Review Article

# Role of secretory clusterin in hepatocarcinogenesis

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**Abstract:** Secretory clusterin (sCLU) is a small stress-induced cytoprotective chaperone protein. Its biological functions are similar to those of a heat-shock protein. The sCLU plays a crucial role in cell proliferation, multiple drug resistance, metastasis, and tumor progression. Abnormal sCLU expression in tumor tissues or sera of patients with primary hepatic cancer has been considered a useful biomarker for diagnosis and surveillance. However, the exact relationship between sCLU overexpression and malignant transformation of hepatocytes is still unknown. The present review examines some novel advances of the knowledge about the oncogenic role of sCLU in hepatocarcinogenesis.

**Keywords:** Hepatocellular carcinoma (HCC); secretory clusterin (sCLU); hepatocarcinogenesis

Received: 18 May 2018; Accepted: 30 July 2018; Published: 01 August 2018.
doi: 10.21037/tgh.2018.07.13
**View this article at:** http://dx.doi.org/10.21037/tgh.2018.07.13

## Introduction

The death of patients with primary hepatic carcinoma (PHC) is one of the most common cancer-related deaths in the world (1). The multiple etiopathogenic factors involved include chronic hepatitis B or C virus (HBV or HCV) infection (2,3), nonalcoholic steatohepatitis, aflatoxin B1 intake (4,5), that induce the activation of oncogenes and the inhibition of oncosuppressor genes (6). PHC prognosis strongly depends on early diagnosis and effectiveness of treatment. However, although great progress of PHC therapy has been made, patients' prognosis remains very poor because of postoperative relapse or metastases. Therefore, the knowledge of PHC markers, to evaluate clinical staging or monitor metastasis (7), is essential to improve the patients' treatment. Recent studies have shown the usefulness of the clinical application of biomarkers such as the evaluation of the presence of circulating tumor cells, as well as of the expression of key signal molecules, long non-coding RNA, and microRNAs involved in PHC pathogenesis (8).

Clusterin (CLU) is considered a molecular chaperone that can be produced under the perturbation of many physical and chemical factors (9). CLU was first described in human liver cancer by Tobe *et al.* (10), that provided evidence that the gene (that they named *CLI* gene) encoding the glycoprotein SP-40,40 (also named apolipoprotein J, sulfated glycoprotein 2, SP-40, or testosterone-repressed prostate message 29) is located in the chromosome 8. Two CLU protein isoforms are present in human cells: a nuclear form (nCLU), proapoptotic, and a secretory form (sCLU) that is prosurvival (11). The predominant sCLU expression locates in the endoplasmic reticulum of certain tumor cells. Recently, circulating sCLU has been shown to be a valuable marker for diagnosis and surveillance of PHC (12,13). However, the exact relationship between abnormal sCLU expression and malignant transformation of hepatocytes is still largely unknown. This paper reviews the new advances of the knowledge of the relationships between sCLU and the malignant transformation of hepatocytes.

## CLU structure and functions

The human *CLU* gene, constituted by 9 exons and 8 introns, is located on chromosome 8p21-p12. It encodes a 2,877 bp mRNA that is translated into a 449-amino acids polypeptide (14). Two CLU isoforms are known: the cytoplasmic sCLU (75~80 kDa) and the truncated nuclear nCLU (55 kDa). The sCLU, the major product of the *CLU* gene, is a highly conserved heterodimeric disulfide-linked polypeptide, widely present in human tissues or body fluids. It plays important roles in various physiological processes as well as in many pathological disturbance states. These include immune regulation, ageing, tissue remodeling, lipid transport, membrane recycling, complements cascade, DNA repair, cell adhesion, and cell-cell interactions, cancer progression, vascular damage, diabetes, kidney and neuron degeneration (15,16).

sCLU is also implicated in the, epithelial-mesenchymal transition (17), malignant transformation of hepatocytes and induction of metastasis. It interacts with oncogenes or suppressor genes, and related signal pathways, and is implicated in multiple drug resistance (MDR) (18,19). Hepatic sCLU is often adaptively overexpressed in hypoxic microenvironment and this contributes to increase tumorigenicity, metastatic potential, and MDR (20). Furthermore, sCLU could inhibit cell apoptosis induced by activated Bax, or protect liver cancer cells from apoptosis induced by endoplasmic reticulum stress, by interacting with the glucose-regulated apoptogenic protein 78 (21,22). sCLU may also interfere with the AKT signaling. Indeed, sCLU by forming a complex with EIF3I (eukaryotic translation initiation factor 3 subunit I) protein, prevents its degradation, thus contributing to the up-regulation of EIF3I/AKT/MMP13 signaling in hepatocellular carcinoma (HCC) (23).

## Alteration of sCLU in hepatocarcinogenesis

Studies on hepatocarcinogenesis, induced in Wistar rats by chemical carcinogens, showed that differences in the expression of blood and liver sCLU could represent specific markers of liver cancer (24). Indeed, circulating and liver sCLU concentrations gradually increase during hepatocarcinogenesis. Furthermore, immunohistochemical determination showed sCLU positivity in hepatocytes four weeks after initiation, that gradually increase around the portal area, at the 8th week, and reached its maximum in liver parenchyma at the 21st week. These findings suggest that sCLU plays a role during liver carcinogenesis (25,26).

As yet, the pathogenesis of PHC has not been fully elucidated. Chronic inflammation and persistent HBV or HCV infection should be implicated in the transformation of liver stem cells (LSC) to cancer stem cells (CSC). Although the pathogenetic role of hepatic sCLU activation in PHC has not yet been fully elucidated, the abnormal sCLU expression could be useful for early diagnosis or could be considered for targeted therapy (27). Also, CLU plays a key role in maintaining the integrity of endoplasmic reticulum during drug-induced stress and drug resistance mechanism of CSC. Hence, down-regulation or suppression of *CLU* gene transcription could significantly alter MDR of liver cancer cells (28).

## Tissues sCLU in human PHC

Great efforts have been made in the past decade to explore the mechanisms of PHC invasiveness and metastasis. The alterations of liver sCLU expression at mRNA or protein level were investigated in PHC and non-tumor surrounding tissue (13). No significant difference of the sCLU mRNA level was found between patients with stage I PHC and the non-tumor controls, but drastic sCLU up-regulation occurred in patients with PHC from staging II to IV. Immunohistochemistry showed sCLU positivity in the cytoplasm of both PHC and non-tumor tissue. However, the positivity in the PHC group, amounting to 73.3% at stage I, 37.5% at stage II, 68% at stage III, and 88.9% at stage IV, was significantly higher than that in the non-tumor group (23.3%) (29,30). Growing evidence indicates that sCLU plays an important role, as a molecular chaperone, in PHC cell proliferation and metastasis formation. The level of circulating sCLU mRNA and protein gradually increased with the PHC clinical stage. These findings strongly suggest that sCLU expression could be a valuable biomarker to distinguish malignant from benign liver nodular lesions (31,32).

## Circulating sCLU expression

The evaluation of the presence of sCLU in PHC patents' serum has shown that high level of circulating sCLU is associated with poor prognosis (33,34). These studies revealed an average serum sCLU level significantly higher in the PHC group than in liver cirrhosis, chronic hepatitis, and normal controls. The receiver operating characteristic (ROC) curve showed that a serum CLU value of 50 μg/

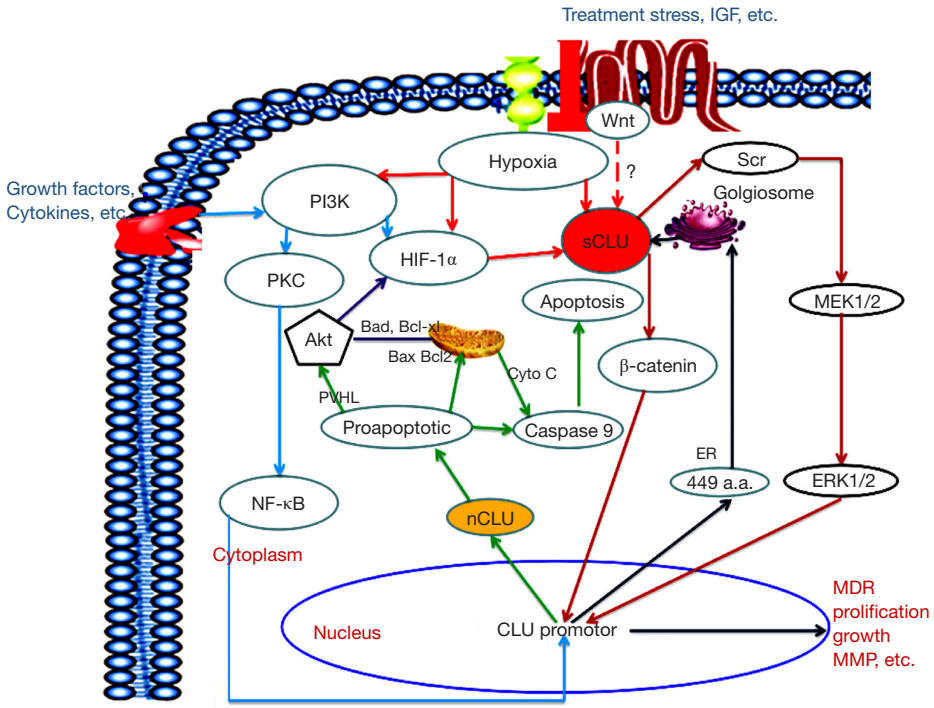


**Figure 1** Potential mechanisms of sCLU in hepatocarcinogenesis. Cyto C, cytochrome c; ER, endoplasmic reticulum; HIF-1α, hypoxia inducible factor-1α; IGF, insulin-like factor; MDR, multiple drug resistance; MMP, matrix metalloproteinase; nCLU, nuclear clusterin; NF-κB, nuclear factor-κB; PKC, protein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

mL produced the best sensitivity (91%) and specificity (83%) for differentiating HCC patients with HBV-related cirrhosis from those with HBV-related cirrhosis alone (33). A comparison with alpha-fetoprotein (AFP), as a PHC marker, showed that serum CLU is more sensitive and specific than serum AFP for differentiating HCC from cirrhosis patients (33). The clinicopathological features of PHC patients with higher sCLU levels in sera indicate a high increase in the serum sCLU in patients with poorly differentiated tumors, portal vein invasion and lymph node infiltration (34). The sCLU is associated with greater ROC area under the curve (0.95) than AFP (0.85) (34). If the cutoff value is 128 μg/mL as a limit, serum sCLU level represents a useful marker for PHC diagnosis with high sensitivity (90%) and specificity (87%) (34). This indicates that the abnormal sCLU level is a useful molecular marker for PHC diagnosis (12,13). Analogous conclusions were reached when sCLU linked to Datura stramonium lectin (DSL; DSL reactive sCLU) was considered (35), indicating that the circulating glycosylated sCLU levels represent a more sensitive PHC marker than AFP.

## Conclusions

In conclusion, the up-regulation of sCLU expression is considered to promote PHC incidence, this could be related to the upregulation of different signaling pathways (*Figure 1*). The circulating sCLU level may be considered a novel biomarker of PHC, useful for the diagnosis and the individualized treatment of the disease. In addition, sCLU could improve MDR of PHC patients (36,37) and could contribute to HCC cell migration, EMT and formation of metastases. Therefore, the inhibition of sCLU expression, by specific CLU inhibitors, could represent a new targeted therapy that could improve the effects of PHC chemotherapy (38,39).

## Acknowledgements

*Funding:* This work was supported by National Natural Science Foundation (No. 81673241 and 81702419); Projects of Jiangsu Medical Science (No. BE2016698) and Graduate innovation (No. KYCX17_1934); Nantong Health and Family Planning Commission (No. WQ2016083);

 

and National Int S. & T. Cooperation Program (No. 2013DFA32150) of China.

## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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doi: 10.21037/tgh.2018.07.13
**Cite this article as:** Yao M, Fang M, Zheng W, Dong Z, Yao D. Role of secretory clusterin in hepatocarcinogenesis. Transl Gastroenterol Hepatol 2018;3:48.

 