SCLT1 Depletion Impair Hepatoma Cell Survival by Suppressing Cell Proliferation and Promoting Apoptosis

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Abstract

SCLT1 (sodium channel and clathrin linker 1), one of the distal accessory structural proteins of the centriole, promotes centriole anchoring to the cell membrane to initiate cilia formation. Cilia-associated proteins have been reported to potentially influence the development of cancer. However, the role of SCLT1 in HCC remains unknown. In this research, we found a positive correlation between SCLT1 and LIHC poor prognostic survival. SCLT1 depletion in hepatoma cells inhibits cell viability and ability of clone formation, which is confirmed in two hepatocellular carcinoma cell lines MHCC97H and SMMC7721. Moreover, drug inhibition experiments showed that SCLT1 knock-down effectively reduced the drug resistance of cancer cells. Western blot results showed that SCLT1 loss upregulated the expression of c-PARP and c-caspase3, which represents an increase of apoptosis. In conclusion, our results suggested that SCLT1 depletion impairs hepatoma cell survival by inhibiting cell proliferation and and drug-resistant of hepatoma cells, which is associated with increased apoptosis.

Keywords

SCLT1; LIHC; Proliferation; Drug Resistance; Apoptosis.

1. Introduction

Liver hepatocellular carcinoma (LIHC) is the most common liver malignancy with the third fatality rate among all types of malignancies. More than 700,000 new cases of LIHC each year in the world[1]. The incidence of LIHC is associated with underlying chronic liver disease, carcinogen exposure and genetic factors. In China, LIHC is the second most lethal malignancy with a fatality rate of over 70%. Surgical resection and liver transplantation are effective clinical treatment measures for early LIHC[2]. However, due to the hidden pathology and the lack of biomarkers for early diagnosis, LIHC patients have already been in the disease progression stage when the diagnosis is clear, resulting in a low 5-year survival rate[3]. Several treatment methods for the mid or late stage of LIHC include interventional therapy, molecular targeted therapy and selective radiotherapy[4]. But these treatments provide only limited survival benefits, and the existing targeted drugs constantly face problems with resistance[5]. Therefore, further study of the potential molecular mechanism of LIHC occurrence and development will provide a theoretical basis for seeking effective new targets or therapeutic measures.

Sodium channel and clathrin connectin 1 (SCLT1) were originally reported as a adaptor protein that connects sodium voltage-gated channel α subunit 10 (SCN10A) and clathrin[6]. Later, it was identified as one of five proteins that form distal appendages of the centriole, which facilitate the docking of the mother centriole to the plasma membrane to facilitate the formation of cilia[7]. There were also studies showing that SCLT1 played a role in many human diseases. Adlyet al. [8] and Naoya et al. [9] found that human SCLT1 mutation caused oral-facial-pointing syndrome (OFD IX) and Bardet-Biedl syndrome (BBS), both of which had similar characterization, as a result they were collectively referred to as "ciliopathies". Li et al. [10] found abnormal cell proliferation and apoptosis in mouse models with SCLT1 gene deletion. In addition, it has been reported that cilia-related proteins are closely related to tumorigenesis. For instance, IFT88 is a centrosome protein required for ciliary formation and maintenance, and IFT88 depletion promotes the cell cycle progression of HeLa cells[11]. Through high-throughput sequencing, Lőrinc Pongor et al. [12] found that SCLT1 gene is

one of the top 10 oncogenic genes in breast cancer patients, indicating that SCLT1 may play an important role in the pathological process of breast cancer.

Here, we investigated the role of SCLT1 in liver hepatocellular carcinoma. SCLT1 depletion by shRNA inhibited the viability of liver cancer cells and the ability of colony formation in vitro. Moreover, SCLT1 knock-down hepatoma cells were more sensitive to chemotherapy drugs. We also demonstrated that SCLT1 depletion was associated with the occurrence of apoptosis, which enhanced cell death of liver cancer by combining with chemotherapy drugs. Thus, inhibiting SCLT1 could represent a novel therapeutic strategy to overcome advanced hepatocellular carcinoma or prolong the prognostic survival of patients with LIHC.

2. Materials and methods

2.1 Cell Culture and Treatment

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2.2 Plasmids, lentivirus production, and transfection

SCLT1 shRNA (5′ GGTAGGAACTGACATATATGC 3′) were inserted into the pLKO.1 vector (a gift from Dr. Song Z. Y, Wuhan University), which was then transfected into 293T cells together with packaging plasmids psPAX2, pMD2.G for producing lentivirus. After 48-72h of transfection, the virus was collected and used to infect target cells. Stable SCLT1 knockdown cell lines were selected using puromycin (Amresco, Pennsylvania, USA, J593) at a concentration of 1 μg/mL.

2.3 Clone formation assay

Cells infected pLKO.1 and sh-SCLT1 lentivirus were inoculated into 6-well plates (1000/well), which was then continued for 2 weeks and was terminated when macroscopic apophyses were found in culture dishes. After washing with phosphate-buffered saline (PBS), fixation with 4% methanol for 10 min, the cells were stained with 0.1% crystal violet solution for 10 min, then the number of clones per well was counted. Cloning formation rate (%) = (number of clones/number of inoculated cells) × 100%.

2.4 Cell proliferation assay

In brief, PLKO.1/shSCLT1 Cells were seeded into 96-well plates at a density of 3×10^3 cells/well and incubated for 24 h. Culture medium containing gradient concentrations of XC-302 (16-0.125 μM) were added to each well, and 0.1% DMSO was set as the negative control. Culture medium containing gradient concentrations of Cisplatin (1.25-20 μM) or Sorafenib (2.5-40 μM) were added to each well, and 0.1% DMSO was set as the negative control. Cell viability was assessed using the Cell Counting Kit (CCK)-8 assay (Beyotime Institute of Biotechnology, Shanghai, China). The absorbance of each well was measured using a spectrophotometer (51119200; Thermo Fisher Scientific, Inc.) at 450 nm. Finally, the cell viability value was calculated using the following formula: (sample - blank)/(control - blank) × 100%.

2.5 Western blot analysis

Following treatment of 10 μM Cisplatin for different cell lines, the total protein were lysed with RIPA buffer (Beyotime, P0013B) with protease inhibitors and sonicated. (Cell Signaling Technology, Beverly, MA, USA). Then the protein concentrations were measured with a Pierce BCA protein assay kit (Thermo Scientific, 23225) according to the manufacturer's protocol. The protein extracts were mixed with SDS and boiled for 10 min in a heating block, and an equal amount of protein (20 μg)

was separated by SDS-polyacrylamide gel electrophoresis (PAGE). Separated protein bands were transferred onto nitrocellulose membranes (4μm pores; Millipore, Billerica, MA, USA). The membranes were subsequently blocked with skimmed milk (5%) and incubated overnight at 4˚C with the following antibodies: Total PARP (1:3,000, Rabbit mAb, #9532), Cleaved PARP (Asp214; 1:1,000, Rabbit mAb, #5625), Caspase-3 (1:2,000, Rabbit mAb, #9662), Cleaved Caspase-3 (Asp175; 1:1,000, Rabbit mAb, #9661), GAPDH (1:10,000, Rabbit mAb, #5174), β-Actin(1:10,000, Mouse mAb, #3700). After that, secondary antibody (anti-rabbit/Mouse IgG; CST) for 1 h at room temperature and the labeled proteins were detected using chemiluminescence reagent and automatic X-ray film. Protein bands were evaluated using Quantity One 1-D analysis software (Bio-Rad, Hercules, CA).

2.6 Statistical analysis

All experiments were carried out in triplicates. The results were expressed as the mean ± standard deviation (SD). The level of statistical significance was set at $p<0.05$ using an unpaired two-tailed Student's t-test. All statistical analyses were performed using GraphPad Prism software.

3. Results

3.1 SCLT1 is highly expressed in LIHC and is associated with poor prognosis

The Cancer Genome Atlas (TCGA) is a National Cancer Institute effort to profile at least twenty different tumor types using genomic platforms and to make raw and processed data available to all researchers[13]. In this study, we analyzed the SCLT1 differentially expressed in LIHC from TCGA, and we found that high expression of SCLT1 was positively associated with poor prognosis of LIHC ($p<0.05$) (Fig. 1A). In addition, SCLT1 was significantly upregulated in the primary hepatic carcinoma samples (N=371) compared to the Normal samples (N=50) from the TCGA LIHC database (Fig. 1B). These results suggested that the high expression of SCLT1 is potentially associated with the tumor progression and poor prognosis of liver hepatic carcinoma.

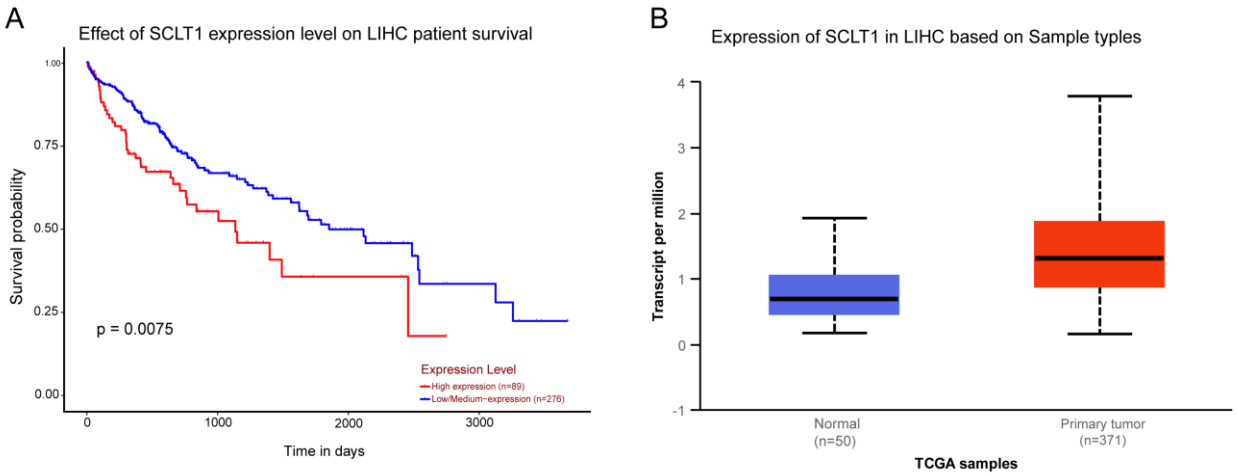

Fig. 1 Correlation between SCLT1 and liver cancer in TGCA database

3.2 Knockdown and identification of SCLT1 expression

MHCC97H and SMCC7721 cell lines were infected with PLKO.1 or sh-SCLT1 lentivirus, and the stably transduced cells were selected by puromycin. Western blot analysis demonstrated that the protein expression levels of SCLT1 in MHCC97H cells were significantly decreased, compared with in the cells of the PLKO.1 control. Similar results were obtained from the SMMC7721 cells ($p<0.01$) (Fig. 2A). Statistical analysis revealed that the protein expression levels of SCLT1 were markedly downregulated, compared with Control group ($p<0.05$) (Fig. 2B). In addition, no significant difference was detected between the Control group and untreated cell groups. These results indicate that SCLT1 knock-down was successfully constructed.

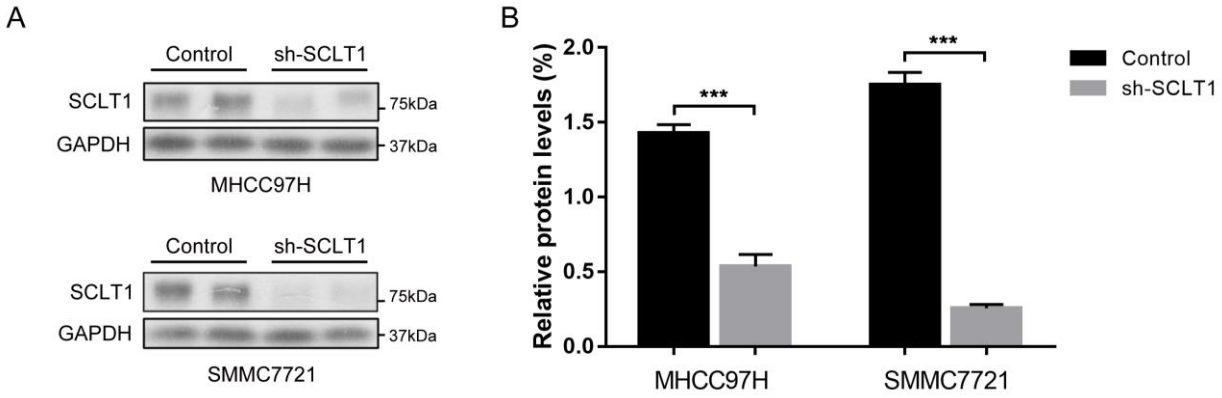

Fig. 2 Construction of SCLT1 knockdown in hepatocellular carcinoma cell lines

3.3 SCLT1 depletion inhibits clone formation and cell proliferation

To determine whether knockdown of SCLT1 expression affected the biological function of hepatocellular carcinoma cells, cellular activities were analyzed using clone formation and CCK-8 assays. SCLT1 knockdown inhibited the clone forming ability of MHCC97H and SMMC7721 cells (Fig. 3A). Statistical analysis demonstrated that clone formation was inhibited by SCLT1 knock down, compared with Control group (p<0.05) (Fig. 3B and C). Cells from the PLKO.1, sh-SCLT1 and untreated groups (WT) were cultured for 5 days. The cell proliferation assay indicated that MHCC97H cell growth in the sh-SCLT1 group was significantly decreased (p<0.05). Statistical analysis revealed that the proliferation rate was significantly decreased, and no significant differences were detected between the PLKO.1 and WT groups (Fig. 3D). Similarly, SCLT1 knockdown inhibited SMMC7721 cell proliferation (p<0.05) (Fig. 3E). These data suggested that SCLT1 depletion inhibited hepatocellular carcinoma cells proliferation, and might have a critical role in LIHC.

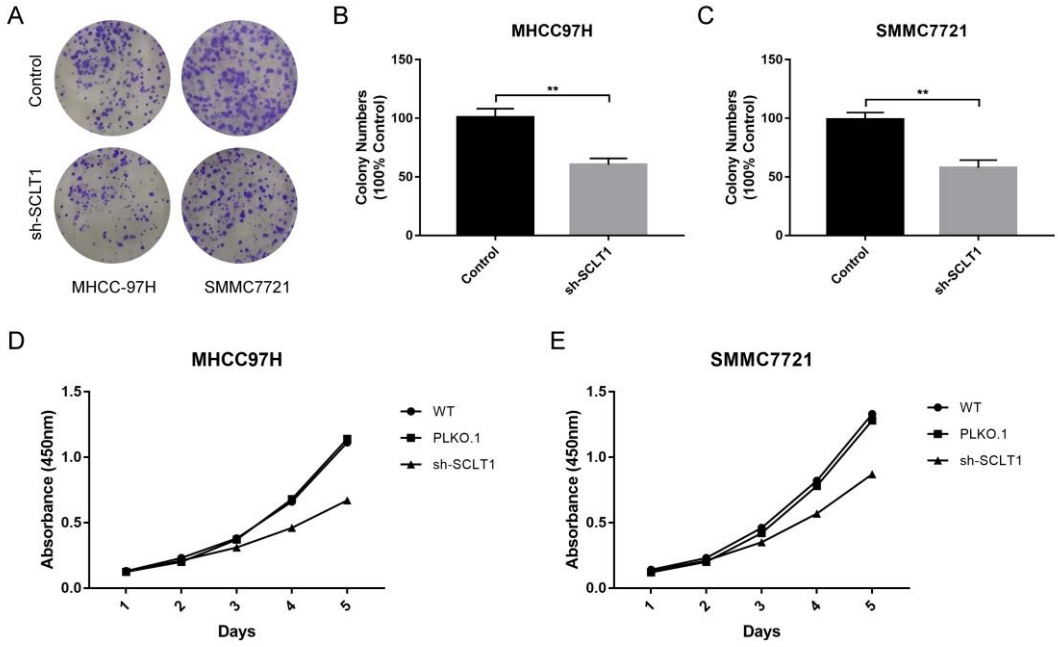

Fig. 3 SCLT1 depletion inhibits clone formation and cell proliferation in LIHC cells

3.4 SCLT1 knockdown attenuated drug resistance in LIHC cells

In order to investigate the effect of SCLT1 on drug resistance of tumor cells, we treated the cells with cisplatin and sorafenib respectively. Cisplatin is one of the most potential and widely used drugs for

the treatment of various solid cancers. It exerts anti-cancer activity via multiple mechanisms which finally lead to apoptosis[14]. Sorafenib, a multikinase inhibitor with anti-proliferative and proapoptotic properties, constitutes the unique target drug approved for the treatment of LIHC[15]. However, drug resistance is the inherent challenges which limit their application and effectiveness. The results of drug inhibition experiments showed that SCLT1 knock-down significantly enhanced the sensitivity of MHCC97H to cisplatin, which was also the same for SMMC7721 (Fig. 4A and B). Moreover, the difference was most pronounced at moderate concentrations. For different concentrations of sorafenib treatment, we also found a decreased cell viability of MHCC97H and SMMC7721 in sh-SCLT1 groups (Fig. 4C and D). These data demonstrate that SCLT1 knockdown attenuated cisplatin or sorafenib resistance in LIHC cells.

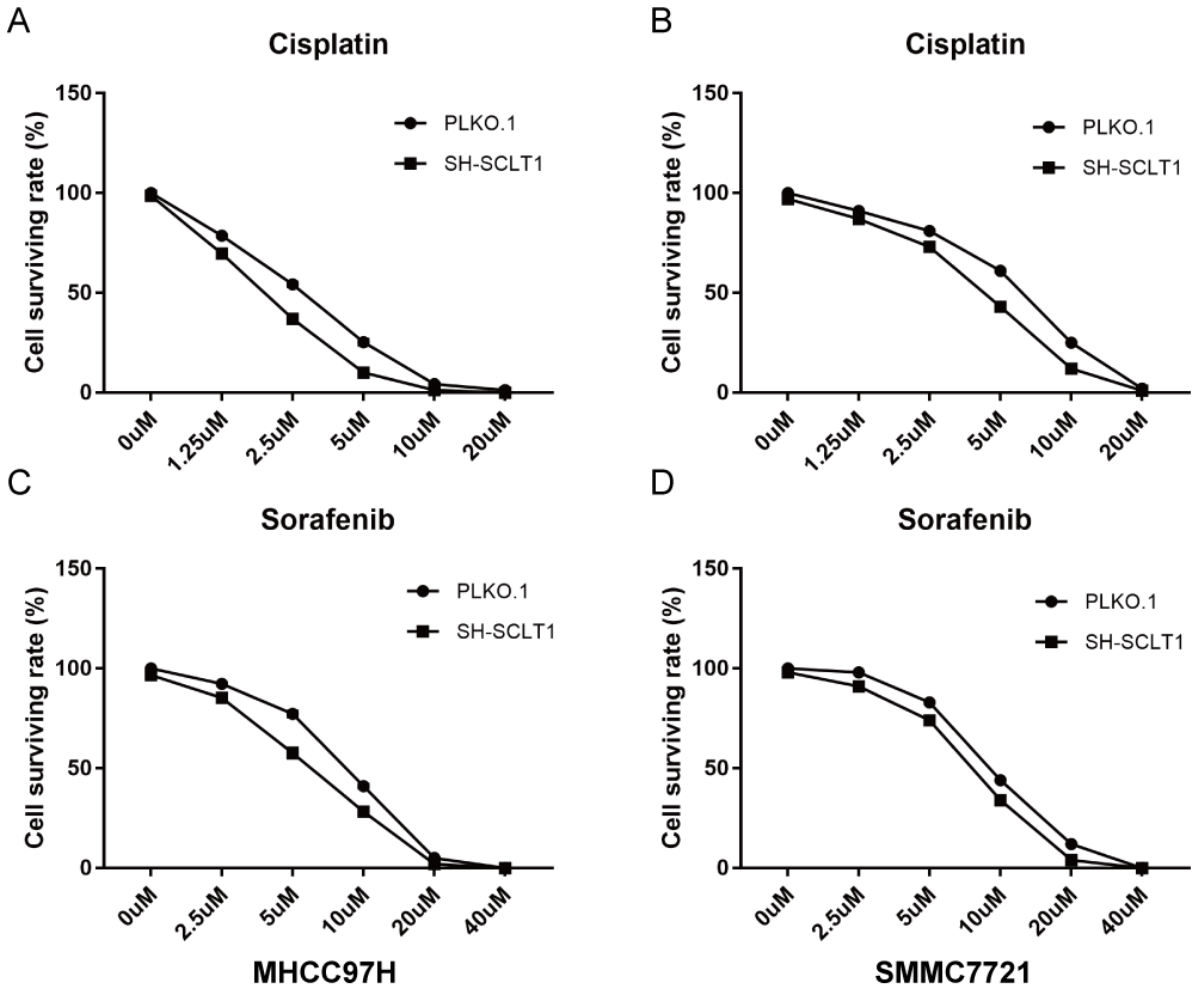

Fig. 4 SCLT1 depletion increases the sensitivity of LIHC cells to therapeutic drugs

3.5 SCLT1 inhibits the proliferation by inducing apoptosis

To assess the effects of SCLT1 on LIHC cell apoptosis in vitro, MHCC97H and SMMC7721 cells were treated with Cisplatin for 24 h and subsequently investigated the expression levels of apoptosis-related proteins. Caspase3 is the central regulatory molecule of apoptosis, which is cleaved at Asp175 and splits into cleaved-caspase3 after apoptosis activation. PARP locates in the nucleus and is the main cleavage target of caspase3, thus it is considered to be an indicator of caspase3 activation. Western blot results showed that SCLT1 knockdown increased the level of c-PARP in MHCC97H, yet there was no significant difference with c-caspase3 between sh-SCLT1 and contriol groups(Fig. 5A). Besides, following exposure to cisplatin, the protein levels of c-caspase3 were markedly increased in both of MHCC97H and SMMC7721 cells(Fig. 5B). These datas revealed that SCLT1 knockdown promotes apoptosis of LIHC cells, which might also play a role in tumor resistance.

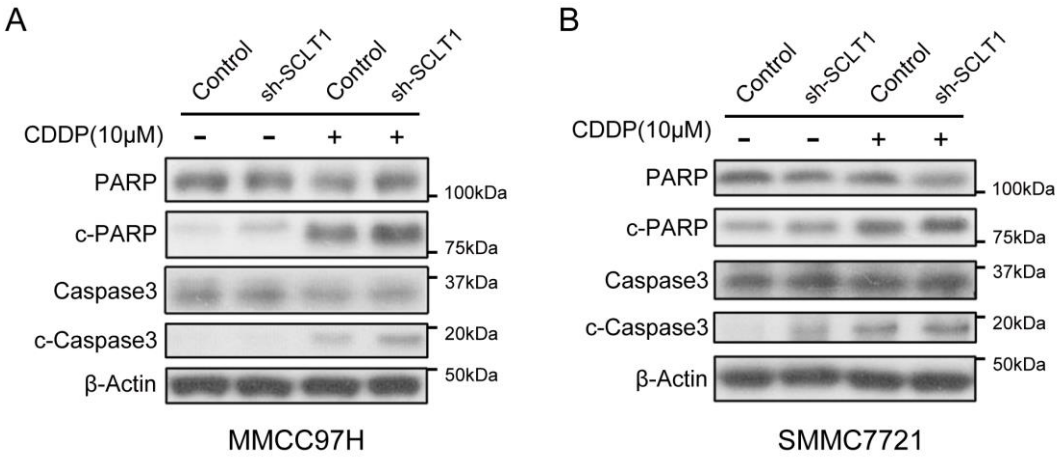

Fig. 5 SCLT1 depletion increases the apoptosis of LIHC cells

4. Conclusion

SCLT1 is an important adaptor protein in cilia formation, which is involved in the development of heritable disease[16]. For a long time, the primary cilia were thought to be a degenerated organelle, but only in recent years has it been discovered that this "antenna-like" organelle can sense changes in chemical signals in the extracellular environment and cause the cell to respond[17]. Due to the function of them in regulating cell movement and signal transduction, cilia defects lead to abnormal development or diseases of various organs, including liver, kidney and skeletal system[18]. Previous studies have shown that cilia-associated proteins have a potential influence on the occurrence of cancer and may be related to DNA damage response[19]. In addition, ciliary assembly is closely related to cell cycle, suggesting that its defects affect cell cycle progression[20].

This study revealed that SCLT1 was functionally involved in LIHC progression and drug resistance. High expression of SCLT1 was present in LIHC and was associated with a poor prognosis. In the contrary, knockdown of SCLT1 suppressed the clone formation and cell proliferation, and attenuated drug resistance in LIHC cell lines. These results indicated that SCLT1 may be an important biomarker and therapeutic target for LIHC.

Apoptosis is regulated by a variety of intracellular signaling pathways, including DNA damage, mitochondrial stress and calcium signal transduction. Based on previous studies, we speculated that SCLT1 induced apoptosis might be related to DNA damage response, while further investigations are needed to confirm these predictions. Besides, SCLT1 depletion may affect cell cycle regulation and lead to inhibition of cell proliferation. More studies are required in the future to elucidate the associations.

Acknowledgments

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