Review

Therapeutic Potential of Clusterin Inhibition in Human Cancer

Desirée Martín-García , Marilina García-Aranda , Maximino Redondo *

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Review

Therapeutic Potential of Clusterin Inhibition in Human Cancer

Desirée Martín-García [1,2,3,4,†], Marilina García-Aranda [2,3,4,†] and Maximino Redondo [1,2,3,4,*]

[1] Surgical Specialties, Biochemistry and Immunology Department, Faculty of Medicine, University of Málaga, 29010, Málaga, Spain; desirermg@uma.es
[2] Red de Investigación en Servicios de Salud en Enfermedades Crónicas (REDISSEC), Red de Investigación en Cronicidad, Atención Primaria y Promoción de la Salud (RICAPPS), Instituto de Investigación Biomédica de Málaga (IBIMA), 29590, Málaga, Spain.
[3] Instituto de Investigación Biomédica de Málaga y Plataforma en Nanomedicina – IBIMA Plataforma BIONAND, 29590, Málaga, Spain.
[4] Research and Innovation Unit. Hospital Costa del Sol, 29602, Marbella, Spain; marilina.garcia.sspa@juntadeandalucia.es
* Correspondence: maximino.redondo.sspa@juntadeandalucia.es
† These authors contributed equally to this work.

Abstract: Clusterin (CLU) protein is involved in various pathophysiological processes including carcinogenesis and tumor progression. In recent years, the role of the secretory isoform has been demonstrated in tumor cells, where it inhibits apoptosis and favors the acquisition of resistance to conventional treatments used to treat cancer. To determine the possible therapeutic potential of inhibiting this protein, numerous studies have been carried out in this field. In this paper, we show the existing knowledge on the inhibition of this protein in different types of cancer and discuss the importance it could have in the development of new therapies directed against this pathology.

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1. Introduction

Clusterin (CLU), also known by various names such as APOJ, CLI, Complement Lysis Inhibitor, SP-40,40, Sulfated Glycoprotein 2, Testosterone-Repressed Prostate Message 2, Apolipoprotein J, Ku70-Binding Protein 1, KUB1, SGP-2, SP-40, or TRPM2, is a multifunctional protein encoded by the CLU gene [1], widely expressed in various human tissues [2,3]. Over the years, CLU has been identified as a key molecule in a variety of physiological processes [3], including cell differentiation, morphogenesis [4], sperm maturation, lipid transport, complement inhibition, tissue remodeling, membrane recycling, cell-cell and cell-substrate interactions, stabilization of stressed proteins, cell proliferation, survival, and apoptosis [5,6].

Alterations in CLU expression has been associated with serious physiological diseases, such as spongiform encephalopathies, hippocampal and heart ischemic injuries, atherosclerosis [7], schizophrenia [8], cardiovascular diseases [9], cancer, vascular damage, diabetes, osteoarthritis [10], and degenerative conditions such as age-related macular degeneration, retinitis pigmentosa [11], Parkinson's disease [12], and Alzheimer's [13,14]. These conditions are often more prominent in advanced aging [13,14], where clusterin overexpression has been observed in phenomena such as enhanced cell migration [15], chemotherapy resistance [16,17], and more aggressive biological behaviors [18] in malignant cells. CLU has been proposed as a cancer biomarker [19] and cellular senescence [20], generating interest in research to better understand its implications and explore its utility in the diagnosis, prevention, and treatment of these pathologies.

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2. Isoforms and Regulation of CLU Expression Gene

The human CLU gene, located on chromosome 8p21-p12, is a highly conserved gene with at least 63 orthologs in different species [21]. It has a structure consisting of 11 exons and spans approximately 16 kilobases. This gene produces at least 17 splice variants, both coding and non-coding [21,22]. However, it generates three main transcription products identified as Variant 1 (NM_001831), Variant 2 (NR_038335), and Variant 3 (NR_045494). These variants share exons 2-11 but differ in exon 1 (1a, 1b, 1c) and in the untranslated terminal regions [23–25], indicating the existence of distinct transcription start sites for each variant, although the biological relevance of the different transcripts generated is still unclear.

The sequence initially encodes a translocation signal to direct the preprotein to the endoplasmic reticulum (ER), where the signal sequence is removed, and N-glycosylation and disulfide bond formation occur [26]. Subsequently, the protein is translocated to the Golgi apparatus, undergoes more complex glycosylation, and reaches a weight of 70 to 80 kDa. The mature form of the protein, known as the secreted isoform (sCLU), is generated by cleavage and disulfide bond formation between residues 227 and 228 (Figure 1). The 449-amino acid sCLU is secreted as a heterodimeric glycoprotein complex and functions as an extracellular chaperone, inhibiting the aggregation of partially unfolded proteins, enhancing phagocytosis and cellular degradation, and exhibiting anti-apoptotic activity in cancer cells [27–29]. This isoform can stabilize the synthesis of the p53 protein, involved in the activation of genes that halt the cell cycle, thus inhibiting cell proliferation [30,31].

In situations of alternative splicing between exons 1 and 3, exon 2 is deleted, generating a non-functional prenuclear isoform of 49 kDa (pnCLU). Under stress, this isoform transforms into a functional nuclear isoform of 55 kDa (nCLU), which, upon translocating to the nucleus and binding to the Ku70 protein, promotes cellular apoptosis [32–35] (Figure 1).

Additionally, there is an almost mature presecreted isoform of 53 kDa (psCLU) that, under stress, binds to the chaperone GRP78 (Bip) in the ER and translocates to the mitochondria (Figure 1). In the mitochondria, it binds to the activated form of the Bax protein, modulating its ability to form homodimers and inhibiting the formation of Bax-Bak complexes [36]. This anti-apoptotic activity is also evident in the cytoplasm, where psCLU can stabilize the Ku70-Bax complex, preventing Bax from reaching the mitochondria and promoting the proteasomal degradation of cytotoxic substances [37,38].

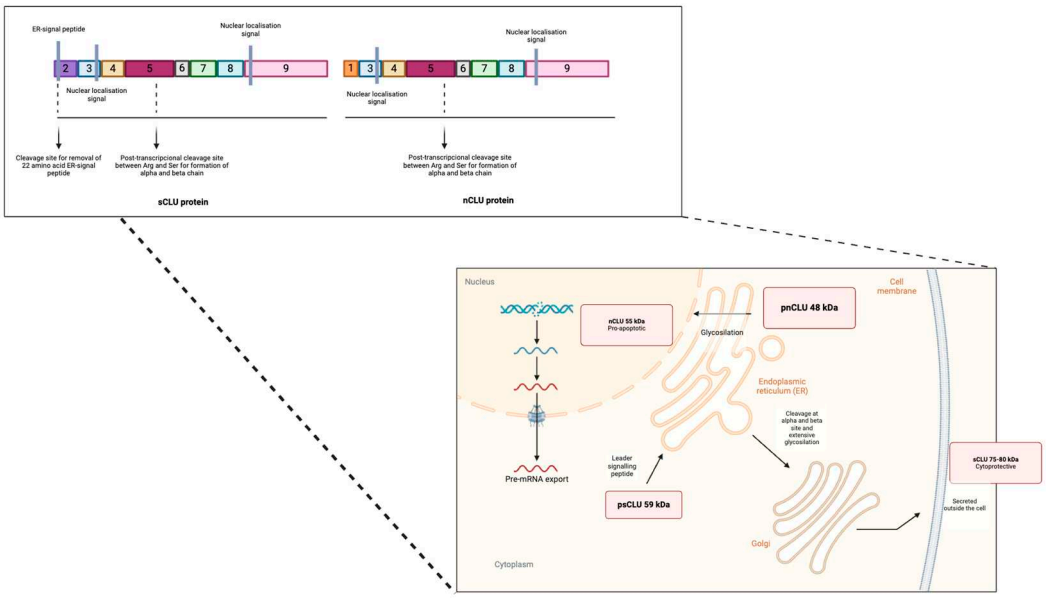

Figure 1. Generation of nCLU and sCLU. psCLU is transported to the ER and by the leader signaling peptide and then cleaved and glycosylated while being transported to the Golgi apparatus. The result is an 80 kDa protein consisting of the alpha and beta subunit by disulfide bonds which is secreted

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outside the cell. pnCLU does not undergo any excision or glycosylation process, it is localized in the cytoplasm of unstressed cells. pnCLU is converted into a mature form inside the nucleus, nCLU.

The expression of CLU is meticulously regulated in both healthy and pathological contexts, where various pathways exert influence on its basal expression in healthy tissues as well as its upregulation in conditions such as apoptosis, pathologies, and aging. In this scenario, two forms of CLU with antagonistic functions are distinguished: sCLU, playing a cytoprotective role, and nCLU, capable of promoting cell death [39,40]. Each CLU variant is subject to different signaling pathways, depending not only on its molecular configuration but also on the cell type in which it manifests. The sophisticated regulation of both CLU isoforms encompasses a broad spectrum of factors, including growth factors such as TGF-β, NGF, EGF, cytokines like TNF-α and IL-1, IL-2, and IL-6, transcription factors such as TCF1, Jak/STAT1, and IGF1, as well as the Wnt signaling pathway [41–45] and epigenetic modifications. Furthermore, expression can be induced by various agents such as oxidative stress, chemotherapeutic agents, ionizing and ultraviolet radiation, estrogen, and androgen deprivation in hormone-sensitive tumors [46,47].Gene expression control in vertebrates is an intricate process orchestrated by numerous regulatory proteins that collaborate to influence the expression of a specific gene or transcript within a cell. Typically, vertebrate gene expression is regulated by the basal promoter, a DNA region spanning from -200bp to the transcription start site (TSS) (+1), facilitating the binding of regulatory proteins, including transcription factors [48]. The variation in CLU expression across different tissues suggests its finely tuned and tissue-specific regulation at transcriptional, translational, and post-translational levels [49]. The human CLU promoter, initially identified as a unique element, contains conserved DNA motifs, including a palindrome (-73 to -87) and a TATAA box (-26), crucial for decoding the genetic sequence. Furthermore, a GCATT box (-93) plays a key role in regulating transcription frequency [50]. Additional studies have uncovered a second promoter region (P2) within the first CLU intron, potentially controlling CLU2 expression [23].

Epigenetics, the study of heritable changes in gene expression without altering the DNA sequence, emerges as a pivotal player in regulating clusterin (CLU) expression during aging and cancer progression [51]. The intricate interplay of DNA methylation and histone acetylation [52,53], influenced by aging and cancer, suggests epigenetic regulation's significant role in controlling CLU [23,51,52,54].

The *Histone Code Hypothesis* outlines the diverse histone modifications governing chromatin structure and transcriptional status [55]. Histone modifications impact gene silencing or activation by modulating DNA accessibility. Notably, histone 3 lysine 9 trimethylation (H3K9me3) and histone 3 lysine 27 trimethylation (H3K27me) contribute to nCLU down-regulation [51] and cell survival. Conversely, histone 3 lysine 4 trimethylation (H3K4me3) or histone 3 lysine 9 acetylation and serine 10 phosphorylation (H3K9AcS10P) lead to nCLU activation [51] and cell death. Epigenetic drug treatments alter histone modifications, impacting CLU1 and CLU2 transcription [23].

Consistent findings across diverse cell types, including cancer cells [23,52], retinal pigment epithelial cells [52], hepatocellular carcinoma cell lines [56], and endothelial tumor cells [53], emphasize the role of histone hyperacetylation induced by histone deacetylase inhibition in promoting CLU expression.

In differentiated mammalian cells, gene repression often involves DNA methylation, co-coordinated with histone modifications. While colon cancer studies indicate predominant regulation of CLU by histone modifications [51], the presence of a G+C-rich region and a CpG mini-island within the CLU promoter suggests epigenetic control via methylation [50]. Promoter methylation is associated with decreased protein expression, observed in breast [54], ovarian epithelial cancer [18], and hormone-refractory prostate carcinoma samples [57]. Induced demethylation of CLU promoters significantly increases CLU1 and CLU2 transcripts, enhancing CLU expression across various colon cell lines [51] and tissues [58].

Furthermore, micro-RNAs (mi-RNAs) contribute to post-transcriptional regulation of CLU expression [59]. In head and neck squamous cell carcinoma, oncogenic miRNA-21 specifically targets

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the CLU1 isoform, down-regulating its growth-suppressive variant [60]. Elevated levels of miRNA-378 inhibit lung adenocarcinoma cell growth and sCLU expression [61].

While this field's studies are recent, the emerging evidence underscores the intricate epigenetic control mechanisms orchestrating CLU expression in diverse cellular contexts. These insights provide a foundation for understanding the molecular intricacies of CLU regulation, offering potential avenues for therapeutic interventions in aging and cancer-related conditions.

3. Clusterin and Its Involvement in Cancer

Despite initially being believed to be linked to biological processes such as scar formation, membrane remodeling, or sperm maturation [50], early studies revealed a correlation between the overexpression of mRNA-CLU and cell death after different toxic signals [62–64]. However, later research presented contradictory results, establishing a relationship between CLU overexpression and cell protection [65,66].

The identification of two sets of CLU isoforms, with opposing roles and cellular locations in terms of cell survival, has contributed to clarifying these contradictions [67,68]. While the expression of the nuclear isoform (nCLU) is associated with healthy tissues, the overexpression of the secretory isoform (sCLU) is related to most malignancies [19]. In tumor cells, a pro-survival environment is observed with a higher sCLU/nCLU ratio [69,70].

Post-translational modifications appear to regulate the distinctive roles of CLU, determining the expression of specific isoforms based on the presence or absence of a leader sequence in the CLU peptide [71]. Inhibition of exon 2 of mRNA-CLU, leading to the silencing of the sCLU isoform, has been crucial for the design of cancer-targeted therapies that are currently under study [72].

Early studies suggest that CLU overexpression persists after tissue degeneration, suggesting that this gene is probably not induced as part of apoptosis that may lead to a degenerative disorder but as a secondary consequence of the disease phenotype [50]. nCLU-mediated apoptosis is associated with the accumulation of mature nCLU protein, while the rapid degradation of CLU and the regulation of its nuclear export/import can explain why highly malignant tumors avoid accumulating nCLU levels [72].The lethality of nCLU is linked to members of the BCL-2 protein family, with nCLU-mediated apoptosis depending on Bax [69]. Additionally, nCLU can sequester the antiapoptotic BCL-XL, promoting apoptosis by forming pores in mitochondrial membranes [73].

The proapoptotic role of nCLU is also related to Ku70, which binds to CLU in response to DNA damage. The formation of a trimeric protein complex (nCLU/Ku70/Ku80) with reduced DNA end-binding activity and interaction with other proteins like Ku70 in the C-terminal coiled-coil domain of nCLU is essential for inducing apoptosis [74].

Although the constant expression of nCLU is related to cell maintenance and apoptosis induction, cells can also express cytoplasmic clusterin (cCLU) or sCLU isoforms in response to different cellular triggers. This results in increased cell survival associated with tumorigenesis, with CLU promoting apoptosis under low or moderate stress conditions but favoring the overexpression of the antiapoptotic isoform [19] under extreme cellular conditions, resulting in increased cell proliferation, viability, and invasiveness [75].

In addition, CLU has garnered attention from the scientific community due to its essential role in various biological processes, such as cell proliferation, apoptosis, epithelial-mesenchymal transition (EMT), metastasis, and resistance to chemotherapy.

3.1. Tumorigenesis

Under normal conditions, CLU expression is low and significantly increases in response to stress, activating the transcription of clusterin mRNA [76,77]. The cytoprotective isoform sCLU, when faced with stress, protects cells through its extracellular chaperone activity, thereby preventing apoptosis and conferring resistance to cytotoxic agents. sCLU plays a crucial role by binding to the Ku70-bax complex, acting as a bax retention factor in the cytosol and inhibiting its proapoptotic function. Inhibition of CLU weakens this complex, increases bax translocation to the mitochondria, triggers cytochrome c release, and activates caspase 9, initiating apoptosis [78]. On the other hand,

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the proapoptotic isoform nCLU induces apoptosis in cancers such as breast and prostate by interacting with proteins like Ku70 and Bcl-XL [79,80].

CLU is also involved in various signaling pathways, such as the transcription factor B-MYB, Akt phosphorylation, and the PI3K/Akt pathway, regulating its apoptotic and antiapoptotic functions [81,82]. The production ratio of both isoforms is crucial for regulating these functions, and CLU overexpression has been associated with the promotion of tumorigenesis and resistance to chemotherapy in cancers such as breast and prostate [83].

3.2. Cell Proliferation

Cell proliferation, a fundamental biological process, involves the increase in the number of cells through repeated cell divisions to maintain a dynamic balance between cell death and promote metabolism in biology. On the other hand, cell growth refers to the physical expansion of cell volume or size as the cell matures [84]. These processes are orderly and strictly controlled by various growth factors and cytokines in normal cells [85]. However, in cancer cells, cell proliferation is uncontrolled and exhibits a sustained proliferation property due to the hyperactivation of proliferative signaling pathways and evasion of growth suppressors [85].

The acquisition of sustained proliferative signaling pathways becomes a distinctive feature of cancer cells, and recent evidence has highlighted the regulatory role of the CLU protein in cell proliferation and growth in various types of cancer [86]. Relevant studies have revealed that c-Myc, a transcription factor encoded by the oncogene MYC involved in tumorigenesis, inhibits the expression of nCLU by upregulating the microRNA cluster miRNA-17 ~ 92 and attenuating the TGF-β axis, thus promoting angiogenesis and tumor growth in colon cancer [87].

Both nCLU and sCLU play a prominent role in regulating the canonical ERK, NF-κB, and AKT pathways, mediating cell proliferation and growth in various types of cancer. Inhibition of sCLU by apocynin, an NADPH oxidase inhibitor, inactivates the MEK-ERK1/2 pathway and suppresses cell proliferation in prostate cancer [88]. Meanwhile, induction of sCLU by DDP promotes the phosphorylation of ERK1/2, thereby driving cell growth and increasing resistance to DDP in OS [89].

The NF-κB axis plays a crucial role in cell proliferation, where activated NF-κB induces the expression of Bcl-2 to promote cell proliferation [90]. Melittin inhibits sCLU, inactivating both the cholesterol/NF-κB/Bcl-2 axis and the cholesterol/p-ERK axis to suppress tumor growth in pancreatic cancer [90]. Interestingly, sCLU exhibits an anti-proliferative property by inactivating the TAK1/NF-κB axis, preventing the transforming growth factor beta receptor 1 (TGFBR1) from recruiting the TNF receptor-activating factor 6 (TRAF6)/TAK1-binding protein 2 (TAB2)/TGF-β-activated kinase 1 (TAK1) complex to inhibit tumor proliferation and growth in human non-small cell lung cancer (NSCLC) [91].

In addition to the ERK and NF-κB pathways, the PI3K/AKT axis is also involved in regulating cell proliferation and growth. Notably, sCLU activates AKT by downregulating the expression of the protein phosphatase 2A catalytic subunit C (PP2AC) to promote the proliferation of PC-3 cells in prostate cancer [92]. This aligns with the finding that silencing CLU gene transcription decreases the phosphorylation level of AKT and GSK-3β, subsequently inhibiting the proliferation of HCCLM3 cells in hepatocellular carcinoma (HCC) [93].

Various external factors also influence CLU expression and, consequently, cell proliferation. Insulin-like growth factor 1 (IGF-1), a crucial component of insulin receptor signaling involved in sustained cell proliferation [94], has been shown in several studies to promote sCLU expression. For example, IGF-1 upregulates sCLU expression and subsequently activates the PI3K/AKT axis to promote the proliferation of A549 cells in NSCLC [95]. Meanwhile, IGF-1 upregulates the transcriptional activity of the CLU gene by activating the STAT-3/Twist-1 axis in prostate cancer [96]. Interestingly, pituitary tumor transforming gene (PTTG) and forkhead box protein L2 (FOXL2), respectively, stimulate sCLU expression and CLU gene transcription by activating the ATM/IGF-1/p38MAPK/CLU axis and directly binding to the CLU promoter. Meanwhile, sCLU exhibits an anti-proliferative property that, in turn, attenuates PTTG expression to restrain cell proliferation in pituitary carcinoma [97].

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Metformin, a classic drug for treating type II diabetes, shows anti-tumor properties, as sCLU suppressed by metformin inhibits tumor growth by inactivating the SREBP-1c/fatty acid synthase (FASN) axis in bladder cancer [98]. Additionally, sCLU promotes cell growth and proliferation by upregulating the expression of the calcium-binding protein S100A4 in renal cancer [99]. Along with metformin and melittin, epigallocatechin-3-gallate (EGCG) and green tea extract (GTE) also induce sCLU expression by suppressing the transcriptional activity of β-catenin, promoting the proliferation of COLO 205 cells in colon cancer [100].

3.3. Epithelial-Mesenchymal Transition and Metastasis

Epithelial-mesenchymal transition (EMT) is a biological process involving significant changes in cell structure and function, such as loss of polarity, cytoskeletal remodeling, and acquisition of invasive properties. EMT facilitates invasion, metastasis, and tumor progression. Clusterin (CLU) has emerged as a key regulator in these events, influencing matrix metalloproteinase (MMP) activity and the ERK1/2 and PI3K/Akt signaling pathways [101]. NF-κB activation by CLU also plays a crucial role in increasing MMP-2 and MMP-9 expression, thus promoting metastasis [102].

In different cancer types, CLU overexpression has been associated with metastasis. In nasopharyngeal carcinoma, CLU is positively regulated by N, N′-dinitrosopiperazine (DNP), inducing MMP-9 and VEGF expression, contributing to metastasis [103]. In breast cancer, CLU collaborates with eHsp90α to activate key signaling pathways, promoting EMT, migration, and tumor metastasis [104]. In colon cancer, CLU interaction with platelets activates the p38MAPK pathway and positively regulates MMP-9, facilitating invasion [95]. Additionally, studies on prostate cancer have shown that miRNA-217-5p regulates invasion and migration by targeting CLU [105].

In patients with pancreatic ductal adenocarcinoma (PDAC), positive regulation of CLU by hepatocyte nuclear factor 1b (HNF1B) correlates with worse survival. Decreased CLU levels, along with reduced HNF1B, contribute to increased cell proliferation, EMT, and chemotherapy resistance, accelerating PDAC progression. These findings suggest that the HNF1B/CLU pathway is crucial for restraining pancreatic cancer progression [106].

3.4. Chemoresistance and Chemosensitivity with Clusterin

Chemoresistance, involving the ability of cancer cells to resist the effects of chemotherapy, is a significant challenge in cancer treatment. This phenomenon can manifest through various mechanisms, including genetic changes, alterations in drug metabolism, and suppression of apoptosis [107]. Chaperone proteins, like clusterin (CLU), play a crucial role in cancer therapy resistance, facilitating the growth of malignant tumors and protecting drug-resistant cells [108].

The stress response induced by treatments such as radiotherapy and chemotherapy lead to the overexpression of sCLU, a cytoprotective chaperone, which, by interacting with activated Bax, blocks the release of cytochrome c and prevents apoptosis [109]. Negative regulation of CLU in certain types of cancer, such as testicular seminoma, has been shown to increase sensitivity to radiotherapy and chemotherapy [110].

In hepatocellular carcinoma (HCC), sCLU is strongly overexpressed, contributing to oxaliplatin resistance by negatively regulating Gadd45a expression and activating the PI3K/Akt pathway. Inhibition of CLU in HepG2/ADM HCC cells restores chemosensitivity to various drugs [111].

4. Clusterin as a Biomarker and Therapeutic Target in Cancer

In the field of medicine, biomarkers have become essential tools to provide valuable information about patient health and the progression of various diseases. From measuring simple protein levels to identifying genetic mutations, these markers play a crucial role in the diagnosis, prognosis, monitoring, and treatment of diseases. In this context, clusterin has been studied as a potential biomarker in various types of cancer due to its tendency to be overexpressed in stressful situations (Table 1).

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Table 1. Clusterin as a potential biomarker in various types of cancer.

Types of cancer	Expression of CLU in vitro	Expression of CLU in vivo
Non-small cell lung	Non-small cell lung cancer cell lines show overexpression upon treatment with chemotherapy or radiotherapy. ASO therapy sensitizes cells to these treatments and decreases their metastatic potential [112]	In patients with positive CLU expression there is a better overall disease-free survival than in negative patients [113].
		More than 80% of the tumors are inmunoreactive for clusterin [113].
Gastric		Overexpression of sCLU correlates significantly with metastasis, tumor invasion and TNM stage. In addition, of with unfavorable survival for advanced-stage gastric cancers [114].
Ovarian		Overexpression of sCLU is inversely correlated with tumor apoptotic index and is more frequently detected in metastatic lesions than in primary tumors [115].
		Increased sCLU expression is associated with increased biological aggressiveness and decreased survival [116].
Endometrial		When clusterin is expressed in endometrial tumors, it is associated with a lower stage, supporting its role in the diagnosis of endometrial carcinoma [117].
		There has been detected higher mRNA expression in both neoplastic and hyperplastic tissues compared to normal endometrium. In this regard, an increase in mRNA expression of the specific sCLU isoform has been observed in neoplastic and hyperplastic endometrial diseases, but an increase in CLU protein was not detected. Furthermore, specific CLU immunoreactivity has been observed in all glandular cells of the endometrium compared to other cellular compartments where CLU immunoreactivity was lower or absent [118].
		Increased CLU expression enhances paclitaxel resistance in endometrial cancer [119,120].
Breast	Studies with the MDA-MB-231 cell line show how sCLU silencing significantly inhibits cell proliferation and drastically reduces cell invasion, cell progression and metastatic potential [121,122].	Atypical hyperplasias, intraductal carcinomas, and invasive carcinomas are characterized by clusterin overexpression, unlike benign lesions [123].
		Overexpression of sCLU is observed in a higher percentage of triple-negative breast cancer [124] and is associated with a negative estrogen and progesterone receptor status [123].
		Likewise, overexpression in the stroma tends to directly correlate with resistance to preoperative neoadjuvant chemotherapy in the primary tumor and inversely with the apoptosis rate,

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		indicating that gene expression may not be necessary for apoptotic cell death [123,125,126].
Colon		sCLU is overexpressed, while nCLU is downregulated [127]. Similarly, sCLU overexpression was mainly shown in the cytoplasm of highly infiltrative tumors and metastatic lymph nodes [128], suggesting that clusterin expression could help identify patients with more aggressive tumors who may benefit from targeted therapies [129].
Bladder	Treatment with the antisense oligonucleotide (ASO) targeting negative regulation of Bcl-2 and clusterin increases the sensitivity of partially resistant bladder carcinoma cells to the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [130].	The recurrence-free survival time of patients with overexpression of clusterin was shorter than that of patients with normal clusterin expression [131,132].
Hepatocellular		High levels of sCLU are associated with migration, invasion, and metastasis [133] due to increased MMP-2 expression and decreased E-cadherin expression [134].
		Furthermore, sCLU overexpression contributes to oxaliplatin resistance [135].
		In peripheral blood mononuclear cells (PBMC) from hepatocellular carcinoma patients, it has been proposed as a prospective detection biomarker along with other genes for its sensitivity and specificity [136]. The combination of CLU and AFP further improves diagnostic performance [137].
		The initial levels of clusterin are initially higher for patients with progressive disease than for those with partial or complete response, respectively [138].
Pancreatic	The inducer of ferroptosis, a type of cell death characterized by the accumulation of reactive oxygen species (ROS), interferes with apoptotic cell death by regulating clusterin. [139].	Clusterin expression in stages I and II is not significantly associated with apoptosis. Moreover, patients with positive clusterin expression present better survival rates [140].
Melanomas	Overexpression is associated with increased drug resistance and prolonged survival of tumor cells, while silencing reduces resistance and reduced survival of melanoma cells both in vitro and in vivo [141].	
Esophageal squamous cells		High CLU expression correlates with poor locoregional, overall, and distant progression-free survival. Moreover, patients with CLU overexpression in both epithelium and stroma have shorter survival times [142].
Head and neck	Overexpression of CLU has been observed, but its implications have not yet been determined [143].	Although is detected in a low proportion of laryngeal carcinomas, it seems to exert a significant role in local invasiveness [144].

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Anaplastic large cell lymphomas		The role of CLU is unknown, but its expression within this lymphoma type provides an additional marker for diagnosis [145].
		Clusterin expression is not related to anaplastic lymphoma kinase-1 (ALK-1) expression, and in reactive lymphoid tissues, only fibroblastic reticular cells and follicular dendritic cells show positive expression [145].
Osteosarcoma		sCLU overexpression is associated with metastasis and chemotherapy resistance [146].
Prostate	The expression of CLU increases in advanced stages of cancer, and its suppression sensitizes cells to chemotherapeutic drugs [147].	It has been observed that clusterin expression decreases considerably compared to benign tissues [147].
Renal	The introduction of the CLU gene enhances the metastatic potential of renal cell cancer [148], while the removal of the CLU gene inhibits growth and migration [149].	

However, further research is needed in this field to determine the exact value of CLU levels and their association with each type of cancer. Currently, it can only be used as a tool to assess risks [150].

As a therapeutic target, the inhibition of CLU has demonstrated excellent therapeutic effects in various cancers both in vitro and in vivo, prolonging the survival of patients. Custirsen (OGX-011), a second-generation antisense oligonucleotide, has proven effective by interacting with the ATG sequence in exon 2 of the secretory isoform of CLU (sCLU), inhibiting its translation and suppressing cancer progression such as prostate cancer [151], renal cell carcinoma [152], bladder [153], liver [154], lung [155], prostate [156], breast [157], lung adenocarcinoma [158], melanoma [159], osteosarcoma [160] and ovary [161].

When combined with other cancer therapies in clinical trials, OGX 011 enhances antitumor activity. In the case of renal cell carcinoma, CLU inhibition by custirsen improves sorafenib cytotoxicity [162]. In ovarian cancer, it has shown to improve the survival of patients treated with paclitaxel by enhancing its response [163], like what happens in castration-resistant prostate cancer with mitoxantrone and docetaxel by reducing sCLU expression [164,165]. Clinical studies have also explored the efficacy of custirsen in metastatic castration-resistant prostate cancer, showing promising results in early-phase trials, although the standard treatment remains prednisone and cabazitaxel [166].

In metastatic breast cancer, similar effects have been shown with combinations of OGX 011 and docetaxel [167], while in lung cancer, combinations with gemcitabine or cisplatin have extended survival [168].

In addition to OGX-011, RNA interference therapies, such as microRNA (miRNA), short hairpin RNA (shRNA), and small interfering RNA (siRNA), have proven effective in inhibiting CLU expression in various cancers. Both miRNA-217-5p and miRNA-195 have negatively regulated CLU expression, inhibiting cell migration and invasion and promoting apoptosis in prostate cancer in vitro [169]. Other therapeutic agents, such as melittin, green tea extract (GTE), apocynin, vitamin D, metformin, and verteporfin, have also shown antitumor activity by blocking CLU protein expression and, therefore, inhibiting cancer progression, as evidenced earlier [170–175].

In the realm of emerging therapies, the drug AB-16B5, a monoclonal antibody targeting sCLU, is being evaluated in a phase II clinical trial in combination with docetaxel in patients with metastatic non-small cell lung cancer [176].

5. Conclusions

CLU plays a crucial role in cancer progression by regulating processes such as programmed cell death, epithelial-mesenchymal transition (EMT), metastasis, and cell proliferation and growth through various signaling pathways. The complexity of its biological role lies in the presence of two alternatively spliced isoforms and the variable localization of their protein products inside and outside the cell. Despite the complexity associated with the functions of these isoforms, it has been observed that the coexistence of nCLU and sCLU in cells, along with the precise regulation of their balance, confers pro or anti-apoptotic properties.

Several potent clusterin inhibitors, such as OGX-011 and RNAi, have been developed for use in cancer therapies and have demonstrated positive therapeutic effects in patients. Given the various regulatory pathways of clusterin in cancer progression and the proven survival benefits of clusterin inhibition in different types of cancer, clusterin emerges as a promising therapeutic target for developing more effective agents and targeted therapies. Considering its carcinogenic properties, the sCLU isoform could be a blood biomarker for cancer diagnosis. However, despite numerous studies on the function of clusterin isoforms, it is not yet fully understood which of them contributes to specific biological effects. Therefore, it is crucial to explore in more detail the specific functional role of the clusterin isoform, especially in the case of sCLU, to address clusterin as a therapeutic target more precisely.

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