The therapeutic and prognostic role of clusterin in diverse musculoskeletal diseases: a mini review

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Abstract:

Purpose: This mini-review aims to introduce the association between Secretory clusterin/apolipoprotein J (sCLU) and diverse musculoskeletal diseases.

Methods: A comprehensive review of the literature was performed to identify basic science and clinical studies, which implied the therapeutic and prognostic role of sCLU in diverse musculoskeletal diseases.

Results: sCLU is a multifunctional glycoprotein that is ubiquitously expressed in various tissues and is implicated in many pathophysiological processes. Dysregulated expression of sCLU had been reported to be assocaited with proliferative or apoptotic molecular processes and inflammatory responses, which participated in many pathophysiological processes such as degenerative musculoskeletal diseases including ischemic osteonecrosis, osteoarthritis (OA) and degenerative cervical myelopathy (spinal cord injury), neoplastic musculoskeletal diseases, inflammatory and autoimmune musculoskeletal diseases including Rheumatoid arthritis (RA), joint damage induced by Brucella abortus, Sjogren's syndrome, idiopathic inflammatory myopathies, muscle glucose metabolism, insulin sensitivity and traumatic musculoskeletal diseases. Recent findings of sCLU in these musculoskeletal diseases provides insights on the therapeutic and prognostic role of sCLU in these musculoskeletal diseases.

Conclusions: sCLU may serve as a promising therapeutic target for ischemic osteonecrosis, OA and spinal cord injury as well as a potential prognostic biomarker for OA and RA. Moreover, sCLU could act as a prognostic biomarker for osteosarcoma (OS) and a promising therapeutic target for OS resistance. Although many studies support the potential therapeutic and prognostic role of sCLU in some inflammatory and autoimmune-mediated musculoskeletal diseases, more

future researches are needed to explore the molecular pathogenic mechanism mediated by sCLU implied in these musculoskeletal diseases.

Keywords: clusterin, ischemic necrosis, spinal cord injury, osteoarthritis, osteosarcoma, rheumatoid arthritis, Brucella abortus, Sjogren's syndrome, idiopathic inflammatory myopathies, muscle glucose metabolism, insulin sensitivity, trauma

Abbreviation Lists

Full name	Abbreviation
Secretory clusterin	sCLU
Osteoarthritis	OA
spinal cord injury	SCI
bone marrow mesenchymal stem cells	BMSCs
Osteosarcoma	OS
Rheumatoid arthritis	RA

1. Introduce

Secretory clusterin (sCLU)/apolipoprotein J was first discovered and isolated in 1979 in rat testis and it was named clusterin after finding its ability to aggregating blood cells in vitro[1, 2]. sCLU is a 70-85 kDa protein ubiquitously expressed in mammalian tissue and its α and β units are translated and cleaved before secretion from the cells[3, 4]. Different isoforms of sCLU could be generated by the initial protein precursor and their functions were diverse. Cleaved sCLU isoforms in the endoplasmic reticulum/Golgi apparatus may be secreted in the form of α-βheterodimer outer the cells[5]. sCLU is considered as a stress-responding protein with multiple biological functions. sCLU acting as a lipid transport and its dysregulation is significantly associated with many pathophysiological processes such as degenerative musculoskeletal diseases including ischemic osteonecrosis, OA and spinal cord injury (SCI), neoplastic musculoskeletal diseases, inflammatory and autoimmune musculoskeletal diseases including RA, joint damage induced by Brucella abortus, Sjogren's syndrome, idiopathic inflammatory myopathies, muscle glucose metabolism,

insulin sensitivity and traumatic musculoskeletal diseases[3, 6-23]. Table 1 summarizes the therapeutic and prognostic value of sCLU in these musculoskeletal diseases.

Table 1. The therapeutic and prognostic role of sCLU in diverse musculoskeletal diseases

Study	Musculoskeletal diseases	Therapeutic/Prognostic role
Yu, B., et al. (2016)	ischemic osteonecrosis	therapeutic target
Abdallah, B. M., et al. (2018)	ischemic osteonecrosis	therapeutic target
Martin-Vaquero, P., et al. (2015)	SCI	therapeutic target
Anjum, A., et al. (2020)	SCI	therapeutic target
Purmessur, D., et al. (2011)	intervertebral disc degeneration	therapeutic target
Huang, H., et al. (2014)	OS	therapeutic target and prognostic biomarker
Lamoureux, F., et al. (2014)	OS	therapeutic target
Ma, J., et al. (2019)	OS	therapeutic target and prognostic biomarker
Rao, U. N., et al. (2013)	OS	prognostic biomarker
Wang, X., et al. (2020)	OS	therapeutic target
Trougakos, I. P., et al. (2004)	OS	therapeutic target
Trougakos, I. P., et al. (2005)	OS	therapeutic target
Liu, K., et al. (2015)	OS	prognostic biomarker
Devauchelle, V., et al. (2006)	RA	therapeutic target
Kropáčková, T., et al. (2021)	RA	prognostic biomarker
Falgarone, G. and G. Chiocchia (2009)	RA	therapeutic target
Falgarone, G., et al. (2012)	RA	therapeutic target
Hughes-Austin, J. M., et al. (2017)	RA	prognostic biomarker
Tedeschi, S. K., et al. (2017)	RA	prognostic biomarker
Yoshizawa, Y., et al. (2022)	RA	therapeutic target and

		prognostic biomarker
Talmon, G., et al. (2013)	RA/OA	prognostic biomarker
Pucci, S., et al. (2019)	osteoporosis related sarcopenia	therapeutic target
Matta, C., et al. (2021)	OA	prognostic biomarker
Tarquini, C., et al. (2020)	OA	therapeutic target
Fandridis, E., et al. (2011)	OA	prognostic biomarker
Kalvaityte, U., et al. (2022)	OA	therapeutic target
Choi, B., et al. (2014)	bone erosion	therapeutic target
Abdallah, B. M., et al. (2018)	osteoporosis	therapeutic target
Aigelsreiter, A., et al. (2013) Klokov, D., et al. (2013)	trauma/wound	therapeutic target
Kwon, M. J., et al. (2014)	insulin resistance	therapeutic target
Seo, J. A., et al. (2020)	muscle glucose metabolism/insulin resistance	therapeutic target
Qiao, L., et al. (2019)	Sjogren's syndrome	therapeutic target
Kropáčková, T., et al. (2021)	idiopathic inflammatory myopathies	therapeutic target
Scian, R., et al. (2013)	joint damage induced by Brucella abortus	therapeutic target

2. sCLU and degenerative musculoskeletal diseases

2.1 sCLU and ischemic osteonecrosis

Ischemic osteonecrosis features the dysregulated differentiation of bone marrow mesenchymal stem cells (BMSCs) including enhanced osteoclast formation and impaired osteogenesis. Ischemic injury promoted the necrotic bone resorption by osteoclasts[24]. Simulated ischemic stimulaiton (hypoxic or energy-starved environment) accelerated the formation of monocyte-derived osteoclasts and suppressed the activity of alkaline phosphatase and calcification in osteoblasts[25]. The maintenance of mitochondrial membrane potential ($\Delta\Psi m$) is vital for cellular homeostasis that can be disrupted by ischemic stimulation. The $\Delta\Psi m$ of mesenchymal stromal cells exposed to hypoxic

environment was maintained and the caspase 3/7 activity was reduced by sCLU in a concentration-dependent manner[26]. Exogenous sCLU treatment significantly decreased LDH release and protected mesenchymal stromal cells against the hypoxic environment[26]. Exogenous sCLU could regulate the osteoblast versus adipocyte differentiation from mouse BMSCs lineage through a mechanism mediated by ERK1/2 signaling[27]. Treating bone marrow macrophages with exogenous sCLU compromised their enhanced proliferative activity elicited by M-CSF and inhibited osteoclast formation from both bone marrow-derived macrophages and osteoclast precursor cells by the suppression of M-CSF-induced ERK activation of osteoclast precursor cells[28]. The levels of exogenous sCLU may be associated with the osteoclast activity and the osteoclast formation in the context of ischemic osteonecrosis, in which process ERK signaling pathway may participates. Application of exogenous sCLU potentially serves as a new therapeutic policy for osteonecrosis of femoral head and prevents ischemic osteolysis through inhibiting osteoclast formation and promoting osteoblast differentiation. Moreover, overexpression of sCLU gene and (or) higher extracellular abundance of sCLU protects BMSCs against ischemic injury, which indicates sCLU as a therapeutic target for ischemic osteonecrosis, fragile fracture and delayed healing of fractures.

2.2 sCLU and Osteoarthritis

OA is one of the common musculoskeletal diseases characterized by the destruction of articular cartilage[29]. OA-induced cartilage degeneration includes inflammation, osteochondral lesions and the hypertrophic chondrocytes[29]. It was confirmed that the expression of sCLU in adult human normal and osteoarthritic cartilage by Northern blot and in situ hybridization analysis[30, 31]. Intensity of immunostaining for sCLU decreased with age in healthy cartilage tissue[32]. sCLU is actively expressed in the repaired human cartilage after autologous chondrocyte implantation, and there is a different distribution of sCLU in repaired tissue compared to healthy cartilage[32], suggesting the possible role of sCLU in the remodeling and regeneration of cartilage tissue. Moreover, the content of sCLU is elevated in human OA cartilage and cultured OA phenotype chondrocytes compared with control groups[29]. sCLU knockdown impaired the proliferative activity of OA phenotype chondrocytes and promoted the apoptosis of hypertrophic articular chondrocytes accompanied with higher MMP13 and COL10A1 content as well as upregulation of

TNF-α, Nox4 and ROS[29]. The production and secretion of sCLU is inhibited in the equine cartilage degradation model and interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α) treatment inhibits the release of secreted sCLU and limits the cytoprotective effects of secreted sCLU on OA phenotype chondrocytes, indicating sCLU as an promising therapeutic target for synovitis and cartilage destruction in OA[33, 34].

The concentration of sCLU in human serum and synovial fluid is higher in OA patient group comparing to healthy group, and the expression of sCLU mRNA and protein in OA phenotype samples is significantly higher than that in healthy tissue samples collected from normal cartilage[7, 34, 35]. Increased sCLU mRNA and protein levels in serum and synovial fluid of knee OA might reflect knee OA severity (disease activity) [34, 36]. Levels of peptides representative of sCLU in plasma are indicative of the severity of OA progression[37]. In summary, the expression levels of sCLU is used as a prognostic biomarker for OA. Differential abundance of sCLU in synovial fluid from OA joints triggers different effects on primary chondrocytes unraveling the association between sCLU and the molecular mechanisms of OA and bringing a new dimension into the pathogenesis of primary OA[38]. The increased expression and secretion of sCLU is indicative of the onset of OA, to some extent, appropriate upregulation of sCLU may in turn protect OA cartilage against further desturction and delay the speed of OA progression. The cytoprotective properties of CLU may support the implementation of regenerative strategies and new approaches for developing targeted therapeutics for OA[39]. The exact role of sCLU in OA has yet to be elucidated. Further studies are needed to support the potentially therapeutic value of sCLU for treating OA and possible prognostic biomarker for OA.

2.3 sCLU and degenerative cervical myelopathy (spinal cord injury)

Higher abundance of sCLU is found in the cerebrospinal fluid from affected dogs diagnosed as degenerative cervical spondylotic myelopathy with clinical signs and abnormal neurologic examination findings[40]. Moreover, corticosteroid treatment can rescue the content of sCLU in cerebrospinal fluid from these affected dogs[40]. As one of the most severe complications of degenerative cervical myelopathy, SCI is a destructive neurological and pathological state that causes major motor, sensory and autonomic dysfunctions[41]. The mRNA and protein expression of

sCLU is increased in neurons located closely to the lesion site within scar tissue in SCI[6], which suggests that sCLU may participate in the spatial and temporal extent of SCI. The increased expression of sCLU is observed both rostrally and caudally from the injury site where acute contusion spinal cord injury induced by using the New York University weight-drop impactor[42]. sCLU is associated with inflammatory responses, tissue degeneration, and cellular apoptosis during acute and subacute traumatic SCI[6, 40, 43].

3. sCLU and neoplastic musculoskeletal diseases

OS is the most common primary malignant bone tumor. The abundance of sCLU is elevated in osteosarcoma sample[9, 10], indicating sCLU as a prognostic biomarker for OS and a monitoring biomarker for chemotherapy resistance[44]. sCLU depletion inhibits the growth of U-2 OS cells exposed to cisplatin and improves the chemosensitivity to cisplatin in vitro and in vivo by inhibiting the activation of ERK1/2[9], which indicates sCLU may be indicative of drug resistance and serve as a prognostic factor for OS patients. Gene knockdown of sCLU suppresses cellular invasion and improves the chemotherapy sensitivity of OS cells to chemotherapeutic drug (gemcitabine and doxorubicin)[10, 45, 46], which implies targeting sCLU can improve the prognosis of OS patients with increased sCLU expression. In OS cell lines, zoledronic acid increases levels of heat shock proteins, especially sCLU by enhancing HSF1 transcription activity[47]. Moreover, sCLU overexpression protects OS sensitive to zoledronic acid against chemotherapy-induced cell death by modulating the expression of multidrug-resistant 1 and farnesyl diphosphate synthase[47]. Zoledronic acid-mediated upregulation of sCLU is attenuated by the antisense drug of sCLU (OGX-011), with synergistic effects on delaying progression of OS and enhanced activity of zoledronic acid on cell growth and apoptosis[47].

Higher expression of sCLU has correlation with metastasis, poor prognosis and chemotherapy resistance in OS patients[45, 48]. The upregulatoin of sCLU after chemotherapy may be the reason of resistance in OS. The expression of sCLU has a positive correlation with the metastatic disease and a negative correlation with the response to chemotherapy[10]. sCLU-silencing in U2OS cells significantly inhibits the invasion and cellular growth of U2OS cells and promotes chemotherapy-induced cellular apoptosis[10, 46]. The intracellular sCLU, at moderate levels, exerts

potent anti-apoptotic effects in KH OS cells[49], which is harmful to OS patients and may be the reason of OS chemotherapy resistance. However, if sCLU accumulates intracellularly too much either by direct synthesis or by uptake from the extracellular milieu, high intracellular abundance of sCLU may become highly cytostatic and/or cytotoxic manifested by growth retardation at the G2/M checkpoint and reduced DNA synthesis[49], which implies sCLU expression is associated with the cellular growth (arrest) and cellular apoptosis (survival) of OS cells. More studies are needed to explore the potential prognostic and therapeutic value of sCLU in OS patients.

4. sCLU and inflammatory and autoimmune musculoskeletal diseases

4.1 sCLU and RA, Sjogren's syndrome-related arthritis

sCLU can be secreted by synoviocytes, and it is detected in the synovial fluid and nonneoplastic synovium[11, 20]. Transgenic overexpression of sCLU promotes the apoptosis of RA fibroblast-like synoviocytes within 24 hour and knockdown of sCLU gene by small interfering RNA promotes the production of IL-6 and IL-8[11]. Moreover, extracellular sCLU in synovial fluid has correlation with the therapeutic effects of RA and the lower remission rate after chemotherapeutic treatment of the low disease activity patients[8, 14], which implies sCLU can serve as the prognostic biomarker for assessing the disease activity and treatment response of RA patients. In vitro experiments show sCLU is involved with the pathophysiological processes of RA simulated by the human fibroblast-like synoviocytes exposed to TNF-α[19]. A localized abnormal tissue expression of sCLU may lead to the progression of a citrullination response of sCLU[50]. Moreover, the dysregulation of citrullinated sCLU participates in the inflammatory and immunological processes of RA[16, 51]. Obesity and elevated expression of citrullinated sCLU may interact to increase RA risk and shorten time to diagnosis[52]. The protein expression of sCLU in labial salivary gland biopsy specimens and saliva samples is higher in Sjögren's syndrome patients[15]. Serum sCLU may act as the potential therapeutic target for primary Sjögren's syndrome patients with neuromyelitis optica spectrum disorder, and sCLU plays an important role in the pathogenesis of the disease acquiring further verification[53].

4.2 sCLU and joint damage induced by Brucella abortus

Brucella abortus infection inhibits the apoptosis of synoviocytes by increasing the expression of

antiapoptotic sCLU[13]. And Brucella abortus infection increases the soluble and membrane RANKL expression in synoviocytes, which then further promotes monocytes to undergo osteoclastogenesis[13], which suggests the expression of sCLU in synoviocytes is vital for the pathogenesis of brucellar arthritis and osteochondral lesions.

4.3 sCLU and idiopathic inflammatory myopathies

Serum levels of sCLU are significantly increased in idiopathic inflammatory myopathies patients compared to controls and positively correlated with myositis disease activity assessment[23]. The sCLU mRNA expression within muscle tissue of idiopathic inflammatory myopathies patients is increased compared to controls and sCLU accumulates in the cytoplasm of regenerating myofibres[23]. From the mentioned above, we can conclude that sCLU plays an important role in the pathogenesis of idiopathic inflammatory myopathies and sCLU may become the potential therapeutic target for idiopathic inflammatory myopathies.

4.4 sCLU and muscle glucose metabolism, insulin sensitivity

Deficiency of sCLU exacerbates high-fat diet-induced insulin resistance in male mice[21]. Deletion of hepatic sCLU causes insulin resistance[22]. In patients with insulin resistance, pioglitazone-induced improvement of insulin action is associated with the increased sCLU expression in muscle[22]. Therefore, sCLU plays an important role in regulating muscle metabolism and insulin sensitivity, and sCLU is vital for the crosstalk between liver and skeletal muscle[22].

5. sCLU and traumatic musculoskeletal diseases

Elastofibroma dorsi is associated with trauma and the abnormal large elastic fibers in elastofibroma dorsi are enveloped by sCLU[18], which indicates the dysregulation of sCLU is associated with trauma-induced elastofibroma dorsi. As one of the pro- and anti-apoptotic agents in muscle tissue[54], sCLU is a pro-survival bystander factor that abrogates TGFβ1 signaling and most likely promotes wound healing[17]. Overexpression of sCLU in muscle biopsies is found in osteoporosis women undergoing surgery for fragility hip fracture, and gene knockdown of sCLU by siRNA can restore the proliferative capability of isolated myoblasts and rescue the repair ability of musculoskeletal tissue[55], indicating sCLU as a promising therapeutic target for muscle degeneration. Therefore, trauma-induced localized upregulation of sCLU may, to some extent, in

turn accelerate tissue regeneration.

6. Conclusion

sCLU is a versatile secreted glycoprotein associated with the complement activation and cell death in the impaired and degenerative tissues[56, 57]. And sCLU plays an important role in preventing a “runaway” inflammatory reaction[6, 42]. Moreover, sCLU has the anti-apoptotic character, which is involved in the regulation of cellular survival (proliferation), lipid transport, extracellular tissue remodeling and apoptosis (necrosis)[27].

Interestingly, the dysregulated expression and abnormal secretion of sCLU is found in SCI, OS, OA, RA, joint damage induced by Brucella abortus, Sjogren's syndrome, idiopathic inflammatory myopathies, muscle glucose metabolism, insulin sensitivity and traumatic musculoskeletal diseases and ischemic osteonecrosis. The involvement of sCLU in bone metabolism provides the theoretic basement that needs more studies to investigate the therapeutic role of sCLU for delayed fracture healing, fragile fracture and osteonecrosis (osteolysis). The association between sCLU and degenerative cervical myelopathy may suggest the therapeutic and prognostic value of sCLU for degenerative spine disorder and shed light on the molecular pathogenesis of acute or chronic SCI. Moreover, sCLU may also act as a prognostic biomarker for metastatic osteosarcoma and a promising therapeutic target for OS resistance. What’s more, the participation of sCLU in OA progression and joint damage induced by Brucella abortus unveils the important role of sCLU in regulating cartilage (chondrocytes) and synovium (synoviocytes), which indicates sCLU as a therapeutic target and a prognostic biomarker for inflammatory and degenerative arthritis. The altered expression of sCLU in RA and Sjogren's syndrome-related arthritis as well as idiopathic inflammatory myopathies and insulin resistance-related sarcopenia implies the therapeutic value of sCLU in autoimmune-mediated musculoskeletal diseases. In summary, more basic experiments and case-control (cohort) studies are needed to explore and confirm the therapeutic and prognostic value of sCLU for these musculoskeletal diseases.

Disclosure statement

No potential conflict of interest was reported by the authors.

Authors’s contributions

Kun Zhang and Yuchen Tang conceived and designed the idea and drafted this paper. Yayi Xia and Bin Geng supervised the framework of the article. Kaixin Liu and Dechen Yu recollected and reanalyzed the references. Peng Xu helped us revise this manuscript by providing important suggestions. All authors read and approved the final version of the manuscript. Kun Zhang is the first author of this article. Peng Xu is the corresponding author of this paper.

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