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Review article

Exploring the translational potential of clusterin as a biomarker of early osteoarthritis

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ABSTRACT

Background: Clusterin (CLU; also known as apolipoprotein J) is an ATP-independent holdase chaperone that prevents proteotoxicity as a consequence of protein aggregation. It is a ~60 kDa disulfide-linked heterodimeric protein involved in the clearance of cellular debris and the regulation of apoptosis. CLU has been proposed to protect cells from cytolysis by complement components and has been implicated in Alzheimer's disease due to its ability to bind amyloid-β peptides and prevent aggregate formation in the brain. Recent studies suggest that CLU performs moonlighting functions. CLU exists in two major forms: an intracellular form and a secreted extracellular form. The intracellular form of CLU may suppress stress-induced apoptosis by forming complexes with misfolded proteins and facilitates their degradation. The secreted form of CLU functions as an extracellular chaperone that prevents protein aggregation.

Methods: In this review, we discuss the published literature on the biology of CLU in cartilage, chondrocytes, and other synovial joint tissues. We also review clinical studies that have examined the potential for using this protein as a biomarker in synovial and systemic fluids of patients with rheumatoid arthritis (RA) or osteoarthritis (OA).

Results: Since CLU functions as an extracellular chaperone, we propose that it may be involved in cytoprotective functions in osteoarticular tissues. The secreted form of CLU can be measured in synovial and systemic fluids and may have translational potential as a biomarker of early repair responses in OA.

Conclusion: There is significant potential for investigating synovial and systemic CLU as biomarkers of OA. Future translational and clinical orthopaedic studies should carefully consider the diverse roles of this protein and its involvement in other comorbidities. Therefore, future biomarker studies should not correlate circulating CLU levels exclusively to the process of OA pathogenesis and progression. Special attention should be paid to CLU levels in synovial fluid.

The Translational potential of this article: There is significant potential for investigating synovial and systemic CLU as a predictive biomarker of osteoarthritis (OA) progression and response to novel treatments and interventions. Given that CLU plays diverse roles in other comorbidities such as rheumatoid arthritis (RA) and obesity, future

Abbreviations: **ACL**, anterior cruciate ligament; **ACR**, American College of Rheumatology; **ApoJ**, apolipoprotein J; **CLU**, clusterin; **CMC-I**, carpometacarpal joint; **COMP**, cartilage oligomeric matrix protein; **ECM**, extracellular matrix; **ELISA**, enzyme-linked immunosorbent assay; **ESCEO**, The European Society for Clinical and Economic Aspects of Osteoporosis: Osteoarthritis and Musculoskeletal Diseases; **hsCRP**, high sensitivity C-reactive protein; **OA**, osteoarthritis; **OARSI**, Osteoarthritis Research Society International; **PsA**, psoriatic arthritis; **qRT-PCR**, quantitative reverse transcription polymerase chain reaction; **RA**, rheumatoid arthritis; **sCLU**, secreted clusterin; **SF**, synovial fluid; **TNF-α**, tumor necrosis factor-α.

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translational and clinical orthopaedic biomarker studies should not directly correlate circulating CLU levels to the process of OA pathogenesis and progression. However, special attention should be paid to CLU levels in synovial fluid. The cytoprotective properties of CLU may support the implementation of regenerative strategies and new approaches for developing targeted therapeutics for OA.

1. Introduction

Osteoarthritis (OA) is the most common form of arthritis globally and a major cause of pain and disability in older adults [1,2]. It is a multifactorial, degenerative, and inflammatory disease of the whole joint, with a rising global burden for health and social care systems across the world [3,4]. The prevalence of OA is highest among the middle-aged and elderly population, particularly in the leading economies of the world [5,6]. OA is characterized by the gradual loss of articular cartilage, synovial inflammation, and structural changes in subchondral bone [7,8]. Inflammation in OA is different from rheumatoid arthritis (RA), psoriatic arthritis (PsA), and other autoimmune joint diseases – it is defined as low-grade inflammation but chronic and persistent [9,10]. All of these changes occurring over time lead to joint destruction, pain, and functional disability [11,12]. The mechanisms and pathways involved in the pathogenesis and progression of OA are not fully understood but senescence [13], abnormal mechanical load [14], metabolic dysfunction [15,16], and low-grade inflammation [10,17] are all thought to be important contributing factors.

Currently, there are no approved disease-modifying treatments that can stop or slow down OA progression [18]. In terms of OA management, the major OA treatment guidelines published recently by OARSI [19,20], ACR [21], and ESCEO [20,22] recommend education, exercise, and weight management for everyone, a limited number of medications, including anti-inflammatory and analgesic drugs for some OA patients, and joint replacement surgery for a much smaller subset of patients. Pharmacological intervention for OA is largely restricted to symptom management with a limited range of anti-inflammatory and analgesic drugs [23,24], many of which have adverse side effects [25].

OA is a chronic but slowly progressing disease with a prolonged asymptomatic molecular phase. It is usually diagnosed clinically and confirmed using X-ray radiography, which can reveal only structural changes in the late stages of the disease after significant extracellular matrix (ECM) degradation and tissue destruction has already occurred. The clinical symptoms may appear only after extensive damage to joint tissues [9,26,27]. For all these reasons, novel approaches are needed for the prevention and early-stage diagnosis of the disease to prevent OA and delay the need for joint replacement surgery.

The identification and analysis of novel biomarkers may provide crucial early information about structural changes in the joint, including the appearance of ECM degradation and remodeling markers in articular cartilage, as well as synovial inflammation, subchondral changes and processes related to OA [22,28,29]. Our research has focused on using omics approaches to identify novel biomarkers of OA in order to accelerate the pace of therapeutic development for this difficult-to-treat disease [30–34]. In this review article, we briefly introduce the biology of clusterin (CLU), a molecular holdase chaperone with multiple cellular protective roles, which also performs moonlighting functions. We review the published literature on CLU in articular cartilage, chondrocytes, and other synovial joint tissues and describe the current state of knowledge related to CLU in the context of joint tissues. We also review recently published translational and clinical studies that have examined the potential for using this protein as a biomarker in synovial and systemic fluids of patients with RA and OA. Since the secreted form of CLU functions as an extracellular chaperone, we propose that it may be involved in chondroprotection of articular cartilage and cytoprotection in other osteoarticular tissues. The ability to quantify CLU in synovial and systemic fluids highlights its potential as a translational biomarker of early repair responses in OA.

2. Clusterin (CLU) – expression and tissue distribution

CLU is a secreted glycoprotein, first discovered in 1983, as an abundant heat-stable, trypsin-sensitive protein in ram rete testis fluid that aggregates cells, and elicits cellular “clustering”, hence it was given the name “clusterin” [35,36]. Based on a wide range of anatomical locations from which it was originally cloned and identified, CLU has many alternative names. It is also known as apolipoprotein J (ApoJ), cytolysis inhibitor (CLI), SP-40, gp80, NA1, NA2, SGP-2, testosterone repressed prostate message 2 (TRPM-2) among many other alternative names [37]. CLU is expressed in a variety of organs and tissues including the ovary, liver, heart, brain, and adrenal gland [37–43]. Although CLU performs homeostatic and physiological functions, it is also involved in pathophysiological processes and is induced by disease and injury [44]. CLU is one of the chaperone-like [45] central stress response proteins [46] that are highly upregulated in inflammation and apoptosis [47]. CLU is thought to be involved in the stimulation of cytokine expression [48], enhancement of lipid metabolism, cell differentiation [49], and tissue remodeling [50].

CLU expression can be triggered as a response to different stimuli, including pro-inflammatory cytokines, hypoxia, stress-inducing, or apoptosis-inducing agents [51,52]. These stimuli induce transcriptional changes in CLU gene expression and lead to elevated levels of CLU mRNA and increased production of CLU protein [53]. After translation, glycosylation, and further processing, mature CLU is secreted [54]. The biogenesis of secreted CLU (sCLU) begins as a pre-proprotein. In human CLU, the first 22 amino acid residues constitute a signal peptide sequence required for its co-translational translocation to the lumen of the endoplasmic reticulum (ER). After the removal of the signal sequence, intramolecular disulfide bonds help fold the ~60 kDa CLU protein into its native form. Following N-glycosylation, CLU translocates to the Golgi apparatus for further glycosylation, followed by enzymatic cleavage resulting in an N-terminal α-chain and a C-terminal β-chain which are interlinked by disulfide bonds. The resulting heterodimeric glycoprotein with a molecular weight of ~80 kDa is then secreted from the cell [44,55]. Due to its extensive glycosylation, the 3D structure of sCLU remains to be determined. Extracellular CLU binds to misfolded protein aggregates and directs them to cell surface receptors which enable internalization. The CLU/misfolded protein complexes are finally degraded by autophagosomes [55].

During cellular stress, an intracellular form of CLU can also be detected, probably as a result of release from the ER/Golgi secretory system to the cytosol [56] (Fig. 1). Intracellular CLU may play an important role in proteostasis as it probably forms complexes with misfolded intracellular proteins and by doing so it helps in their elimination via the proteasome or autophagy. Since CLU released from different stations of the secretory system may not have fully undergone complete glycosylation, it appears in conventional SDS-PAGE analysis to have variable molecular masses [55]. This may have fueled previous proposals of organelle-specific intracellular CLU isoforms such as nuclear CLU (nCLU) [57] or mitochondria-associated CLU [58]. However, the existence of these hypothetical CLU isoforms has not been experimentally confirmed and is now therefore considered outdated. Based on currently available data, CLU in various cellular locations has likely been released from the ER/Golgi apparatus and therefore lacks organelle-specific functions [44,55] (Fig. 1).

3. CLU as a translational biomarker of OA

Recent studies suggest that CLU has the potential to be a clinically important biochemical marker associated with carcinogenesis and neoplasia [59], neurodegeneration [60], obesity, and inflammation [61]. CLU is also gaining more attention in the context of OA development and progression. Publications on this topic so far have proposed a role for CLU in OA development, cartilage metabolism, inflammation, and oxidative stress [29,62]. Being a regulatory molecule involved in inflammation, redox environment, and energy homeostasis, CLU may be a potential marker to investigate in the context of OA development and responses to experimental interventions [62].

An increasing number of studies are reporting altered levels of CLU expression in OA, either focusing on joint tissues or systemic fluids (Table 1). For this narrative review, the publications have been selected from PubMed, based on the search results using keywords "clusterin", "osteoarthritis" and "cartilage".

3.1. CLU mRNA expression in healthy and OA cartilage and synovial tissues

The firstly published article in this context showed a similarly high CLU transcript abundance in normal *vs*. OA cartilage, with an elevated CLU gene expression in early OA and reduced in advanced OA, compared to the normal cartilage samples [63]. CLU was found to be expressed by the cells located at fluid–tissue interfaces of articular tissues, suggesting that CLU potentially protects the cells exposed to tissue damaging factors in the extracellular environment [64]. There was a moderate (1.2-fold) up-regulation of CLU mRNA in chondrocytes of early stage OA compared to normal cartilage. Using *in situ* hybridization, normal adult articular cartilage expressed low-to-moderate levels of CLU mRNA in the superficial zone chondrocytes, while in early OA cartilage, high levels of CLU mRNA were detected in the upper mid-zone chondrocytes. Advanced OA cartilage demonstrated low CLU expression in the upper mid-zone and insignificant in the lower mid-zone and deep zones. In advanced OA cartilage with deep fissuring and proteoglycan loss, CLU mRNA was reduced in all chondrocytes compared to early OA, but it was still detectable. This could be explained by two mechanisms, either by correlation of CLU expression with the altered levels of shear stress in OA cartilage, or by an attempt of chondrocytes to minimize the damage caused by oxidative stress, resulting in up-regulation of CLU expression in early OA [64]. Both proposed mechanisms suggested that the chondrocytes in early OA may be able to enter a protective phase to slow the loss of articular cartilage, and CLU may play an important role during this process. However, the study included a relatively low number of samples which could indicate that the results may not be directly applicable to all stages of OA development and in all patients [64].

Another study determined the expression of CLU in *ex vivo* synovial tissues of RA, OA, and healthy patients [48]. The study included both full-length and spliced isoforms of CLU mRNA. While there was no difference in CLU protein levels between RA and OA in synovial fluid (SF) samples, there was a significant decrease of both forms of CLU in RA,

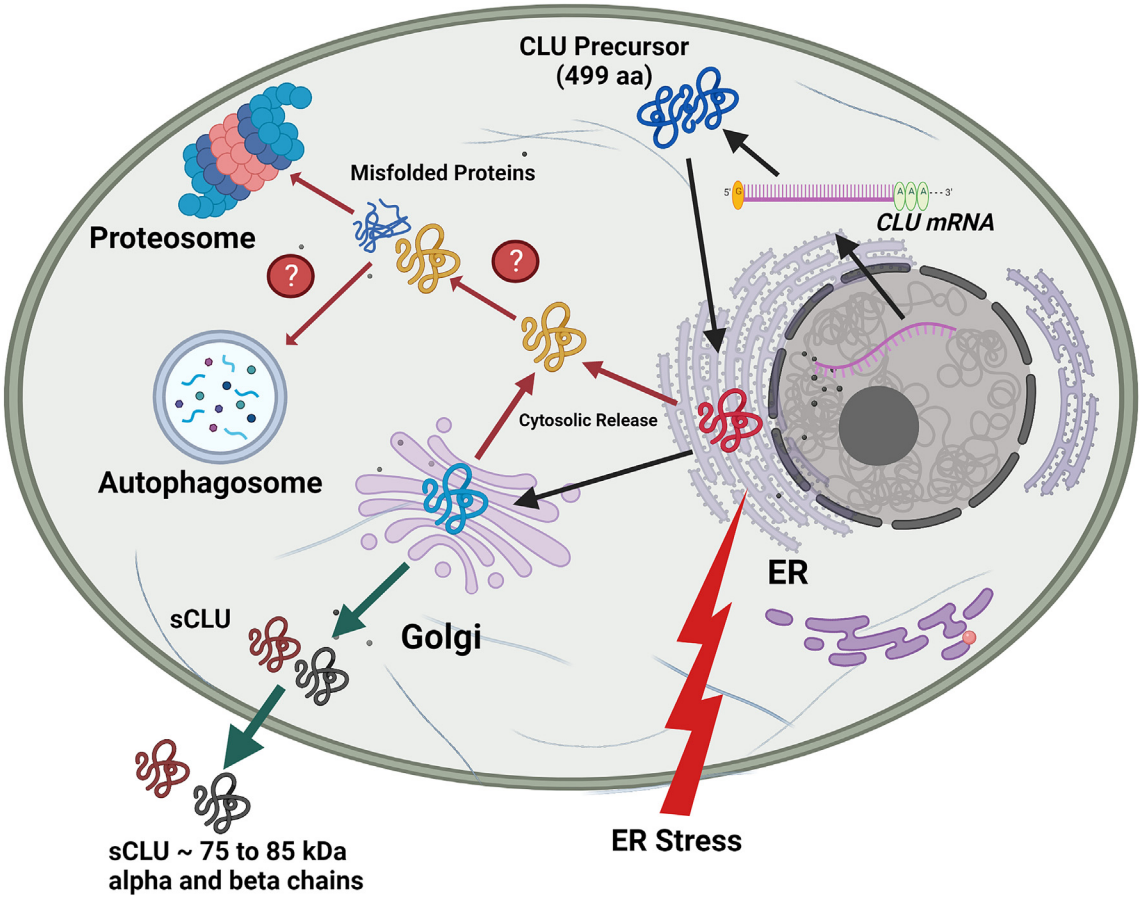

Figure 1. Schematic illustration of clusterin synthesis and processing within the chondrocyte. Clusterin is coded by its gene on chromosome 8. A precursor consisting of 449 amino acids (AA) is synthesized. The first 22 AAs code a signal peptide, which helps its translocation to the endoplasmic reticulum (ER) for post-translational modification. Following disulfide bond formation in the ER, CLU precursor translocates to the Golgi apparatus, where it is further glycosylated and then processed into alpha and beta subunits, bonded by disulfide bonds, resulting in the mature, secreted sCLU. As a result of ER stressors, CLU is probably released from the ER/Golgi complex into the cytosol, where it may form complexes with misfolded proteins. These complexes may then be targeted to the proteasome/autophagosome for degradation. Adapted from Refs. [55,81] and created with Biorender.com.

Table 1
Summary of the experimental models, methodology, the CLU form(s) analysed and the main findings of primary research papers focused on the analysis of CLU in the context of osteoarthritis which are included and discussed in this review article.

#	Experimental model	Method	CLU form analysed	Outcome	Reference
1	cDNA libraries from normal *vs*. OA adult human articular cartilage	~5000 ESTs sequenced from cDNA libraries and analysed using bioinformatic tools, northern blot and *in situ* hybridisation	CLU mRNA	similar CLU transcript abundance in normal and OA cartilage; CLU gene expression ↑ in early OA and ↓ in advanced OA vs. normal cartilage	Kumar et al., 2001 [63]
2	Normal *vs*. OA adult human articular cartilage	qRT-PCR, cDNA microarrays, *in situ* hybridisation	CLU mRNA	CLU expression ↑ in early OA vs. normal	Connor et al., 2001 [64]
3	Blood plasma and SF levels of CLU in knee OA patients; CLU mRNA expression in knee OA FLSs	Sandwich ELISA, qRT-PCR	sCLU; CLU mRNA	CLU expression ↓ in advanced OA vs. early OA	Ungsudechachai et al., 2020 [62]
4	Plasma and serum samples, and knee radiographs from subjects with primary knee OA (progressors and non-progressors)	N-glycoproteomic 2D-LC-MALDI analysis, western blot, ELISA, *in situ* hybridisation	CLU glycosylation	Plasma CLU levels ↑ in OA vs. paired SF samples	Fukuda et al., 2012 [66]
5	Serum concentrations of CLU in patients with hand OA (erosive *vs*. non-erosive) *vs*. healthy controls	ELISA	sCLU	Positive association of plasma and SF CLU levels with radiographic severity of OA (joint space narrowing)	Kropáčková et al., 2018 [69]
6	CLU expression in synovial tissue from patients with RA or OA *vs*. healthy controls	qRT-PCR, western blot, northern blot, *in situ* hybridization and immunohistochemistry	full length and spliced isoforms of CLU mRNA	Direct correlation between plasma CLU and hs-CRP	Devauchelle et al., 2006 [48]
7	Post-traumatic porcine OA model (surgical ACLT)	LC MS/MS-based proteomics to determine protein profile of SF samples	sCLU	CLU mRNA expression ↑ in OA FLSs vs. no synovitis samples	Kiapour et al., 2019 [70]
8	Immunolocalization of clusterin in repair cartilage of patients with ACI	Immunostaining of biopsy samples	CLU	CLU is a potentially relevant biomarker; level of glycosylation may increase during disease progression	McCarthy et al., 2013 [71]
9	Human SF samples obtained from CMC-I OA and knee joint of OA patients	Label-free quantitative LC-MS/MS, ELISA	sCLU	Serum CLU levels ↓ in OA vs. healthy subjects	Barreto et al., 2018 [72]
10	Secretome of equine cartilage explants, osteochondral biopsies, and isolated unpassaged chondrocytes, following treatment with IL-1β and TNF-α	qRT-PCR, western blot	sCLU and total CLU	Serum CLU levels ↓ in erosive hand OA vs. non-erosive hand OA patients	Matta et al., 2021 [73]

Abbreviations: 2D-LC-MALDI, 2-dimensional liquid chromatography matrix-assisted laser desorption/ionization; ACI, autologous chondrocyte implantation; ACLT, anterior cruciate ligament transection; cDNA, complementary DNA; CLU, clusterin; CMC-I, carpometacarpal joint; ELISA, enzyme-linked immunosorbent assay; ESTs, expressed sequenced tags; hs-CRP, high-sensitivity C-reactive protein; IL-1β, interleukin-1β; FLSs fibroblast-like synoviocytes; mRNA, messenger RNA; OA, osteoarthritis; qRT-PCR, quantitative reverse transcription PCR; RA, rheumatoid arthritis; sCLU, secreted clusterin; SF, synovial fluid; TNF-α, tumour necrosis factor α

compared to OA samples. It was concluded that CLU mRNA was under-expressed in RA, and over-expressed in knee OA as compared to synovium of patients with traumatic ligament lesions, and could be a marker for differentiating between RA and OA [48].

3.2. Correlation of CLU levels in plasma and SF samples to OA severity

Body fluids including blood, plasma, serum, urine and SF contain soluble factors, many of which may be potential biomarkers of chronic diseases such as OA. Biomarkers provide useful diagnostic information by detecting cartilage degradation in OA, reflecting disease-relevant biological activity, and predicting the course of disease progression [65]. Putative biomarkers of joint tissue turnover (i.e. ECM fragments), cytokines and chemokines, and other molecules have been studied in the context of OA in different cohort studies. CLU has been identified as a secreted factor with altered levels in healthy *vs*. inflammatory samples, but its role as a potential biomarker is yet to be established.

When plasma and SF CLU levels were quantified using ELISA in OA patients, plasma CLU levels were significantly higher compared to the corresponding SF samples [62]. Also, a correlation between systemic inflammation measured by high sensitivity C-reactive protein (hsCRP) levels and plasma CLU levels was observed, as well as significant associations between plasma and SF CLU levels with radiographic severity of OA. In patients with advanced-stage OA, both plasma and SF CLU levels were higher compared to early-stage OA patients [62]. This was in contrast to the concept proposed in earlier studies where CLU mRNA levels were found to be lower in advanced-stage OA [64]. The authors hypothesized that during the early stages of OA, up-regulation of CLU expression indicates a protective mechanism of the chondrocytes [64]. Given the correlation between CLU levels in patient plasma samples and systemic inflammation, the fact that CLU mRNA was up-regulated in the knee OA-derived fibroblast-like synoviocytes (FLS) stimulated with TNF-α suggested that CLU may be a relevant marker of synovial inflammation. As OA development and symptoms associated with it progress, synovial inflammation also increases and some patients with obesity and metabolic disease develop systemic inflammation. The authors of the study proposed that increased CLU levels possibly constitute a part of a defensive mechanism to maintain structural integrity of the ECM to manage the imbalance between anabolic and catabolic processes [62].

An *N*-glycoproteomic analysis was employed to determine potential plasma biomarkers for knee OA in patients diagnosed with primary OA [66]. The patients were split into two groups – progressors (subjects undergoing disease progression) and non-progressors (subjects in a stable condition). Among over 6800 biomarkers identified in the glycoproteomic analysis, only four were selected to have the potential of being a relevant biomarker for evaluating the progression of knee OA. These four markers included CLU, hemopexin, macrophage stimulating protein (MSP), and α-1 acid glycoprotein-2 (AGP-2). Even though ELISA analysis results did not show a significant difference of CLU and hemopexin in the serum samples between the progressors compared to non-progressors, western blotting analysis showed the presence of both biomarkers at substantial levels in synovial tissues. As the chosen *N*-glycoproteomic analysis measures not only the amount of protein itself but also the level of protein glycosylation, the authors hypothesized that the level of glycosylation of CLU and hemopexin probably increases with the progression of the disease [66].

Proteomic analysis of SF has shown that CLU is produced by both healthy chondrocytes as well as chondrocytes isolated from OA cartilage. Using mass spectrometry-based selected reaction monitoring (SRM) analysis, CLU and lubricin were detected in both SF and serum of samples of patients with late knee OA, suggesting that their levels in plasma are as

predictive of OA progression as age [67]. In a peptidomic study of endogenous peptides released from articular cartilage, three neopeptides belonging to CLU and one from cartilage oligomeric matrix protein (COMP) showed a disease-dependent decrease specifically in hip OA [68].

A study was performed to analyze serum CLU concentrations in patients with hand OA using ELISA [69]. This study also included a comparison of CLU levels in different forms of hand OA (erosive and non-erosive) and examined associations with clinical and laboratory parameters. Interestingly, the serum concentrations of CLU were significantly lower in hand OA patient samples compared to healthy subjects. Significantly lower levels of CLU were observed in the erosive hand OA compared to non-erosive hand OA patients, and these results were not affected by the concurrent presence of knee or hip OA. This study also indicated a negative correlation between pain and CLU levels in erosive hand OA patients. This contradicts the results of the previously discussed reports [62,64], which linked rising CLU levels with inflammatory processes, because erosive hand OA is associated with a higher degree of inflammation. However, the authors proposed to link their findings to a potentially protective role of CLU against bone erosions, which are also present in RA.

Translational study conducted in a validated Yucatan minipig model of anterior cruciate ligament (ACL) injury and post-traumatic knee OA used proteomics analysis of knee SF in pigs undergoing untreated ACL transection and augmented ACL repair *versus* the controls [70]. CLU was among the top 20 most abundant proteins at 6 months (in both ACL transection and repair groups) and at 12 months (only in the repair group). They also reported moderate (1.4–1.7-fold) increases in CLU levels in ACL transection and repaired knees compared to controls at all time points. There was a significant negative correlation between CLU levels and macroscopic cartilage damage as measured by total tibiofemoral cartilage lesion area. These observations are complementary to the abovementioned studies and reinforce the protective role of CLU, particularly in early stages of injury and OA, revealing how reduced levels of CLU may contribute to the disease progression. The results of another study, characterizing the localization of CLU in the repaired cartilage after autologous chondrocyte implantation also support the protective role of CLU [71], based on differences in distribution of CLU between healthy and repaired cartilage.

SF samples of non-erosive OA from the carpometacarpal joints (CMC-I) and of primary idiopathic knee OA patients were investigated by quantitative liquid chromatography mass spectrometry (LC-MS), and revealed multiple peptide differences, including CLU, paraoxonase/arylesterase 1 (PON1) and transthyretin [72]. In the knee OA group, 28 proteins were up-regulated and 12 down-regulated, as compared to CMC-I group, while 16 of those up-regulated and 3 of down-regulated proteins were linked to inflammatory processes in the joint and lipid transport. Higher CLU concentrations (~1.7-fold increase) were detected in SFs from knee OA patients than in ones from CMC-I OA. These differences in CLU levels might have occurred because the weight-bearing (knee OA) and non-weight bearing (CMC-I) joints were analysed and compared [72]. Changes in CLU levels in healthy *vs.* inflammatory SF or serum samples are summarized in Table 1.

3.3. Roles of CLU in models of inflammatory joint diseases in vitro

A recent *in vitro* study published by our group was the first to specifically demonstrate the secreted form of CLU and focus on sCLU identification in three different *in vitro* models to investigate its role in cytokine-stimulated cartilage degradation [73]. The *in vitro* systems used in that study represent the low-grade inflammatory microenvironment in early OA. We determined sCLU secretion with or without the pro-inflammatory cytokines interleukin-1β (IL-1β) and TNF-α in the secretome of equine cartilage explants, osteochondral biopsies, and primary isolated (unpassaged) chondrocytes. In addition to sCLU, we also measured the release of sulfated glycosaminoglycans, COMP, and the catabolic matrix metalloproteinases (MMP) 3 and 13 in the secretomes of these *in vitro* models by western blotting. To address the complication of pre-existing sCLU in the cartilage matrix, the primary chondrocyte secretome was analysed. An important aspect that differentiates this study from previous publications is that numerous control steps were included to ensure the culture models are reflective of the processes that occur during the early stages of OA; even the chosen pro-inflammatory cytokine concentration was pathophysiologically relevant to OA, which ensures the reliability and applicability of the findings for further studies. The results of this study indicated a significant reduction of sCLU in three *in vitro* models of OA, as compared to the healthy tissues. The levels of COMP, a cartilage degradation marker, in the explant secretome were increased two-fold under IL-1β and TNF-α stimulation versus the control. In contrast to the above results, human OA articular chondrocytes cultured *in vitro* were reported to express higher CLU mRNA and protein levels (detected in total cell lysates) compared to untreated controls [12] We propose that lower sCLU secretion by OA cartilage could be caused by an interruption at the transcriptional level, or by CLU being retained intracellularly during stress, rather than by degradation of extracellular sCLU.

4. Conclusions and future directions

CLU is emerging as a unique molecular chaperone performing critical and synergic roles in both intracellular and extracellular proteostasis [55], with involvement in multiple pathologies. The consensus emerging from the literature is that clusterin CLU is a moonlighting protein with many functions. The protein plays a key role in neurodegeneration and Alzheimer's disease by protecting neurons against intracellular proteotoxicity [74,75]. There is also substantial published literature on CLU in cancer and inflammation [44]. In contrast, however, there has been less research on CLU in arthritic and rheumatic diseases with a special focus on cartilage, chondrocytes, and other synovial joint tissues and cells. There are only two published studies that have examined the potential for CLU as a biomarker of cartilage lesions [48,70]. The published research suggests that in patients with erosive hand OA, lower serum CLU levels are associated with more pain [69]. Proteomic studies of SF from a minipig model of ACL surgery have shown increased levels of CLU after ACL injury and in early OA stages, and negative associations between CLU levels and cartilage lesion area [70]. However, the number of patient biospecimens included in data analyses in clinical studies is sometimes low. Furthermore, studies do not always stratify patients or specify the grade of OA and therefore the results do not reflect a broader biological understanding of the role of CLU in the pathogenesis of OA. In the majority of OA biomarker studies the focus is on catabolic aspects with less attention paid to anabolic and repair processes. More basic, translational and clinical research is needed in this area, particularly focusing on the role of CLU as a cytoprotective protein and extracellular chaperone. More studies are needed to investigate the roles of the secreted and intracellular forms of the protein in osteoarticular tissues. It is clear that CLU is secreted by articular cartilage [76] and chondrocytes [77], and is a robust marker of synovial inflammation (Fig. 2) [78]. CLU mRNA is increased in joint tissues from primary knee and hip OA samples, which has led researchers to suggest that the protein is involved in disease progression [79]. However, CLU may have cytoprotective effects that have not been studied in the context of OA and therefore this should be the focus of future studies. From a clinical perspective, several proteomic studies have shown that CLU is one of several promising glycoprotein biomarkers of OA [66]. Furthermore, sCLU may play a key role as an molecular chaperone in the ECM of articular cartilage protecting against proteotoxicity as it does in neurodegenerative diseases [80].

In conclusion, due to the paucity of predictive biochemical markers in the OA biomarker toolbox, there is significant potential for investigating synovial and systemic CLU as biomarkers of OA. Future translational and clinical orthopaedic studies should carefully consider the diverse roles of this protein and its involvement in other comorbidities. Therefore, future

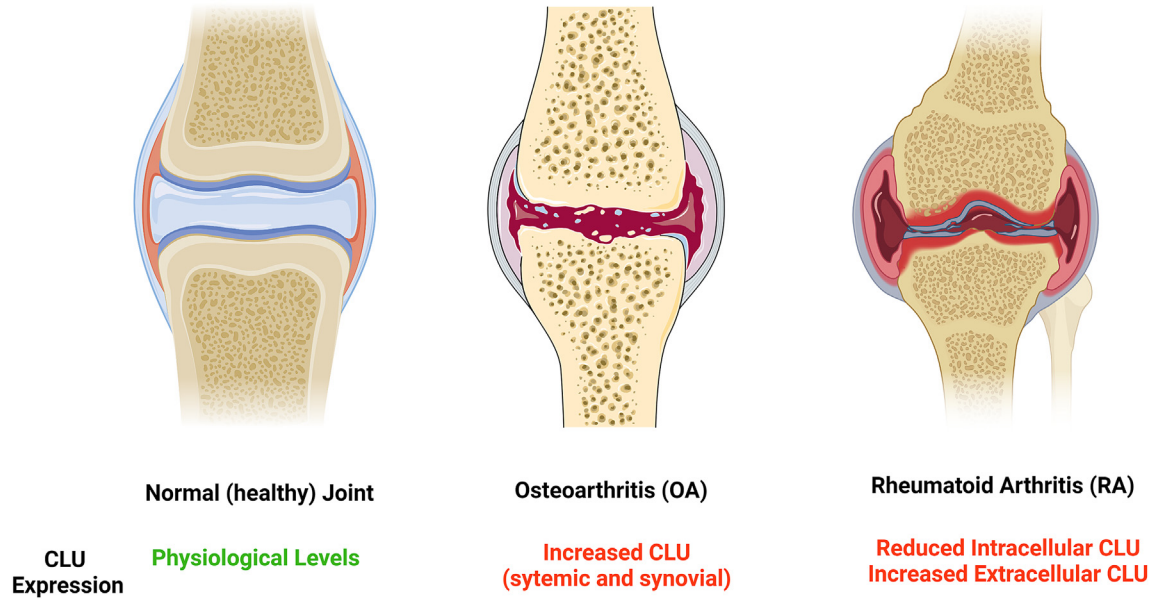

Figure 2. Clusterin expression in healthy joints, osteoarthritis (OA), and rheumatoid arthritis (RA). Figure created with Biorender.com using images from SMART Servier Medical Art.

biomarker studies should not correlate circulating CLU levels exclusively to the process of OA pathogenesis and progression. Special attention should be paid to CLU levels in SF.

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Author contributions

Conceptualization: U.K. and A.M.; Writing – Original Draft Preparation: U.K. and A.M., R.L.; Writing – Review & Editing: all authors; Supervision, A.M. and E.B. Project Administration: A.M. and E.B.; Funding Acquisition, A.M. and E.B.

Declaration of competing interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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