Abstract. Osteoarthritis (OA) is a slowly progressive degenerative joint disease that is associated with joint space narrowing, osteophyte formation and subchondral sclerosis. Despite extensive effort actual breakthroughs in the field of genetic or biochemical biomarkers of OA are limited. As secretory apolipoprotein J/clusterin (sCLU) has been implicated in both inflammatory and apoptotic molecular processes which contribute to the OA phenotype, the sCLU concentration in human serum and synovial fluid during advanced primary knee and hip OA was analysed. Elevated sCLU protein levels were shown in these two biological fluids. sCLU mRNA expression was also studied in normal cartilage and in advanced primary knee and hip OA samples. A significant up-regulation of sCLU mRNA expression (~25-fold) was found in samples collected from the tibial bone that was osteotomized during total knee arthroplasty in patients with primary knee OA, as compared to healthy tissue samples collected from the femoral head of macroscopically normal cartilage during the surgical treatment of subcapital fractures. By studying sCLU mRNA expression levels in samples collected during total hip arthroplasty in patients with advanced primary hip OA, an additional up-regulation of the sCLU mRNA expression (~4-fold), as compared to advanced primary knee OA, was found. Taken together, these observations indicate that the sCLU protein or mRNA expression level may be of a significant diagnostic and/or prognostic value during OA progression.

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Increased Expression Levels of Apolipoprotein J/Clusterin during Primary Osteoarthritis

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target in phase II clinical trials in prostate cancer patients (14), while CLU gene variants were recently found to be associated with Alzheimer’s disease (15, 16). Existing studies referring to sCLU in degenerative joint diseases are limited. CLU mRNA has been reported to be up-regulated in early osteoarthritic versus normal cartilage (17), while CLU expression levels in rheumatoid arthritis (RA) tissues were lower as compared to osteoarthritic or healthy synovium (18).

In the current report for the first time, both sCLU serum and synovial fluid content were analysed in patients with advanced primary OA, as well as sCLU mRNA expression levels in healthy tissue and osteoarthritic cartilage.

Patients and Methods

Patient profile and sample collection. Patients were included in the study on the basis of the following criteria: advanced primary knee or hip OA, no prior surgery for the last 12 months, no immunosuppressive medication and no corticosteroids or anti-inflammatory drugs for the last 2 months. Patients with advanced primary OA and excessive varus or valgus knee deformity were excluded from the study. Venous blood from fasting subjects with advanced primary knee (n=30) or hip (n=16) OA was collected in the absence of anticoagulant into plastic tubes and allowed to clot for 30 min at 37°C. The clot was removed by centrifugation and the serum was aliquoted and stored at −80°C. Minimal amounts of synovial fluid were collected from the same subjects during total knee or hip arthroplasty and stored at −80°C until usage. The study gained approval from the KAT Hospital Ethics Committee. Informed consent was obtained from all donors.

Paraffin embedding of collected biopsies. Biopsies were collected from the tibial bone that was osteotomized during total knee arthroplasty in patients with primary knee OA (n=11); from the femoral head of macroscopically normal cartilage during the surgical treatment of subcapital fractures (not related to osteoporosis) (normal tissue; n=22), as well as from patients with primary advanced hip OA during arthroplasty (n=3). Paraffin embedding of healthy or OA cartilage for standard pathoanatomical examination was carried out as described previously (19). Tissue sections were floated in a water-bath at 48°C, collected on poly-lysine-coated glass slides and stained with haematoxylin and eosin.

sCLU ELISA. Quantitative measurement of relative sCLU concentration in serum or synovial fluid was performed by using a sCLU-specific ELISA previously developed by us (20). In both cases, the starting biological fluid input in the ELISA assay was equal [5.5 μl; see also (20)] and thus the results regarding the relative sCLU concentration were comparable.

Total RNA isolation. The total RNA from cartilage tissue was isolated from 10 mg of finely minced paraffin-embedded tissue by using NucleoSpin® RNA II and NucleoSpin RNA/DNA buffer set kits (Macherey-Nagel Inc., Bethlehem, USA) as per the manufacturer’s instructions. Gene expression was analysed with the Roche Diagnostics (Basel, Switzerland) LightCycler2.0 and the Sybr Green method. The sCLU-specific primers used were: forward 5’-ATGATGAAAGACTGCTGTGCTG-3’ and reverse 5’-TCTCTGG AGCTCATGTTCTG-3’. Actin gene expression was used as a normalizer. The PCR amplicons were also analysed in agarose gels to verify specificity of the cDNA amplification.

Statistical analysis. The level of significance in the collected data was analysed by single factor ANOVA and the Student’s t-test. Values are reported as mean±standard deviation (SD).

Results

sCLU protein concentration in the serum and synovial fluid. Pathologoanatomical comparative examination of representative biopsies from healthy and OA cartilage verified that the selected samples displayed all the morphological features of advanced primary OA (Figure 1). Specifically, in the healthy cartilage (Figure 1A), tissue cells in the superficial zone were small, elongated in shape, parallel to the surface and lacked an extensive pericellular matrix, while the middle zone was distinguishable by rounded cells with random orientation being embedded in the extracellular matrix; cells in the deep zone exhibited an extensive pericellular matrix deposition, with chondrons in groups of three or more cells arranged in columns perpendicular to the surface. In contrast, the OA cartilage (Figure 1B) was characterized by significant disorganisation of both the (heavily collagenised) joint cartilage and bone surfaces.

As the applied ELISA procedure was identical to that in our reported studies, where OD 492 values for healthy donors were in the range of ~200-300 (20, 21), the observed mean OD 492 values of ~360 indicated an increase in the serum sCLU content in advanced primary OA (Figure 2A); no significant difference was found between knee and hip OA. As shown in Figure 2B, sCLU concentration in the human OA synovial fluid was found to be relatively high (mean OD492 values of ~270); again, no significant difference was found in the synovial fluid sCLU content between knee or hip OA.

sCLU mRNA expression. As shown in Figure 3A, a significant up-regulation of the sCLU mRNA expression level (~25-fold) was found in the samples derived from the knee OA. To cross-verify this finding, sCLU mRNA expression was also assayed in samples collected during total hip arthroplasty in patients with advanced hip OA. As shown in Figure 3B, an additional up-regulation of the sCLU mRNA expression of ~4-fold, as compared to primary knee OA, was observed. Apart from primary OA, the sCLU mRNA expression levels in the cartilage tissue showed no correlation with other pathophysiological or lifestyle parameters of the patients (data not shown).
Discussion

In the current study, the sCLU-specific assays were designed in order to discriminate between additional CLU gene isoforms that can be produced by alternative splicing (22), and showed that sCLU protein content increased in the serum of patients with OA. sCLU also accumulated in the osteoarthritic synovial fluid, indicating the sCLU is constitutively secreted by the various cell types (e.g. synovial cells and chondrocytes) that line the articular cavity. Moreover, the sCLU mRNA expression was found to be significantly up-regulated in the OA cartilage compared to the healthy tissue. These findings corroborate previous studies showing CLU mRNA expression in human cartilage (23) and CLU gene up-regulation in the human OA cartilage (17). Moreover, microarray analyses have shown that CLU gene expression was higher in OA as compared to RA synovial tissues (18). On the basis of this finding, it was suggested that the measurement of CLU gene expression is a highly interesting test for distinguishing between OA and RA tissues (24). Considering that inflammation also contributes to progression of OA (2), the specific functional role of sCLU in pathogenesis of OA has to be clarified.

According to the present data, the sCLU content in the OA synovial fluid is relatively high. We hypothesise that synovial fluid sCLU most probably originates from the chondrocytes of the cartilage. In support of this suggestion, the upper mid-zone chondrocytes in early stage OA cartilage have been found to express high levels of CLU mRNA (17). Moreover, the sCLU mRNA abundance in cartilage in cDNA libraries was equivalent to that of genes which have been more commonly associated with cartilage (17), indicating that sCLU is among the abundantly expressed genes in this tissue.

Almost invariably, sCLU has been found to exert a cytoprotective function (14); these observations mostly relate to the property of sCLU to chaperone unfolded damaged proteins (7). Thus, we speculate that the sCLU mRNA up-regulation in OA and the protein accumulation in the synovial fluid may relate to a cytoprotective and tissue repair attempt by the surrounding cells. Recent studies have shown that arthritis developed faster and lasted longer in CLU knock-out as compared with CLU wild-type mice (9). Moreover, mice with a null mutation of the latent transforming growth factor (TGF)-β binding protein-3, which regulates the activation of TGF-β, a positive regulator of the sCLU gene (25), developed both osteosclerosis and OA (26).

In summary, although it should be noted that the predictive value of sCLU in OA has yet to be proven in larger clinical trials, these observations provide evidence that the sCLU protein or mRNA expression levels in OA may be used as a marker for cartilage changes in this chronic degenerative condition.

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Declaration of Interest

The Authors report no conflicts of interest.
References


Figure 2. Relative sCLU content (OD_{402}) in the serum (A) or the synovial fluid (B) of patients with advanced primary OA of knee (n=30) or hip (n=16). Error bars denote standard deviation.

Figure 3. sCLU mRNA level in advanced primary OA of knee and of hip. (A1) Real-time PCR mRNA expression curves, showing a clear separation between the amplification curves of the healthy and osteoarthritic tissue. (A2) Mean values of sCLU mRNA expression levels. (B) Comparative quantitative real-time PCR analysis of the sCLU mRNA expression levels in advanced OA of knee versus that of hip. Error bars denote standard deviation; *significant at p<0.05.


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