Chapter 6

Oncogenic Secretory Clusterin: A Promising Therapeutic Target for Hepatocellular Carcinoma

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Abstract

Oncogenic secretory clusterin (sCLU) is a stress-induced molecular chaperone that confers proliferative and survival advantages to hepatocellular carcinoma (HCC), plays a crucial role in cell proliferation, multiple drug resistance, metastasis, and tumor progression. However, the targeted effects and molecular mechanisms of sCLU for malignant tumor are still unknown. This chapter aims to review some progression of oncogenic sCLU as a promising therapeutic target for HCC. An English-language literature search was conducted using bibliographic databases on some valuable articles in focused review questions to analyze the interventions and findings of included studies using a conceptual framework. The positive rate of hepatic sCLU expression in cancerous tissues was significantly higher more than that in their surrounding non-cancerous ones at gene transcription level or at protein level, with increasing according to tumor-node-metastasis (TNM) staging. Abnormal expression of oncogenic sCLU associated with poor differentiation degree and TNM stage of HCC also has been considered as a valuable diagnostic or independent prognostic biomarker for HCC. Furthermore, silencing sCLU at mRNA level by specific shRNA or inhibition by OGX-011 suppressed the colony formation and proliferation of tumor cells with apoptosis increasing, cell cycle arrested, alterations of cell migration and invasion behaviors, decreasing phosphorylation level of Akt and GSK-3β in vitro, and significantly suppressing the xenograft tumor growth with decreasing expression of β-catenin, p-GSK3β, and cyclinD1 in vivo. The oncogenic sCLU expression was closely associated with tumor progression, and it should be a novel potential molecular-targeted therapy for HCC.

Keywords: hepatocellular carcinoma, secretory clusterin, targeted therapy

1. Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide [1]. Growing understanding of the multiple pathogenic factors including hepatitis B or C virus (HBV or HCV) infection, toxic, lipid accumulation, aflatoxin B1 intake, and so on with complex molecular mechanisms underlying HCC reveal that hepatocarcinogenesis is a multistep process including lots of activated or suppressed oncogenes or anticancer genes [2–4]. Some techniques for HCC therapy have experienced great progress. However, the prognosis of HCC patients is still very poor due to the high rates of tumor recurrence and metastasis. Effective therapy of HCC is dependent on early specific diagnosis, therefore, to provide optimal treatment for patients, more precise and effective markers are urgently needed in all phases of management from early detection to staging, treatment monitoring, and prognosis [5–7]. Numerous studies have shown the clinical utility of novel blood-based markers, such as circulating tumor cells, key signal molecules, long non-coding RNA, and microRNA with great potential for HCC [8, 9].

Molecular chaperones are proteins that response to cellular stresses including genotoxic agents, nutrient starvation, and heat shock, with cellular stresses-induced protein misfolding, aggregation, and denaturation [10, 11]. To date, only few specific markers such as hepatoma-specific γ-glutamyl transferase [12, 13], oncofetal antigen glypican-3 (GPC-3) [14, 15], hepatoma-specific alpha-fetoprotein (HS-AFP or AFP-L3) [16], member 3a of Wingless-type MMTV integration site family (Wnt3a) [17, 18], and molecular chaperones like heat shock proteins (Hsp27 or Hsp90) [19] and clusterin have been developed as valuable biomarkers for primary hepatocellular carcinoma (PHC) diagnosis and surveillance. The clusterin (CLU) that was first detected in HCC tissues by Tobe et al., who found that SP40-40 gene in hepatoma cells was located in human chromosome 8, also designated as apolipoprotein J (APOJ), SP-40, sulfated glycoprotein 2 (SGP2), and testosterone-repressed prostate message 2 (TRPM2) [20]. Following the detection of their complete cDNA cloning, sequencing and comparison, secretory CLU (sCLU) is found to be the mature isoform of cytoplasm endoplasmic reticulum (ER)-Golgi CLU, which is over-expressed in a wide variety of tumors with oncogenicity [21, 22]. Recently, the mechanisms of abnormal sCLU expression and its targeted effects for HCC have been explored [23, 24]. This article summarizes some progression of sCLU as a promising target for HCC gene therapy.

2. Gene structure and functions of sCLU

Human CLU gene is a single-copy gene on chromosome 8p21-p12 including 9 exons and 8 introns, encoding an mRNA of 2877 bp and translating to a polypeptide with 449 amino acids (a.a.) [25]. The secretory glycoprotein (1st a.a.) is a signal sequence of hydrophobic leader, and targets the ER protein. CLU gene encodes two isoforms with distinct functions as a result of alternative splicing and post-translational modifications: cytoplasm sCLU (75–80 kDa) and nuclear CLU (nCLU, 55 kDa), which is mainly located in the nucleus. The sCLU molecule is a

highly conserved heterodimeric disulfide-linked 449 amino acid polypeptide that represents the major product of CLU gene. The ER-Golgi sCLU is considered to influence immune regulation, transformation, tissue remodeling, lipid transport, membrane recycling, complements cascade, DNA repair, cell adhesion, and cell-cell interactions, indicated that sCLU is widely distributed in tissues and body fluids involved in various physiological processes [11, 26, 27].

Multiple reports have shown that the cytoplasm sCLU is cytoprotective and anti-apoptotic [28, 29], whereas nCLU protein is proapoptotic. Abnormal oncogenic sCLU expression was reported to correlate closely with HCC progression, such as inducing epithelial-mesenchymal transition (EMT) [30], formation of multiple drug resistance (MDR) [31], distal metastasis of tumor cells, and malignant transformation of hepatocytes, interaction with oncogenes or suppressor genes, and related signal pathways (**Figure 1**) [32, 33]. Because of ischemic or hypoxic microenvironment existence in cancerous tissues, the sCLU are often adaptively over-expressed and closely related with increased tumorigenicity, metastatic potential, and MDR to chemotherapy. As a stress-induced chaperone that inhibits protein misfolding and aggregation in a manner similar to small heat shock proteins (HSPs) [34, 35], its promoter

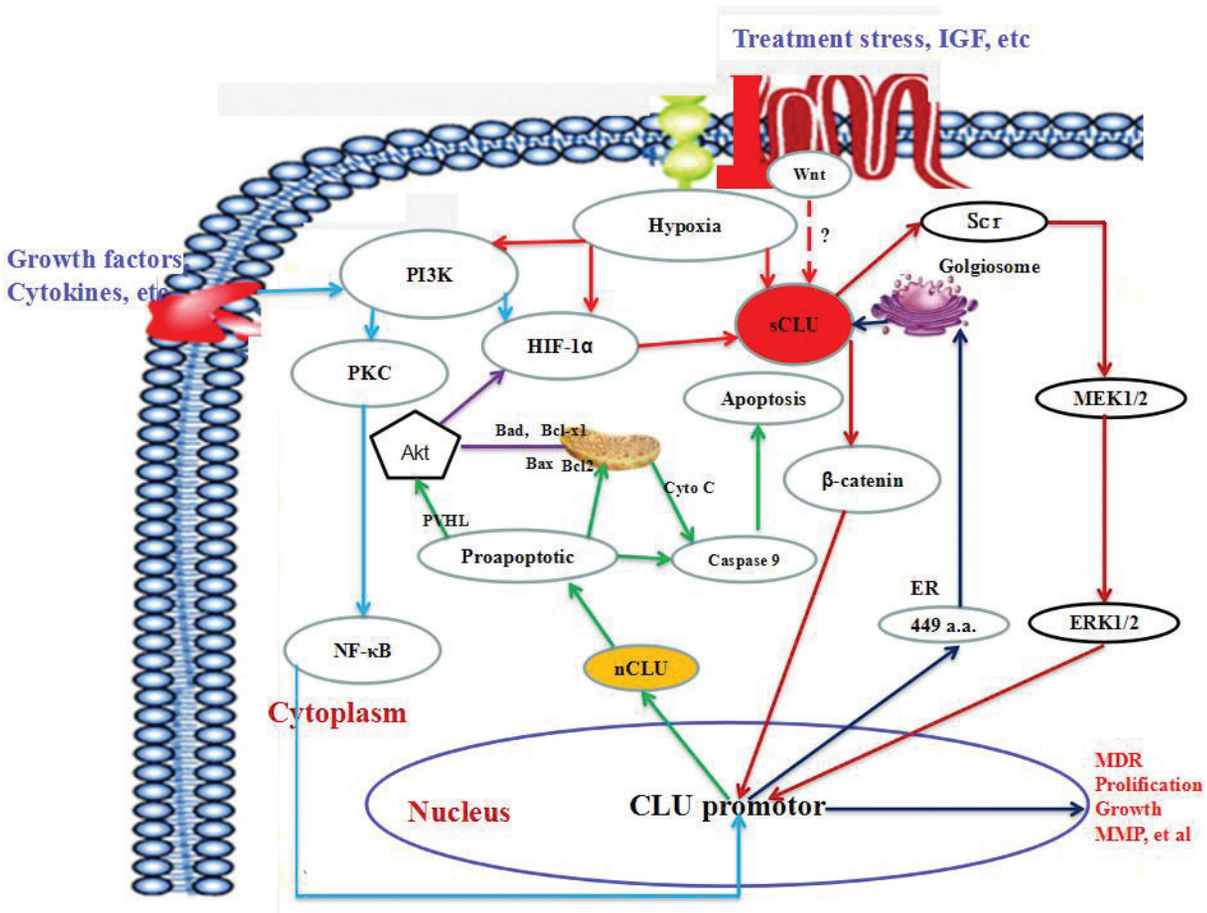

Figure 1. Potential mechanisms of hepatic sCLU in hepatocellular carcinoma. Cyto C, cytochrome c; ER, endoplasmic reticulum; Gene Reg., gene regulation; HIF-1α, hypoxia inducible factor-1α; IGF, insulin-like factor; MDR, multiple drug resistance; MMP, matrix metalloproteinase; nCLU, nuclear clusterin; NF-κB, nuclear factor-κB; PKC, protein kinase C; PI3K, phosphatidylinositol 3-kinase; Scr, sarcoma gene; sCLU, secretory clusterin.

region contains an element recognized by heat shock factor 1 (HSF-1) [36]. Cytoplasm sCLU-inhibited apoptosis by interacting with activated Bax, and protects HCC cells from ER stress-induced apoptosis through a physical interaction with glucose-regulated prptein78 (GRP78) [29, 37].

Hepatic sCLU has been confirmed that it was physically associated with eukaryotic translation initiation factor 3 subunit I (EIF3I), and might protect EIF3I protein from degradation. A positive correlation was founded between sCLU and EIF3I, and both of their functions might be as a cooperative unit in HCC. The levels of sCLU and EIF3I expression were investigated in HCC using tissue microarray (TMA) and the patients with high EIF3I level exhibited poor prognosis. After silenced EIF3I, Akt phosphorylation was significantly inhibited. The EIF3I-Akt complex could prevent PP2A-mediated dephosphorylation, which in turn led to a constitutive Akt signal activation, suggesting that the CLU-EIF3I complex might prevent EIF3I degradation, and then contribute to Akt upregulation [33, 38].

3. Biological behaviors of sCLU expression in cancerous tissues

Although great efforts have been made to explore molecular mechanism of HCC invasion and metastasis in the past decade [39–41], the mechanism of HCC remains incompletely understood. The alterations of sCLU expression at messenger RNA (mRNA) or protein level were investigated in HCC- and their non-tumorous tissues (NT) with self-control [42]. The overall level of sCLU mRNA in the HCC group was 75% up-regulated, 7.5% down-regulated, and 17.5% non-changed. Although no significant difference of the sCLU mRNA level at staging I was found between NT and HCC, they were drastically up-regulated expression from staging II to IV. The staining of sCLU mainly presented in the cytoplasm at protein level in HCC and their NT tissues were analyzed by tissue microarray (TMA) with immunohistochemistry (IHC). Its incidence in the HCC group (73.3%), with 37.5, 68 and 88.9% at staging I, II and III & IV, was significantly higher than that in the NT group (23.3%), respectively. The levels of sCLU protein consistent with their mRNA expression were gradually upregulation with increasing HCC staging [42, 43], indicated that high sCLU should be a valuable biomarker to distinguish malignant from benign liver nodular lesions [44].

The sCLU as a functional homolog of HSPs is a stress-induced chaperone that confers proliferative and closely associates with poor prognosis of HCC. Recurrence and metastasis are the most causes of poor prognosis of HCC. Clinicopathological features of sCLU revealed that its high expression was significantly linked to poor differentiation and advanced TNM stage [45]. There was a trend toward a poorer overall survival in HCC with high sCLU expression. Besides, survival time of HCC with high TNM stage was significantly shorter than that of cases with low stage. Moreover, in the subset of HCC patients with III and IV stage, high sCLU expression was prone to result in a shorter survival time (**Table 1**). There is a closely positive correlation between abnormal sCLU expression and HCC. High sCLU expression has more invasive phenotype for HCC [46]. The upregulation of sCLU is associated with HCC progression by contributing to angiogenesis, chemoresistance, cells survival, and metastasis.

Group		n	Pos. n (%)	χ^2 value	*P* value
AFP (μg /L)	≤50	37	31 (83.78)	1.733	0.118
	>50	38	27 (71.05)		
Portal vein invasion	With	7	6 (85.71)	0.309	0.578
	Without	68	52 (76.47)		
HBsAg	Negative	46	36 (78.26)	0.058	0.809
	Positive	29	22 (75.86)		
Tumor size	≤5 cm	45	33 (73.33)	1.027	0.311
	>5 cm	30	25 (83.33)		
Liver cirrhosis	With	57	45 (78.95)	0.353	0.552
	Without	18	13 (72.22)		
Lymph node metastasis	With	23	22 (95.65)	6.351	0.012*
	Without	52	36 (69.23)		
Differentiation	Well & moderate	58	43 (74.14)	1.491	0.222
	Poor	17	15 (88.24)		
Gross classification	Unifocal	62	46 (74.19)	1.744	0.187
	Multifocal	13	12 (92.31)		
TNM	I & II	45	30 (66.67)	7.683	0.021*
	III & IV	30	28 (93.33)		
Child degree	A	44	30 (68.18)	5.086	0.024*
	B&C	31	29 (90.32)		
5 years' survival	No	51	43 (84.31)	4.430	0.035*
	Yes	24	15 (62.50)		

Pos. n (%), positive number (%). *P<0.05

Table 1. Clinicopathological features of hepatic sCLU expression in HCC.

Growing evidences showed that sCLU with molecular chaperones played an important role in MDR formation, cells proliferation, metastasis of HCC [47]. Furthermore, univariate and multivariate analyses indicated that sCLU might be an independent prognostic indicator, in line with the factor of lymph node metastasis.

4. Circulating sCLU as diagnostic marker

The observations were in accordance with the early literature showing that upregulation of sCLU-positive expression might be associated with poor clinical outcome in HCC

patients [21, 48]. According to previous clinical studies, average serum sCLU level was significantly higher in the HCC group more than that in any of groups with cirrhosis, chronic hepatitis, or healthy control (**Table 2**). The area under receiver operating characteristic (ROC) curve and diagnostic sensitivity were 0.75 and 74.7% in serum sCLU, and 0.74 and 58.7% in serum AFP, respectively. The incidence of both combining detection rose up to 90.7% for HCC diagnosis (**Table 3**). High-circulating sCLU levels were observed in HCC patients, consistent with a recent study using a three-step serum proteome analysis, which showed that serum sCLU levels in HCC were significantly higher than those in benign liver diseases [42].

CLU is related to reverse cholesterol transport, platelet degranulation and human immune response pathways [49]. Protein-protein interaction analysis and pathway assessment showed a closed molecular relationship between cirrhosis and HCC [50]. Serum samples collected from HCCLM3-R metastatic HCC tumor model at specific stages of metastasis (1., 3 and 6 weeks) were subjected to iTRAQ labeling followed by 2DLC-ESI-MS/MS analysis. Circulating sCLU was significantly up-regulated during cancer progression and metastasis. The expression of sCLU was significantly higher in metastatic HCC cells and samples from HCC patients. Serum sCLU was highly increased with tumor size, tumor number, lymph node infiltration (**Table 4**) [42], and showed that the ROC area under curve value was 0.95 in sCLU more than that (0.85) in AFP. If 128 μg/mL as a cutoff value, the sensitivity or specificity of serum sCLU level for predicting HCC was 90 or 87%, respectively. The data indicated that circulating sCLU is a promising molecular marker of diagnosis or predicting metastasis for HCC [22, 51, 52].

Group	n	Mean ± SD	Positive n (%)	χ^2 value	*P* value
sCLU		**μg/mL**			
HCC	75	119.21 ± 16.67	56 (74.67)		
LC	30	97.78 ± 19.06	8 (26.67)**	20.744	<0.001
CH	30	106.30 ± 19.22	12 (40.00)**	11.285	<0.001
NC	36	89.96 ± 7.27	0 (0.00)**	54.249	<0.001
AFP		**ng/mL**			
HCC	75	2177.32 ± 3757.99	44 (58.67)		
LC	30	126.84 ± 244.76	10 (33.33)*	5.505	0.019
CH	30	30.27 ± 50.09	5 (16.67)**	15.188	<0.001
NC	36	7.1 ± 3.50	1 (2.78)**	31.520	<0.001

Serum sCLU values >104.0 μg/mL or AFP values >50 ng/mL were considered positive.

sCLU, secretory clusterin; LC, liver cirrhosis; CH, chronic hepatitis; NC, normal control; and AFP, α-fetoprotein.

*$P < 0.05$, compared with HCC group.

**$P < 0.01$, compared with HCC group.

Table 2. Serum sCLU or AFP levels among patients with chronic liver diseases and comparative analysis of both diagnostic values for HCC.

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	sCLU level > 104.2 μg/mL (%)	AFP level > 50 ng/mL (%)	Both (%)
Sensitivity (%)	74.67	58.67	90.67
Specificity (%)	66.67	75.00	60.00
Accuracy (%)	71.11	65.93	77.03
Positive predictive value (%)	73.68	74.58	73.91
Negative predictive value (%)	67.80	59.21	83.72

AFP, α-fetoprotein; sCLU, secretory clusterin.

Table 3. Assessment diagnostic validity of serum sCLU or AFP level for HCC.

Group	n	Average (μg/mL)	*t* value	*P* value	Positive, n (%)	χ^2 value	*P* value
Age (years)							
≤60	47	118.09 ± 16.45	0.751	0.455	33 (70.21)	1.320	0.251
>60	28	121.09 ± 17.16			23 (82.14)		
AFP (μg/L)							
≤50	31	120.15 ± 17.12	0.405	0.686	24 (77.42)	0.212	0.645
>50	44	118.55 ± 16.50			32 (72.73)		
Portal vein invasion							
With	28	119.34 ± 14.52	0.050	0.960	22 (78.57)	0.360	0.548
Without	47	119.14 ± 17.97			34 (72.34)		
Tumor size							
≤5 cm	34	114.63 ± 18.10	2.221	0.029[a]	21 (61.76)	5.473	0.019
>5 cm	41	123.00 ± 14.52			35 (85.37)		
Lymph node metastasis							
Without	44	114.84 ± 15.21	2.826	0.006[b]	29 (65.91)	4.316	0.038
With	31	125.40 ± 16.91			27 (87.10)		
Differentiation							
Well & moderate	57	117.43 ± 16.34	1.662	0.101	42 (73.68)	0.121	0.728
Poor	18	124.84 ± 16.90			14 (77.78)		
Gross classification							
Unifocal	36	114.02 ± 18.57	2.698	0.009[c]	23 (63.89)	4.251	0.039
Multifocal	39	124.00 ± 13.20			33 (84.62)		

Group	n	Average (μg/mL)	*t* value	*P* value	Positive, n (%)	χ^2 value	*P* value
TNM stage							
I & II	31	113.93 ± 14.36	2.375 0.02[d]		20 (64.52)	2.878	0.09
III & IV	44	122.93 ± 17.31			36 (81.82)		
Child classification							
A	55	117.60 ± 14.56	1.395	0.167	40 (72.73)	0.410	0.522
B&C	20	123.63 ± 21.23			16 (80.00)		

AFP, α-fetoprotein; TNM, tumor node metastasis.
[a]With the tumor size ≥5 cm group.
[b]The lymph node metastasis group.
[c]With the multifocal group.
[d]With TNM III & IV group.

Table 4. Clinicopathologic features of serum sCLU expression in HCC patients (Mean ± SD).

5. Application prospect of targeting CLU gene

5.1. Antisense oligonucleotide (ASO) therapy

ASO is a useful technique to inhibit specific-targeted CLU genes, with a small synthetic natural nucleic acid analogue, that can complementary to CLU mRNA that induce degradation or inhibit translation into protein [52]. It is considered to be a potent inhibitor of sCLU expression in vitro, in vivo, and in human clinical trials, with no apparent effect on the expression of nCLU. Custirsen (OGX-011, 5′-CAGCAGCA GAGTCTTCATCAT-3′, 50 nM) is a novel 2′-methoxy-ethyl-modified phosphorothioate ASO, which is a 21-nucleoside complement to target the translation initiation site of CLU gene exon II mRNA translation initiation site with one CpG motif [53]. Hence, OGX-011 plays the role of chemosensitization by influencing the anti-apoptotic protein sCLU instead of the proapoptotic protein nCLU. Xiu et al. have reviewed the current state of research on clusterin, to predict future research directions and to analyze the potential of the clinical application of custirsen in HCC [54]. However, the median overall survival of HCC cases were ms 23.4 mo in the group of the OGX-011 combining anti-cancer drugs (docetaxel/prednisone) and ms 22.2 mo in the other group of cases with docetaxel/prednisone alone. No significant difference was found between two groups. Some potential key factors might contribute to its results, and still want to do more clinical trials to be ongoing [55].

5.2. Reversal MDR by specific shRNA-targeted sCLU

Resistance of tumor cells to chemotherapy continues to be a major clinical obstacle to extend the survival rate of patients with HCC. Recently, one of the major strategies for liver cancer is surgical resection with adjuvant anti-HCC drug chemotherapy [10]. However, the HCC patients always tend to acquire MDR during tumor progression. MDR-related P-glycoprotein (P-gp), encoded by MDR1 gene is positively linked closely with chronic liver diseases, because of its

drug efflux via ATP-binding cassette (ABC) family transporters, which can decrease intracellular concentration of anti-HCC drugs. Targeting sCLU to sensitize cancer cells to chemotherapy has become an attractive new strategy for cancer treatment. Previous studies found sCLU expressed in line with P-gp via immunohistochemical staining. The sCLU expression was analyzed in human hepatoma cells and chemoresistant counter-part HepG2/ADM cells. After transfection of shRNA-1 (5′-GTAAGTACGTC AATAAGGA-3′) into HepG2/ADM cells, the inhibition of CLU expression was 73.68% at mRNA level with obvious enhancement in chemosensitivity, and increasing apoptosis induced by doxorubicin [56]. Knockdown CLU also significantly decreased the drug efflux pump activity through the depression of MDR1/P-gp. Moreover, silencing CLU led to downregulation of β-catenin, suggesting that downregulation of CLU might be a key point to reverse MDR of HepG2/ADM cells [57].

5.3. Blockade-related pathway

5.3.1. PI3K/Akt/NF-κB pathway

Previous data revealed that CLU promoted cell survival through the PI3K/Akt pathway and induced MMP-9 expression via ERK1/2 and PI3K/Akt/NF-κB pathways [32]. CLU could increase p-Akt and MMP13 expression. A positive correlation between CLU expression and p-Akt level was observed in cohort of HCC tissues. Where CLU knockdown using OGX-011 significantly decreased p-Akt and MMP13 levels and suppressed HCC metastasis in two metastatic models through inhibiting EIF3I/Akt/MMP13 signaling. The related signaling molecule blockade of the PI3K-Akt pathway could significantly inhibited MMP13 expression in human HepG2-CLU or HCCLM3 cells [38, 58]. Decreased level of CLU accompanied with downregulation of MMP13 and p-Akt was observed in tumors derived from HCCLM3-shCLU group, revealed that p-Akt level was significantly correlated with poor prognosis and indicated that CLU may play a crucial role in HCC metastasis [38].

5.3.2. Wnt/β-catenin pathway

Wnt canonical pathway is often constitutively active in neoplastic cells, although, normally β-catenin is negatively regulated by GSK-3β that phosphorylates β-catenin to drive it for proteosomal degradation [30, 59]. Previous data emphasized sCLU modification after being exposed to Wnt/β-catenin inhibitor, and expressions of the crucial β-catenin and GSK-3β genes were detected in cases of sCLU depletion. The data indicated that sCLU suppression might lead to the inhibition of Wnt/β-catenin pathway in reverse. Hence, sCLU might play an important role in chemoresistance of HepG2/ADM cells together with Wnt/β-catenin signaling molecules [31, 56].

5.3.3. IGF-1/IGF-1R/Src/Mek/Erk pathway

Hepatic sCLU is a general genotoxic stress-induced, prosurvival gene product implicated in cancer [36, 37]. The regulatory signal transduction processes that control sCLU expression, the induction of sCLU is delayed, peaking 72 h after low doses of ionizing radiation, and is dependent on up-regulating IGF-1 and phosphorylation-dependent IGF-1R activation [23, 27] that stimulates the downstream Src-Mek-Erk signal transduction cascade to ultimately

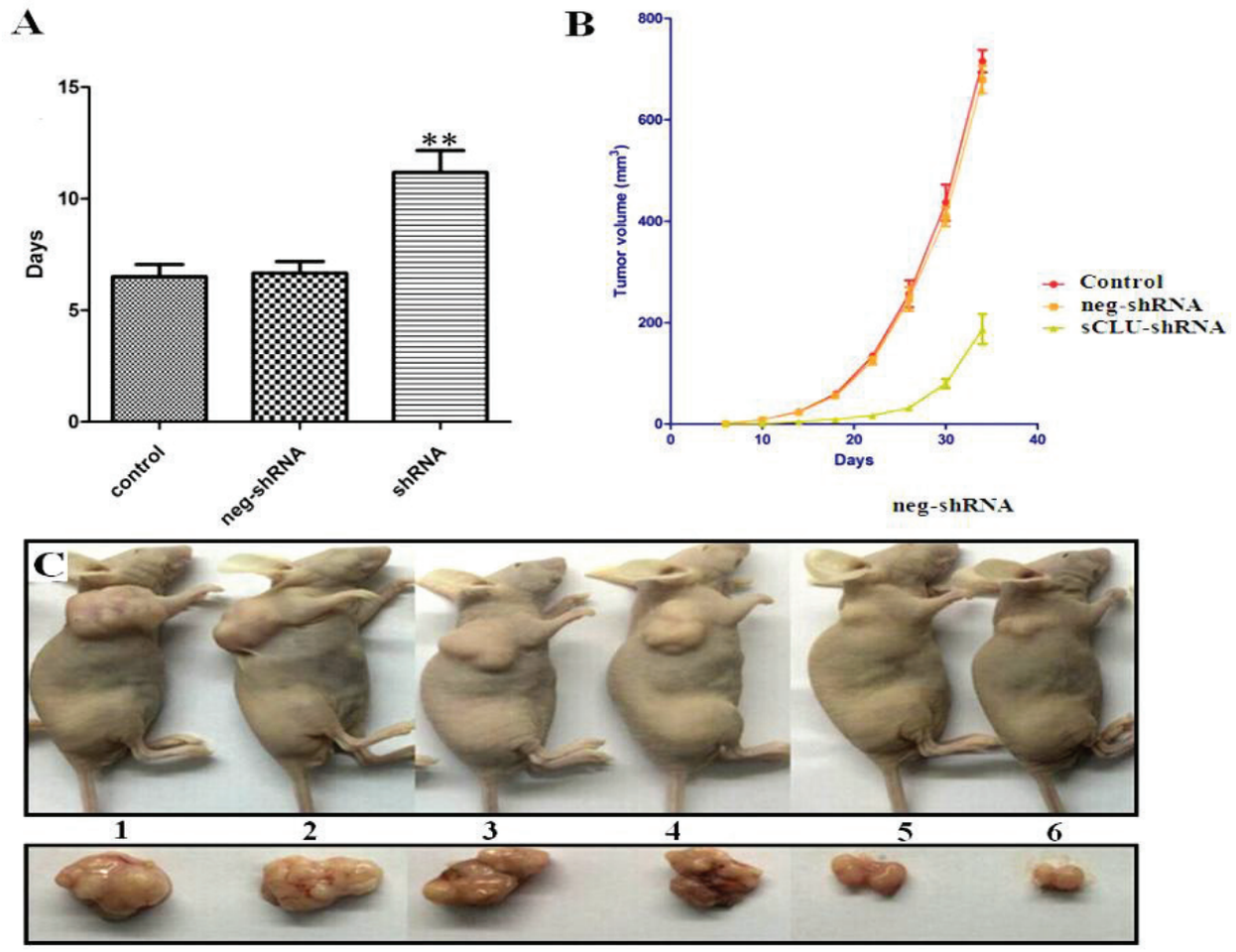

Figure 2. Inhibition of sCLU gene transcription by specific shRNA-1 on effects of forming time, growth curve and size of orthotopic xenograft tumors. The control, neg-shRNA, or sCLU-shRNA groups were injected with the plasmid contained specific shRNA-1 into nude mice. (A) The forming time of the orthotopic xenograft tumors from each group. (B) The growth curves of the xenograft tumors from each group. And the tumor volumes were measured at the indicated time point. (C) The representative photographs of the nude mice and corresponding dissected tumors from each group. 1 and 2, the xenografts in the control group; 3 and 4, the xenografts in the neg-shRNA group; 5 and 6, the xenografts in the sCLU-shRNA group. The data are presented as the mean ± SD. Compared with the control group, $^{**}P < 0.01$.

transactivate the early growth response-1 (Egr-1) transcription factor. Thus, the ionizing radiation exposure causes stress-induced IGF-1R-Src-Mek-Erk-Egr-1 activation that regulates the sCLU prosurvival cascade pathway for radiation resistance in HCC therapy [60].

5.4. Suppressed HCC growth in vivo by silencing sCLU

The inhibition of sCLU gene transcription by specific shRNA-1 on effects of forming time, growth curve, and size of orthotopic xenograft tumors after sacrifice of the mice at the 34th day with injection are shown in **Figure 2**. The mean weight of the xenograft tumors in the shRNA-1 group was significantly less than that of the control or NC-shRNA group, respectively. The curves of xenograft tumor growth indicated that tumor sizes in the shRNA-1 group with lower mRNA level were significantly smaller less than those of the control or NC-shRNA group [31, 46]. Consistently, the sCLU protein expression in the shRNA-1 group was also

lower than that in the control or the NC-shRNA group; and the sCLU staining in the control or NC-shRNA group was stronger than that in the shRNA-1 group by immunohistochemistry [46]. Specific shRNA-mediated downregulation of sCLU resulted in a reduced migratory capacity in HCC cell lines, as well as a reduction in pulmonary metastasis *in vivo* [38]. Overexpression of sCLU in HepG2 cell line showed increased cell migratory ability. In addition, sCLU also plays an important role in the regulation of TGF-β1-smad3 signaling pathway, suggested that oncogenic sCLU might promote HCC metastasis via the induction of EMT process and could be a promising candidate target for HCC therapy [32, 61–63].

6. Conclusions

In conclusion, the upregulation of sCLU expression at early staging of HCC is considered to promote tumor development, which may be related to the phosphorylation of AKT/GSK-3β. An increasing number of reports have provided evidence that sCLU level could be a novel biomarker for HCC diagnosis and prognosis, and there will be of great significance for the individualized treatment in HCC patients. The sCLU regulating signaling pathways could be critical to unraveling the solution for MDR in HCC. Therefore, silencing sCLU gene transcription and inhibiting sCLU expression by specific Custirsen inhibition have provided a new mechanism insight into molecular-targeted therapy for HCC in injected- or orthotopic model, indicated that sCLU gene would be a potential molecular-targeted for HCC therapy. Further study found that sCLU contributed to HCC migration and EMT *in vitro*, and metastasis *in vivo*. Although additional preclinical and clinical trials are necessary to explore the sCLU role in HCC, targeting the oncogenic sCLU could validate the approach as a systemic therapy to increase chemotherapy sensitivity.

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Abbreviations

AFP alpha-fetoprotein

HCC hepatocellular carcinoma

IHC immunohistochemistry

MDR multiple drug resistance

miRNA	microRNA
sCLU	secretory clusterin
shRNA	short hairpin RNA
TMA	tissue microarray
VEGF	vascular endothelial growth factor
Wnt	Wnt/β-catenin signaling pathway

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