12 group I candidate biomarkers, 7 (58%) were detected in this study, and 5 displayed similar differential gene expressions (Table 5). Of the proposed 9 group II candidate biomarkers, 5 (56%) were detected in our study (Table 5). The consistency between our findings and the previous report[16] suggests that human telomerase-transduced OA FLS display a disease-specific gene expression profile. In addition, this supports the application of selected differential gene expressions as disease markers of OA.

Our findings are consistent with many clinical observations and previous experimental data; however, unexpected findings were also uncovered. OA is a degenerative disease, whereas RA is an abnormal immune response disease. Naturally, one would expect that the number of immune response genes displaying elevated expressions in RA FLS would outnumber the number of immune response genes displaying elevated expressions in OA FLS. However, we observed the opposite. A possible explanation for this unexpected finding is that OA 13A FLS possess certain traits of macrophage-like synoviocytes (MLS). The elevated expression of HLA-DPA1, HLA-DRB4, HLA-DQA1, and carboxylesterase 1/monocyte-macrophage serine esterase 1 (CES1) in hTERT-OA 13A FLS provides support for this explanation[55]. Recently it has been shown that synovial macrophages mediate the formation of osteophyte in OA[56]. It is worth noting that the association of osteophytes with OA may be partially explained by our finding that OA FLS possess certain traits of MLS. OA-FLS, together with OA-MLS, may promote the formation of osteophyte. However, further study is needed to examine the role of OA FLS in the formation of osteophytes.

Galligan, *et al*[17] recently reported that 287 differentially expressed genes were detected between OA FLS and RA FLS. Among these genes, 67 genes displayed significant differential gene expressions from control base line. Of the 67 genes, 23 (34%) are detected in our study. However, only 10 genes (44%) among the 23 displayed similar differential gene expressions; 7 had decreased expression and 3 had elevated expression in OA FLS compared to RA FLS

(Table 6, the 3 columns from the left). The other 13 genes display opposite differential gene expressions (Table 6, the 3 columns from the right). One possible explanation for this "inconsistency" is that primary FLS of passages 2−3 may contain up to 5% of other types of cells, but telomerase-transduced FLS contain no other types of cells. The elevated expression of HLA-DRA and HLA-DRB4 in primary passage RA FLS compared to primary early passage OA FLS, especially in passage 2 FLS, might result from the elevated expressions of these genes in RA MLS rather than in RA FLS[56]. In contrast, the elevated expression of HLA-DRA and HLA-DRB4 in hTERT-OA 13A FLS compared to hTERT-RA 516 FLS might actually reflect the intrinsic characteristics of the OA 13A FLS.

Our study has limitations. All gene expressions were examined at transcription levels. In order to draw a more definite conclusion, confirmation at the protein level is needed. Another limitation is that the gene expressions were compared between one telomerase-transduced OA FLS and one telomerase-transduced RA FLS. Although many of our findings are consistent with findings reported from other laboratories where a large number of primary OA synovium/ FLS and RA synovium/FLS was used, it is still uncertain how many of the differentially expressed genes (Table 2) are disease-specific rather than individual-specific. It is also unclear whether longterm cultures of telomerase-transduced OA FLS can be classified into different subgroups according to their gene expression profiles. A larger number of telomerase-transduced OA and RA FLS are needed to address these issues. We plan to establish a larger number of telomerase-transduced OA and RA FLS, and will carry out further studies to address these limitations.

Acknowledgments

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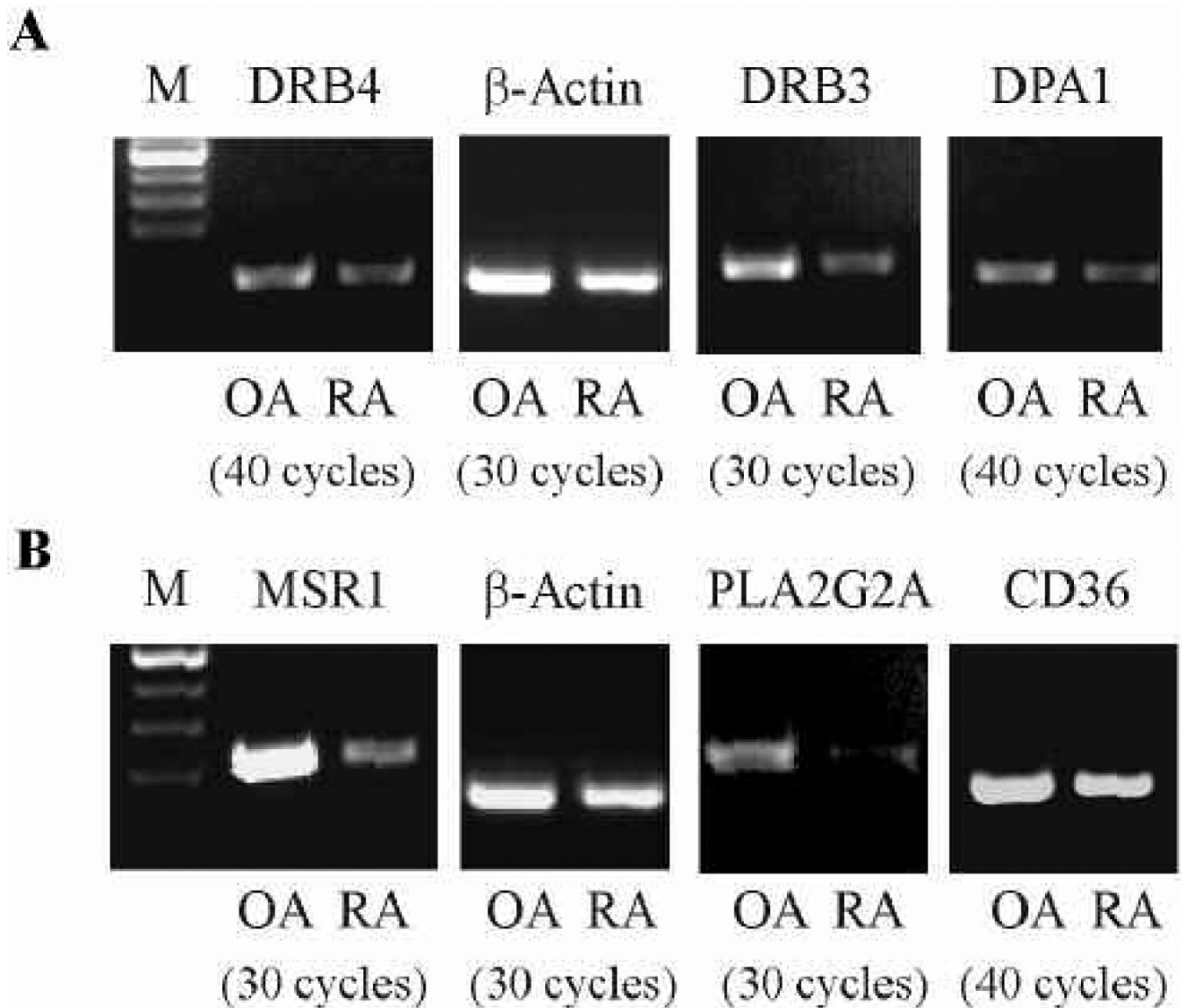
Figure 1.
Validation of the differential expression of selected genes. hTERT-OA 13A FLS (passage 8) and hTERT-RA 516 FLS (passage 8) were plated in 100 mm plates at 60% confluence. On the second day, medium containing 10% serum was added and the cells cultured for another 24 h. RNA samples were extracted and examined by semiquantitative RT-PCR. A. The mRNA levels of HLA-DRB4, HLA-DRB3, HLA-DPA1, and ß-Actin. B. The mRNA levels of MSR1, PLA2G2A, CD36, and ß-Actin. Experiments were repeated at least 3 times and used 2 different RNA batches. Similar results were obtained.

Table 1

Selected differentially expressed genes detected by Agilent microarray.

Funtional Family	Gene Name	Differ Expre (fold)[*]	Description
Immune response			
	HLA-DR beta 4	6.1 (35.6)	Beta 4 chain of HLA-DR, a subunit of MHC class II molecules that bind peptides and present them to CD4+ T lymphocytes
	DPP4	3.7 (5.4)	Dipeptidylpeptidase IV, a serine type peptidase, removes N-terminal X-Pro and X-Ala dipeptides from regulatory peptides
	HLA-DR beta 3	2.6 (0)	Major histocompatibility complex class II DR beta 3, a class II major histocompatibility complex antigen
	INDO	2.6 (0)	Indoleamine-pyrrole 2−3 dioxygenase, catabolizes tryptophan, reduces tryptophan abundance and thereby suppresses T cell
	HLA-DR beta 5	2.5 (11.7)	Major histocompatibility complex class II DR beta 5, subunit of an MHC class II molecule
	HLA-DPA1	2.5 (12.7)	MHC class II DP alpha 1, subunit of the HLA-DP dimmer that functions as a major histocompatibility complex class II protein
	IL-7	6.8 (8.9)	Interleukin 7, a hematopoietic growth factor required for nomral growth and development of B cells and T cells
	SEMA3C	2.5 (1.9)	Semaphorin 3C (semaphorin E), a member of the semaphorin family involved in neuronal growth cone guidance
	IGJ	−2.8 (−14.9)	Immunoglobulin J polypeptide, regulates polymer formation of immunoglobulins IgA and IgM, and helps promote their secretion
	CCL26	−2.7 (0)	Eotaxin-3, a CC class chemokine and chemoattractant for basophils, eosinophils, T cells, and monocytes
	F3	−2.7 (−65)	Coagulation factor III (tissue factor), integral membrane protein and receptor for factor VIIa (F7)
	CCL2	−4.4 (−4.8)	Cytokine A2, CC chemokine that attracts monocytes, memory T-cells, natural killer cells and endothelial cells, plays a role in the inflammatory
Lipid transport, catabolism and metabolism			
	MSR1	7.3 (48.4)	Macrophage scavenger receptor type I, binds and internalizes low-density lipoprotein and activates protein kinase C
	SCARA5	3.3 (75.3)	Scavenger receptor class A, member 5
	CD36	4.3 (22.8)	An integral membrane receptor, binds thrombospondin, collagen, oxidized low density lipoprotein, long chain fatty acids
	HSD11B1	11.1 (12.7)	11-beta-hydroxysteroid dehydrogenase type 1, catalyzes the interconversion of inactive cortisone and active cortisol
	PLA2G2A	4.6 (4.7)	Phospholipase A2, group IIA (platelets, synovial fluid), nuclear gene encoding mitochondrial protein
	AKR1C2	3.3 (1.8)	Aldo keto reductase family 1 member C2, functions in bile transport, steroid metabolism, and xenobiotic metabolism
	AKR1C3	3.3 (2.2)	Aldo keto reductase family 1 member C3, a type 2 3 alpha (17 beta)-hydroxysteroid dehydrogenase
	NR2F2	3.0 (25.9	Nuclear receptor subfamily 2 group F member 2, binds to hormone-responsive elements in APOB, APOA2, apoCIII

Funtional Family	Gene Name	Differ Expre (fold)[*]	Description
	ALDH1A3	−4.7 (−3.6)	Aldehyde dehydrogenase 1 family member A3, catalyzes the oxidation of all-trans-retinaldehyde to retinoic
	PTGIS	−2.2 (−1.8)	Prostaglandin 12 synthase, a member of the cytochrome P450 family of proteins, converts prostaglandin H2 to the vasodilator
Phosphate transport			
	MSR1	7.3 (48.4)	Macrophage scavenger receptor type I, binds and internalizes low-density lipoprotein and activates protein kinase C
	SCARA5	3.3 (75.3)	Scavenger receptor class A, member 5
	COL8A1	−2.5 (−2.5)	Alpha 1 subunit of type VIII collagen, contains a non-triple helical (NC1) domain which
Miscellaneous			
	ENPP2	4.4 (5.2)	Ectonucleotide pyrophosphatase-phosphodiesterase 2, hydrolyzes nucleotides, lipids, generating pyrophosphate/phosphate.
	ENTPD1	2.8 (18.3)	Ectonucleoside triphosphate diphosphohydrolase 1, hydrolyzes extracellular ATP and ADP to AMP, generating pyrophosphate/phosphate
	ADCY3	2.8 (2.6)	Adenylyl cyclase 3, a membrane-associated ATP to cAMP-converting enzyme, generating pyrophosphate
	RAB27B	4.3 (5.9)	Ras-related GTP-binding protein 27b, GTP-binding protein and GTPase, may be involved in exocytosis
	CES1	3.9 (6.2)	Carboxylesterase 1 (monocyte-macrophage serine esterase 1), hydrolyzes aromatic and aliphatic esters, expressed only in the monocyte-macrophage
	ADA	3.2 (3.8)	Adenosine deaminase, plays a role in immune response, binds to CD26 (DPP4); deficiency causes a form of severe combined immunodeficiency

[*] Positive number indicates elevated expression (fold) in hTERT-OA 13A FLS (passage 8) compared to hTERT-RA 516 FLS (passage 8). Negative number indicates decreased expression (fold) in hTERT-OA 13A FLS (passage 8) compared to hTERT-RA 516 FLS (passage 8) or indicates elevated expression (fold) in hTERT-RA 516 FLS (passage 8) compared to hTERT-OA 13A FLS (passage 8). The number inside the parenthesis indicates the differential gene expressions detected using Affymetrix microarray between 25 passage hTERT-OA13A FLS and 25 passage hTERT-RA 516 FLS.

Table 2

Selected differentially expressed genes detected by Affymetrix microarray.

Funtional Family	Gene Name	Differ Expre (fold)[*]	Description
Proteolysis			
	MMP3	4.0	Matrix metalloproteinase 3
	MMP1	1.7	Matrix metalloproteinase 1
	CTSC	4.9	Cathepsin C
	CTSF	2.1	Cathepsin F
	CTSH	3.7	Cathepsin H
	CTSL	2.5	Cathepsin L
	CTSL	1.8	Cathepsin-L-like
	ADAM10	1.9	Disintegrin and metalloproteinase domain 10
	ADAM23	1.8	Disintegrin and metalloproteinase domain 23
	ADAM32	1.9	Disintegrin and metalloprotease domain 32
	PAPPA2	6.6	Pappalysin 2
	CFB	2.2	Complement factor B
	CFD	2.7	Complement factor D (adipsin)
	C1R	1.8	Cmplement component 1, r subcomponent
	C1S	1.9	Human complement subcomponent C1s, alpha- and beta-chains
	C5	3.8	Complement component 5
	PSMB9	2.7	Proteasome (prosome, macropain) subunit, beta type, 9
	PSMB10	2.7	Proteasome (prosome, macropain) subunit, beta type, 10
	FBXO6	3.6	F-box protein 6
	DPP4	5.4	Dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)
	MME	4.2	Membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase)
	XPNPEP3	9.5	X-prolyl aminopeptidase (aminopeptidase P) 3
	CD55	3.3	CD55 molecule, decay accelerating factor for complement
	FBXL2	4.8	Lucine-rich repeat-containing F-box protein FBL2 mRNA
	UCHL1	4.5	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)
	SERPING1	2.9	Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1
	NAALADL1	2.6	N-acetylated alpha-linked acidic dipeptidase-like 1
	HTRA3	2.5	HtrA serine peptidase 3
	TMPRSS6	2.4	Transmembrane protease, serine 6
	LRP8	2.4	Low density lipoprotein receptor-related protein 8, apolipoprotein e receptor
	AI671186	2.4	Regeneration associated muscle protease
	CAPN7	2.2	Calpain 7
	SIAH1	2.2	Seven in absentia homolog 1 (Drosophila)
	OMA1	2.1	OMA1 homolog, zinc metallopeptidase (S. cerevisiae)
	AI671186	2.4	DKFZP586H2123 protein
	UBE2E1	2.3	Homo sapiens cDNA FLJ12000 fis, clone HEMBB1001531

Funtional Family	Gene Name	Differ Expre (fold)[*]	Description
	AK000110	2.3	Homo sapiens cDNA FLJ20103 fis, clone COL04793.
	SIAH1	2.1	Seven in absentia (Drosophila) homolog 1
	ESPL1	1.7	Cluster Incl. D79987:Human mRNA for KIAA0165 gene
	MASP1	1.7	ESTs, Highly similar to JN0883 Ra-reactive factor
	AI828007	1.8	ESTs, Weakly similar to serin protease with IGF-binding motif
	CASP8	1.7	Cspase 8, apoptosis-related cysteine protease (CASP8)
	CTRB1	1.7	Cymotrypsinogen B1
	USP32	1.8	Homo sapiens mRNA for TL132
	CLN8	1.8	Putative transmembrane protein mRNA
	SENP1	1.8	SntrinSUMO-specific protease 1
	SENP7	1.7	SntrinSUMO-specific protease 7
	PYCARD	1.8	Homo sapiens, clone MGC:10332
	UBE2C	1.8	Uiquitin carrier protein E2-C
	ADAMTS1	−4.7	ADAM metallopeptidase with thrombospondin type 1 motif 1
	ADAMTS3	−23.6	ADAM metallopeptidase with thrombospondin type 1 motif 3
	ADAMTS5	−4.1	ADAM metallopeptidase with thrombospondin type 1 motif 5
	ADAM12	−2.4	ADAM metallopeptidase domain 12 (meltrin alpha) Adam 12
	CPA4	−2.5	Carboxypeptidase A4
	CPXM2	−11.4	Carboxypeptidase X (M14 family), member 2
	CPZ	−3.0	Carboxypeptidase Z
	CTSD	−3.6	Cathepsin D
	CFH	−3.9	Complement factor H
	CFI	−2.6	Complement factor I
	C3	−2.4	Complement component 3
	CASP2	−2.1	Caspase 2, apoptosis-related cysteine peptidase
	CASP8	−5.0	Caspase 8, apoptosis-related cysteine peptidase
	AGC1	−8.6	Aggrecan 1
	AK023795	−4.5	Homo sapiens cDNA FLJ13733 fis, clone PLACE3000147
	IBRDC2	−7.4	ESTs, Moderately similar to ALU1_HUMAN ALU SUBFAMILY J
	PPP4R2	−3.7	ESTs, Moderately similar to ALU7_HUMAN ALU SUBFAMILY SQ
	ABHD5	−3.2	Abhydrolase domain containing 5
	QPCT	−3.5	Glutaminyl-peptide cyclotransferase (glutaminyl cyclase)
	ENC1	−2.7	Ectodermal-neural cortex (with BTB-like domain)
	AEBP1	−8.2	AE binding protein 1
	PCSK1	−2.9	Proprotein convertase subtilisin/kexin type 1
	PLAU	−2.5	Plasminogen activator, urokinase
	LRAP	−2.6	Leukocyte-derived arginine aminopeptidase
	C9orf3	−2.6	Chromosome 9 open reading frame 3
	SERPINE1	−2.4	Serpin peptidase inhibitor, clade E
	SERPINE2	−2.0	trinucleotide repeat containing 3

Funtional Family	Gene Name	Differ Expre (fold)[*]	Description
	PCSK5	−4.6	Proprotein convertase subtilisin/kexin type 5
	PRSS12	−2.1	Protease, serine, 12 (neurotrypsin, motopsin)
	DPP7	−2.2	Dipeptidyl-peptidase 7
	KLKB1	−2.1	Homo sapiens cDNA FLJ12052 fis, clone HEMBB1002042
	USP3	−1.7	Homo sapiens cDNA FLJ14136 fis, clone MAMMA1002744
	USP12	−1.8	Ubiquitin specific protease 12
	USP25	−1.8	Homo sapiens ubiquitin-specific processing protease
	USP53	−2.6	KIAA1350 protein
	U13022	−1.8	Cluster Incl. U13022:Human negative regulator of programmed cell death
	MASP1	−1.8	Mannan-binding lectin serine protease 1
	UCHL3	−1.8	Ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase)
	NLN	−1.7	KIAA1226 protein
	RNF11	−1.7	Homo sapiens mRNA for Sid1669p, complete cds
	FBXO11	−1.7	Homo sapiens hypothetical protein FLJ12673
	MALT1	−1.7	Homo sapiens clone 24631 mRNA
	FBXL7	−1.7	F-box and leucine-rich repeat protein 7
Immune response			
	HLA-DRA	65.0	Major histocompatibility complex, class II, DR alpha
	HLA-DRB1	50.2	Major histocompatibility complex, class II, DR beta 1
	HLA-DRB4	35.6	Major histocompatibility complex, class II, DR beta 4
	HLA-DRB5	11.7	Major histocompatibility complex, class II, DR beta 5
	HLA-DRB6	2.9	Major histocompatibility complex, class II, DR beta 6
	HLA-DPA1	12.7	Major histocompatibility complex, class II, DP alpha 1
	HLA-DPB1	4.7	Major histocompatibility complex, class II, DP beta 1
	HLA-DMA	9.2	Major histocompatibility complex, class II, DM alpha
	HLA-DQA1	7.1	Major histocompatibility complex, class II, DQ alpha 1
	CD77	4.7	CD74 molecule, major histocompatibility complex, class II invariant chain
	IL1R1	2.1	Interleukin 1 receptor, type I
	IL-7	8.9	Interleukin 7
	IL-7R	2.4	Interleukin 7 receptor
	NFIL3	2.3	Nuclear factor, interleukin 3 regulated
	CXCL6	3.9	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)
	CLCL2	2.8	Chemokine (C-X-C motif) ligand 2
	CCR1	2.3	Chemokine (C-C motif) receptor 1
	LST1	3.1	Leukocyte specific transcript 1
	IFIH1	2.2	Interferon induced with helicase C domain 1
	IFIT3	2.1	Interferon-induced protein with tetratricopeptide repeats 3
	C5	3.8	Complement component 5
	CFB	2.2	Complement factor B

Funtional Family	Gene Name	Differ Expre (fold)[*]	Description
	CFD	2.7	Complement factor D (adipsin)
	CD55	3.3	CD55 molecule, decay accelerating factor for complement
	CD55	3.3	CD55 molecule, decay accelerating factor for complement
	PSMB10	2.7	Proteasome (prosome, macropain) subunit, beta type, 10
	PSMB9	2.7	Proteasome (prosome, macropain) subunit, beta type, 9
	BST1	3.7	Bone marrow stromal cell antigen 1
	BST2	3.7	Bone marrow stromal cell antigen 2
	COLEC12	2.5	Collectin sub-family member 12
	KIR3DL1	3.9	Killer cell immunoglobulin-like receptor, three domains
	KIR2DL1	4.5	Killer cell immunoglobulin-like receptor, two domains
	OAS1	2.3	2,5-oligoadenylate synthetase 1, 40/46kDa
	TRIM22	2.1	Tripartite motif-containing 22
	DPP4	5.4	Dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)
	SECTM1	5.7	Secreted and transmembrane 1
	CTSC	5.0	Cathepsin C
	ADA	3.8	Adenosine deaminase
	ETS1	3.0	V-ets erythroblastosis virus E26 oncogene homolog 1 (avian)
	IGHG1	2.1	Immunoglobulin heavy constant gamma 1 (G1m marker)
	IL-11	−2.7	Interleukin 11
	IL-26	−9.9	Interleukin 26
	TIRAP	−2.0	Toll-interleukin 1 receptor (TIR) domain containing adaptor protein
	IL1RL1	−3.3	Interleukin 1 receptor-like 1
	TGFB1	−2.5	Transforming growth factor, beta 1
	TGFB2	−2.7	Transforming growth factor, beta 2
	CFH	−3.9	Complement factor H
	CFI	−2.6	Complement factor I
	C3	−2.4	Complement component 3
	TNFRSF9	−2.6	Tumor necrosis factor receptor superfamily, member 9
	TNFRSF11A	−3.3	Tumor necrosis factor receptor superfamily, member 11a, NFKB activator
	CD24	−16.5	CD24 molecule
	IFI27	−12.5	Interferon, alpha-inducible protein 27
	F3	−6.5	Coagulation factor III (thromboplastin, tissue factor)
	PTGER4	−5.8	Prostaglandin E receptor 4 (subtype EP4)
	CCL2	−4.8	Chemokine (C-C motif) ligand 2
	PDCD1LG2	−3.6	Programmed cell death 1 ligand 2
	PAWR	−3.4	PRKC, apoptosis, WT1, regulator
	IGSF4	−2.9	Immunoglobulin superfamily, member 4
	TRPM4	−2.3	Transient receptor potential cation channel, subfamily M, member 4
	SMAD3	−2.1	SMAD family member 3

Funtional Family	Gene Name	Differ Expre (fold)[*]	Description
	ARL6IP2	−2.0	ADP-ribosylation factor-like 6 interacting protein 2
	OAS2	−2.3	2−5-oligoadenylate synthetase 2, 69/71kDa
Phosphate transport			
	MSR1	48.4	Macrophage scavenger receptor 1
	SCARA5	75.3	Scavenger receptor class A, member 5 (putative)
	COL1A2	2.2	Collagen, type I, alpha 2
	COL3A1	2.6	Collagen, type III, alpha 1
	COL5A3	11.3	Collagen, type V, alpha 3
	COL8A1	−2.5	Collagen, type VIII, alpha 1
	COL8A2	6.2	Collagen, type VIII, alpha 2
	COL11A1	−4.0	Collagen, type XI, alpha 1
	COL12A1	2.4	Collagen, type XII, alpha 1
	COL13A1	−2.1	Collagen, type XIII, alpha 1
	COL15A1	5.9	Collagen, type XV, alpha 1
	CCBE1	2.4	Collagen and calcium binding EGF domains 1
	COLEC12	2.5	Collectin sub-family member 12
	GLDN	−6.2	Gliomedin
	ANKH	−3.2	Ankylosis, progressive homolog (mouse)
Lipid catabolic process			
	PLA2G2A	4.7	Phosphatase A2, group IIA (platelets, synovial fluid)
	PLA2G4C	3.5	Phospholipase A2, group IVC (cytosolic, calcium-independent)
	PPT1	3.0	Palmitoyl-protein thioesterase 1
	WNT1	3.0	WNT1 inducible signaling pathway protein 2
	LIPC	2.6	Lipase, hepatic
	FLRT2	2.5	Fibronectin leucine rich transmembrane protein 2
	PLCB4	−3.8	Phospholipase C, beta 4
Lipid transport			
	APOL6	2.8	Apolipoprotein L, 6
	SLC27A3	3.3	Solute carrier family 27 (fatty acid transporter), member 3
	OSBPL1A	2.2	Oxysterol binding protein-like 1A
	STARD3	2.5	START domain containing 3
	APOL3	2.3	Apolipoprotein L, 3
	STARD5	−2.3	START domain containing 5
	ATP8A1	−2.2	ATPase, aminophospholipid transporter, Class I, type 8A, member 1
Fatty acid metabolic process			
	CD36	22.8	CD36 molecule (thrombospondin receptor)
	PNPLA8	3.7	Patatin-like phospholipase domain containing 8
	CYB5A	2.4	Cytochrome b5 type A (microsomal)
	HADHA	2.1	Hydroxyacyl-Coenzyme A dehydrogenase, alpha
	ACSL5	−3.1	Acyl-CoA synthetase long-chain family member 5

Funtional Family	Gene Name	Differ Expre (fold)[*]	Description
	ACSL1	−2.1	Acyl-CoA synthetase long-chain family member 1
Cytoskeleton-dependent intracellular transport			
	KIF14	2.2	Kinesin family member 14
	KIF15	2.3	Kinesin family member 15
	KIF18A	2.4	Kinesin family member 18A
	KIF23	2.5	Kinesin family member 23
	MYO1E	2.5	Myosin IE
	MYO9B	2.4	Myosin IXB
	RHOT1	2.3	Ras homolog gene family, member T1
	UCHL1	4.5	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)
	MYRIP	−15.8	Myosin VIIA and Rab interacting protein
	TUBA1	−3.5	Tubulin, alpha 1
	KIFI13	−2.1	Kinesin family member 1B
Positive regulation of cell proliferation			
	IL-7	8.9	Interleukin 7
	LAMA1	2.9	Laminin, alpha 1
	LAMC1	2.8	Laminin, gamma 1 (formerly LAMB2)
	ADRA2A	10.5	Adrenergic, alpha-2A-, receptor
	1RS2	4.0	Insulin receptor substrate 2
	SSR1	2.5	Signal sequence receptor, alpha (translocon-associated protein alpha)
	CDC25B	2.9	Cell division cycle 25 homolog B (S. cerevisiae)
	LRP5	2.6	Low density lipoprotein receptor-related protein 5
	PBEF1	2.3	Pre-B-cell colony enhancing factor 1
	P21	2.0	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)
	FGF2	−3.3	Fibroblast growth factor 2 (basic)
	FGF9	−19.1	Fibroblast growth factor 9 (glia-activating factor)
	FGF10	−2.0	Fibroblast growth factor 10
	TGFB1	−2.5	Transforming growth factor, beta 1
	TGFB2	−2.6	Transforming growth factor, beta 2
	TGFBR1	−2.6	Transforming growth factor, beta receptor I
	ID4	−4.1	Inhibitor of DNA binding 4, dominant negative helix-loop-helix protein
	LYN	−5.1	V-yes-1 Yamaguchi sarcoma viral related oncogene homolog
	J05008	−4.8	Endothelin 1
	CSF1	−3.3	Colony stimulating factor 1 (macrophage)
	PDCD1LG2	−3.6	Programmed cell death 1 ligand 2
	CLEC11A	−3.8	C-type lectin domain family 11, member A
	PTHLH	−4.3	Parathyroid hormone-like hormone
	BNC1	−3.2	Basonuclin 1
	PTN	−3.3	Pleiotrophin (heparin binding growth factor 8)

Funtional Family	Gene Name	Differ Expre (fold)[*]	Description
	ERPB2	−3.7	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2
	VEGFC	−3.6	Vascular endothelial growth factor C
	TNFRSF11A	−3.3	Tumor necrosis factor receptor superfamily, member 11a, NFKB activator
	HBEGF	−3.0	Heparin-binding EGF-like growth factor
	IL-11	−2.7	Interleukin 11
	EDG3	−2.7	Endothelial differentiation, sphingolipid G-protein-coupled receptor, 3
	IGF1R	−2.4	Insulin-like growth factor 1 receptor
	STAMBP	−2.1	STAM binding protein
	LAMB1	−2.1	Laminin, beta 1
Anti-apoptosis			
	ATF5	4.9	Activating transcription factor 5
	SERPINB9	4.7	Serpin peptidase inhibitor, clade B (ovalbumin), member 9
	HRK	3.1	Harakiri, BCL2 interacting protein (contains only BH3 domain)
	BRE	2.1	Brain and reproductive organ-expressed (TNFRSF1A modulator)
	BIRC6	2.0	Baculoviral IAP repeat-containing 6 (apollon)
	SERPINB2	−17.3	Serpin peptidase inhibitor, clade B (ovalbumin), member 2
	CCL2	−4.8	Chemokine (C-C motif) ligand 2
	TNFRSF10D	−3.1	TNF receptor superfamily, member 10d, decoy with truncated death domain
	SFRP1	−3.1	Secreted frizzled-related protein 1
	ERC1	−3.1	ELKS/RAB6-interacting/CAST family member 1
	U37546	−3.4	Baculoviral IAP repeat-containing 3
	IGF1R	−2.3	Insulin-like growth factor 1 receptor
	BCL2A1	−2.4	BCL2-related protein A1
	DHCR24	−2.3	24-dehydrocholesterol reductase
	POGK	−2.3	Pogo transposable element with KRAB domain
	BNIP3	−2.2	BCL2/adenovirus E1B 19kDa interacting protein 3
	CASP2	−2.1	Caspase 2, apoptosis-related cysteine peptidase
	TNFAIP3	−2.1	Tumor necrosis factor, alpha-induced protein 3
	STAM	−2.1	STAM binding protein
	HDAC1	−2.1	Histone deacetylase 1
	HTATIP2	−2.1	HIV-1 Tat interactive protein 2, 30kDa
	HSPB1	−2.0	Heat shock 27kDa protein 1
Angiogenesis			
	SERPINF1	6.3	Serpin peptidase inhibitor, clade F, member 1
	COL15A1	5.9	Collagen, type XV, alpha 1
	ARTS-1	5.8	Type 1 tumor necrosis factor receptor shedding aminopeptidase regulator
	PLXND1	2.5	Plexin D1
	TMPRSS6	2.4	Transmembrane protease, serine 6
	ANGPT1	−3.5	Angiopoietin 1

Funtional Family	Gene Name	Differ Expre (fold)[*]	Description
	VEGFC	−3.6	Vascular endothelial growth factor C
	EDNRA	−3.0	Endothelin receptor type A
	FGF1	−2.1	Fibroblast growth factor 1 (acidic)
	FGF2	−3.3	Fibroblast growth factor 2 (basic)
	CTGF	−4.7	Connective tissue growth factor
	RUNX1	−2.1	Runt-related transcription factor 1
	THBS1	−3.3	Thrombospondin 1
	TGFB2	−2.6	Transforming growth factor, beta 2
	ERBB2	−3.7	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (avian)
	HBEGF	−3.0	Heparin-binding EGF-like growth factor
	SEPPINB8	−2.1	Serpin peptidase inhibitor, clade E, member 1
	HTATIP2	−2.1	HIV-1 Tat interactive protein 2, 30kDa
	JAG1	−2.0	Jagged 1 (Alagille syndrome)
	BMP4	−2.0	Bone morphogenetic protein 4

[*] Positive number indicates elevated expression (fold) in hTERT-OA 13A FLS (passage 25) compared to hTERT-RA 516 FLS (passage 25). Negative number indicates decreased expression (fold) in hTERT-OA 13A FLS (passage 25) compared to hTERT-RA 516 FLS (passage 25) or indicates elevated expression (fold) in hTERT-RA 516 FLS (passage 25) compared to hTERT-OA 13A FLS (passage 25).

Table 3

Differential expression detected by microarray versus that detected by real-time RT-PCR between hTERT-OA 13AFLS and hTERT-RA 516 FLS. Numbers are the ratio of the relative expression level of a gene in hTERT-OA 13A FLS to the relative expression level of a gene in hTERT-RA 516 FLS.

Gene	Microarray (passage 8)	Microarray (passage 25)	RT-PCR (passage 25)	Description
HLA-DRA	2.5	82	89.6	Major histocompatibility complex, class II, DR alpha
MGST1	4.5	164	230.1	Microsomal glutathione S-transferase 1
RAB27B	4.3	6.8	16.6	Ras-related GTP-binding protein 27b, GTP-binding protein and GTPase
MMP1	—	1.74	5.2	Matrix metalloproteinase 1
COL5A3	—	11.38	12.41	Collagen, type III, Alpha 1
COL8A2	—	8.04	12.28	Collagen, type V, alpha 3
ADAMTS1	0.16	0.20	0.36	ADAM metallopeptidase with thrombospondin type 1 motif 1

Table 4

Genes harboring susceptibility for OA, and their differential expressions between hTERT-OA 13A FLS (25 passages) and hTERT-RA 516 FLS (25 passages).

Gene	Differential Expression
AACT (1)	
ADAM12 (1)	−2.4
ADAMTS-5 (2)	−4.1
ASPN (3)	2.1
BMP2 (1)	
CALM1 (3)	
CLIP (1)	
COL2A1 (3)	
COMP (3)	−3.3
COX2 (1)	
DIO2 (4)	−3.6
ENPP1 (5)	1.5
ESR1 (1)	1.6
FRZB (3)	
GDF5 (6)	−1.5
IDH1 (7)	1.6
IL-1 (8)	
ILR4 (9)	
NRP2 (7)	−1.6
MATN3 (2)	−2.1
OPG (1)	
PLA2G4A (10)	
PTGS2 (10)	
RHOB (11)	
TNA (1)	
TXNDC3 (11)	
VDR (1)	

Genes harboring susceptibility for OA from the literature: (1) Arthritis Rheum 2006; 54:533−9. (2) Arthritis Rheum 2008; 58:435−41. (3) Arthritis Rheum 2007; 56:137−46. (4) Hum Mol Genet 2008; 17:1867−75. (5) Arthritis Res Ther 2005; 7:R1082−90. (6) Hum Mol Genet 2008; 17:1497−504. (7) Eur J Hum Genet 2007; 15:791−9. (8) Arthritis Rheum 2004; 50:1179−86. (9) Expert Rev Mol Med 2005; 7:1−12. (10) AM J Hum Genet 2008; 82:1231−40. (11) Am J Hum Genet 2006; 78:793−803.

Table 5

Comparison of the differential expressions of putative disease markers.

Gene	Differential Expression [*] (hTERT-OA 13A FLS/hTERT-RA 516 FLS) (passage 25)	Gene Name	Differential Expression [**] (OA synovium/RA synovium)	Mean Absolute Deviation [**][†]	Description
Group I					
IL1R1	2.05	IL1R1	2.15		Interleukin 1 receptor, type I
P4HA2	2.37	P4HA2	1.93		Procollagen-proline, 2-oxoglutarate 4-dioxygenase
COL3A1	2.35	COL3A1	1.96		Collagen, type III, alpha 1
SORD	0.49	SORD	0.59		Hyman L-iditol-2 dehydrogenase
PGK1	0.58	PGK1	0.24		Phosphoglycerate kinase (alternatively spliced)
EXT1	2.47	EXT1	0.61		Homo sapiens insert cDNA clone YP87D03.
LIMK2	0.36	LIMK2	1.38		LIM domain kinase 2, transcript variant 2a
Group II					
RPL15	2.23	NA	NA	1.4	ESTs, AI939308
CXCL2	2.82	NA		1.32	Human gro-beta mRNA, complete cds
GSTT1	1.78	NA		1.30	Glutathione S-transferase theta 1
PPP2R5C	1.65	NA		1.42	Homo sapiens mRNA; cDNA DKFZp761H0317
FN1	0.40	NA		1.40	Homo sapiens mRNA for MSF-FN70 (FN gene)

[*] This study. Numbers are the ratio of the relative expression level of a gene in hTERT-OA-13A FLS to relative expression level of a gene in hTERT-RA 516 FLS.

[**] Previously reported[16]. Numbers are the ratio of the relative expression level of a gene in OA synovium to relative expression level of a gene in RA synovium.

[†] Mean absolute deviation of the expression levels of a gene in OA synovium from the mean of the expression levels of a gene in normal synovium. Group I: The genes that displayed either elevated expression or decreased expression in all OA synovium samples compared to all RA synovium samples. Group II: The genes that displayed greater differential gene expression (judged by mean absolute deviation) between OA synovium and RA synovium, although in some OA synovium samples expression of the gene of interest was up and in other OA synovium samples expression was down compared to RA synovium samples[16].

Table 6

Comparison of the differential expressions of genes detected in our study with those previously reported[17].

Gene	Differential Expression[*]	Differential Expression[**]	Description
LILRB1	↓	↓	Leukocyte immunoglobulin-like receptor, subfamily A
C9orf47	↓	↓	Endo differentiation, sphingolipid G-protein-coupled receptor, 3
NRN1	↓	↓	Homo sapiens neuritin
PLCB4	↓	↓	Phospholipase C, beta 4
GATA6	↓	↓	Homo sapiens mRNA for GATA-6
THY1	↓	↓	Thy-1 cell surface antigen
LRRC15	↓	↓	Leucine-rich repeat containing 5
MAB21L2	↑	↑	Homo sapiens MAB21L2 protein
CES1	↑	↑	Carboxylesterase
SFRP2	↑	↑	Secreted frizzled-related protein 2
SEMA3A	↓	↑	Sema domain, immunoglobulin domain (Ig), (semaphorin) 3A
SFRP1	↓	↑	Secreted frizzled-related protein 1
LMO2	↓	↑	LIM domain only 2
CRLF1	↓	↑	Cytokine receptor-like factor 1
TMOD1	↓	↑	Homo sapiens, tropomodulin
PRG4	↓	↑	Proteoglycan 4
HLA-DQA1	↑	↓	MHC class II, DQ alpha 1
HLA-DRA	↑	↓	MHC class 22 HLA-DR-alpha
HLA-DRB4	↑	↓	MHC class II DR beta 4
CTSC	↑	↓	Cathepsin C
TBX5	↑	↓	ESTs, AW269421
HOXD13	↑	↓	Homeo box D13
BST1	↑	↓	Bone marrow stromal cell antigen 1

↑ indicates elevated expression in hTERT-OA 13A FLS (passage 25) compared to hTERT-RA 516 FLS (passage 25) or in primary OA FLS (passage 2−3) compared to primary RA FLS (passages 2−3). ↓ indicates decreased expression in hTERT-OA 13A FLS (passage 25) compared to hTERT-RA 516 FLS (passage 25) or in primary OA FLS (passages 2−3) compared to primary RA FLS (passages 2−3).

[*] This study.

[**] Previously reported[17].