Functional validation of Cell Division Cycle Associated 8 (CDCA8) as a novel therapeutic target in Human Hepatocellular Carcinoma

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Directed by Professor Young Nyun Park

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This certifies that the Master's Thesis of Tae-Won Jeon is approved.

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ABSTRACT

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Hepatocellular carcinoma (HCC) is the third most lethal cancer worldwide and remains a major challenge due to poor prognosis and limited treatment options. Cell Division Cycle Associated 8 (CDCA8) is known as a component of a chromosomal passenger complex required for stability of the bipolar mitotic spindle and it is commonly overexpressed in human HCC. However, the role of CDCA8 in HCC remains indefinable. In this study, we tested the hypothesis that specific targeting of CDCA8 is sufficient to inhibit HCC progression. Small interfering RNA (siRNA)-mediated silencing of CDCA8 inhibited HCC cell

growth by blocking cell-cycle progression, inducing apoptosis. Next generation sequencing (NGS) expression analysis of CDCA8 knockdown signature showed that CDCA8 blockade caused anti-proliferative effects. These effects were also associated with dysregulation of CDCA8-regulated genes that control cell cycle and apoptosis. Anti-proliferative effects were driven by a subset of molecular alterations including the upregulation of ATF3 and GADD34, whereas a key regulator of cell growth and invasiveness BGLAP was repressed. Subsequent Western blotting revealed that CDCA8 silencing also decreased the level of pro-caspase 3 and PARP-1, accelerating apoptotic signaling in HCC cells. Taken together, these findings offer a preclinical proof-of-concept that CDCA8 can be a promising molecular target for systemic therapy of HCC.

Key words: CDCA8, siRNA, Hepatocellular carcinoma, Apoptosis, Cell cycle

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I. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common neoplasms worldwide and the third leading cause of cancer-related mortality with annual death exceeding 600,000.[1,2] Similarly, about 14,000 annual incidence of HCC has been reported in South Korea. Even with advanced treatment, HCC usually has a poor prognosis, with a 5-year survival rate of patients as low as 25%-39% after curative treatments such as surgical resection, liver transplantation, chemotherapy, and radiotherapy.[3,4] Generally, only 30%-40% of HCC patients

are diagnosed as eligible for current curative treatment due to late diagnosis, high recurrence rate, underlying liver disease and a lack of effective treatment options.[5] It is widely accepted that environmental factors and additional genetic and epigenetic alterations associated with development of HCC.[6] Although the risk factors for HCC are well characterized and there are ongoing efforts, the molecular mechanism of HCC carcinogenesis is not well understood. Therefore the identification of efficient new targets for the inhibition of HCC must be urgently needed.

Cell cycle deregulation is one of the hallmarks in various cancer types and, thus, recognition of targets underlying regulatory mechanisms for cell cycle regulation is critical in the development of effective cancer therapies.[7,8] In a previous study, cell division cycle associated 8 (CDCA8) is associated with in cell cycle progression.[9] CDCA8, also called as Borealin or Darsa B, is known as a component of a chromosomal passenger complex, which acts as a key regulator of mitosis, required for stability of cell division and it is commonly overexpressed in a series of solid tumors, including HCC.[10,11] However, the underlying molecular mechanisms of CDCA8 on HCC cells have not yet been clearly demonstrated.

In the present study, our aim was to investigate the role of CDCA8 on Huh-1

and Huh-7 HCC cells and to elucidate the molecular mechanism. CDCA8 knockdown via RNAi markedly suppressed these phenotypes in Huh-1 and Huh-7 HCC cell lines. CDCA8 knockdown inhibited HCC cell growth and long-term colony formation. It also induced apoptosis and cell cycle arrest. We also performed RNA sequencing and subsequent protein analysis to understand the molecular mechanism on a whole genome scale. The growth inhibitory effects were induced through a subset of molecular alterations, including the upregulation of ATF3 and GADD34, which play a pivotal role in apoptotic progression. These results may provide further insight into HCC progression and suggest that CDCA8 may be a potential therapeutic target in HCC.

II. MATERIALS AND METHODS

1. Cell culture and siRNA transfection

Liver cancer cell lines, Huh-1 and Huh-7 were purchased from the Japanese Cellection of Research Biosources Cell Bank (JCRB). Huh-1 and Huh-7 cells were cultured in DMEM medium (HyClone, South Logan, UT, USA) supplemented with 10% fetal bovine serum (HyClone) and 1% penicillin/streptomycin solution (HyClone) at 37℃ with 5% CO_2. The phenotypes of these cell lines have been authenticated by the JCRB. Cells were plated at 30% density 24hr before transfection. Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was mixed with siRNA molecules in Opti-MEM (Invitrogen, Carlsbad, CA, USA). The medium was replaced 24hr after transfection. CDCA8 siRNA duplexes were chemically synthesized by Ambion (Austin, TX, USA) (CDCA8: siRNA ID# s30269). The negative control siRNA (NC siRNA) molecules that do not target any endogenous transcript were used for control experiments. The sequences of NC siRNA (Bioneer, Daejeon, South Korea) were as following : 5'-ACGUGACACGUUCGGAGAA(UU)-3'(sense) and 5'-UUCUCCGAACGUGUCACGU-3'(antisense).

2. Measurement of cell proliferation and apoptotic cell death

The cell growth was measured using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Medison, WI, USA) according to the manufacturer's instructions. For the measurement of cell proliferation and apoptosis, cells were seeded at 30% confluence in 96-well plates in culture media without antibiotics one day before transfection. The cells were transfected as described above and incubated for 2hr at 37℃ in a humidified, 5% CO_2 atmosphere. The colored formazan products were determined by measuring the absorbance at 490 nm using a VersaMax microplate reader (Molecular Device, Sunnyvale, CA, USA). The level of caspase-cleaved keratin 18 (ccK18) fragments was detected using M30 Apoptosense ELISA (PETIVA, Bromma, Sweden) as recommended by the manufacturer.

3. Clonogenic assay

Cells transfected with siRNA were seed in 6-well culture plates at $1x10^3$ cells per well. Cells were maintained in culture for 12 days without medium change to let the viable cells propagate to sizable colonies for quantification. The colonies were fixed with methanol and then stained with 0.5% crystal violet for 30min at room temperature. The number of colonies formed in each well was

counted under the microscope.

4. Real-time RT-PCR

The changes in target gene expression on mRNA level were detected using real-time quantitative RT-PCR. Total RNA was isolated using TRIzol (Ambion Inc., Texas, USA) and synthesized to cDNA by using 1st strand cDNA Synthesis Kit (Takara Biotech, Kusatsu, Shiga, Japan) according to the manufacturer's instruction. cDNA of CDCA8 gene GAPDH were amplified using corresponding pair of primers (CDCA8 forward, 5'-GCAGGAGAGCGGATT TACAAC-3'; CDCA8 reverse, 5'-CTGGGCAATACTGTGCCTCTG-3' and GAPDH forward, 5' GGGAGCCAAAAGGGTCATCATCTC-3'; GAPDH reverse, 5'-CCATGCCAGTGAGCTTCCCGTTC-3') synthesized by Macrogen Inc. (Seoul, South Korea). The relative quantification of mRNA was measured by LightCycler 96 (Roche, Basel, Switzerland) according to the manufacturer's instructions and quantified using LightCycler 96 software version 1.1, comparing with the Ct (threshold cycle) values of each target gene. The mRNA levels of GAPDH were used for normalization.

5. Cell cycle analysis

Cells were cultured in 60mm culture dishes and harvested 48hr later after siRNA transfection. Cells were washed with cold PBS, and then fixed 24hr with 70% cold ethanol at -20℃. Cells were washed with cold PBS again and incubated in the dark with in Propidium Iodide (PI) staining solution with RNase A (BD Biosciences, SanDiego, CA) for 30min at room temperature. The cell cycle was measured by FACSVerse flow cytometry (BD Bioscience, SanDiego, CA) according to the manufacturer's instructions and quantified using FlowJo software program.

6. Detection of apoptosis

Annexin V and PI dual staining was used to detect apoptosis cells in a similar procedure as described above but the cells were not fixed with 70% ethanol. Apoptotic cells were stained using FITC Annexin V Apoptosis Detection Kit I (BD Bioscience, SanDiego, CA) following the manufacturer's instruction. The cell death was measured by FACSVerse flow cytometry (BD Bioscience, SanDiego, CA) and quantified using FlowJo software program.

7. RNA sequencing

Total RNA was extracted 48hr after siRNA transfection using the RNeasy mini kit (Qiagen, Valencia, CA, USA). The quantity of the total RNA was evaluated using RNA electropherograms (Bio-Rad Experion, Hercules, CA, USA); RNA quality was assessed based on the RNA quality indicator (RQI). The total RNA from each sample with a RQI value of 8.0 or higher was used. The resulting mRNA samples were processed for sequencing libraries using the Illumina TruSeq Stranded mRNA sample preparation kit (Illumina, San Diego, CA, USA) according to the manufacturer's protocols. RNA sequencing was performed using the Illumina HiSeq 2500 to generate non-directional, paired-end 100-base-pair reads. Quality-filtered reads were mapped to the human reference genome sequence hg19 (UCSC Genome Bioinformatics, https://genome.ucsc.edu) using tophat2 (http://ccb.jhu.edu/software/tophat). The relative transcript abundance was estimated by counting the fragments per kilobase of the exon model per million mapped sequence reads (FPKM), and differentially expressed genes were evaluated using the cufflinks package (http://cole-trapnell-lab.github.io/cufflinks). The significantly overlapping pathways and Gene Ontology categories with differentially expressed genes were analyzed using DAVID (http://david.abcc.ncifcrf.gov).

8. Western blot analysis

Cells were suspended in RIPA buffer (Thermo Scientific, Rockford, IL, USA) containing 0.01% of a protease and phosphatase inhibitor cocktail (Thermo Scientific) 48hr after siRNA transfection. The amount of protein was quantified by using the Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). Equal amounts (50 ug) of total proteins were fractionated by SDS-PAGE on a 10% gel and transferred to PVDF membranes (Roche, Basel, Switzerland). The membrane was blocked with 5% milk/Tris-buffered saline plus Tween 20 (TBST) and incubated with primary antibodies against human ATF-3 (sc-188), GADD34 (sc-8327), p-cdc2 (sc-12341), pro-caspase 3 (sc-7272), PARP-1 (sc-8007) (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA), cyclin B (ab72) and CDCA8 (ab70910, Abcam, Cambridge, UK). HRP goat anti-mouse IgG and HRP goat anti-rabbit IgG (Santa Cruz) were used as the secondary antibodies. Immunoreactive bands were visualized with an LAS-3000 Imager (Fujifilm Corporation, Tokyo, Japan). Equal loading was assessed by probing the same membrane with a β-actin antibody (Santa Cruz).

9. Statistical analysis

All data were obtained at least three independent experiments and are presented as mean ± SE (Standard Error), unless otherwise indicated. Statistical analysis was performed using one-way ANOVA and Student's *t*-test. Data were considered significant if *p<0.05, **p<0.01.

III. RESULTS

1. CDCA8 knockdown inhibits HCC cell proliferation and long-term colony formation

To examine the biological effects of CDCA8 knockdown, two HCC cell lines, Huh-1 and Huh-7, were transfected with CDCA8-specific siRNA. To select the siRNA eliciting the highest treatment efficacy and target gent knockdown, we tested a 10 to 20 nmol/L dose range for CDCA8 siRNA at optimal experimental conditions. Cells were plated at 30% confluence 24hr before transfection and treated with siRNA mixed with the lipofectamine. After evaluating the efficiency of siRNA, CDCA8 siRNA at a concentration of 15 nmol/L was considered appropriate for CDCA8 knockdown. Notably, CDCA8 siRNA at a concentration of 15 nmol/L caused a maxium growth suppression of approximately 70% in both the Huh-1 and Huh-7 cells after 4 days of treatment (Figure 1A). This dose was, therefore, selected for all subsequent studies. Consistent with the phenotypic result, CDCA8 siRNA was also effective in silencing CDCA8 mRNA in both of the examined HCC cells, when compared with NC siRNA which was not able to knock down expression of any gene (Figure 1B). CDCA8 knockdown was specific as transfection with NC siRNA did not affect target gene expression. The decrease in cell viability was affirmed

by microscopic observation in 4 days of target gene silencing (Figure 2). To further investigate the long-term effect of CDCA8 knockdown, colony formation experiment was performed. Consistent with the results of the short-term phenotypic assay, CDCA8 knockdown was also effective in inhibiting long-term colony formation (Figure 3A). CDCA8 siRNA caused a strong reduction of the growth about 70% and 80% in Huh-1 and Huh-7 cells, respectively (Figure 3B). Taken together, these data indicated that siRNA-mediated knockdown of CDCA8 in HCC cells inhibit cellular proliferations and colony formation of HCC cells.

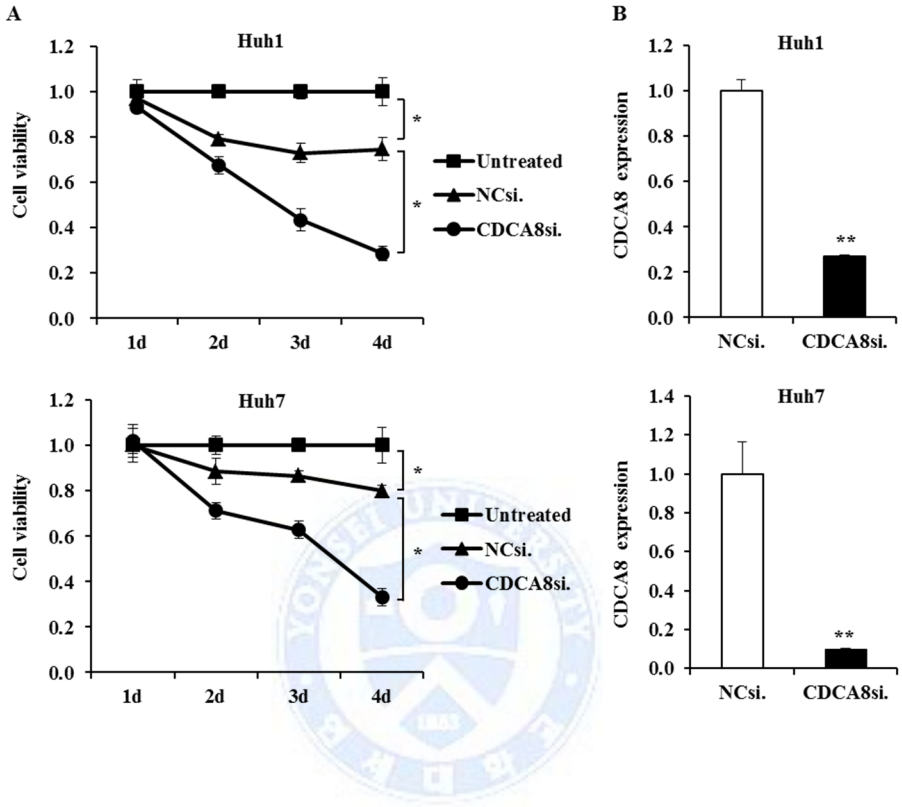


Figure 1. siRNA knockdown of CDCA8 suppresses HCC cell proliferation. **(**A) Kinetics of growth inhibition in Huh-1 and Huh-7 cells treated with 15nmol/L NC siRNA and CDCA8 siRNA. The data represent three independent experiments. (B) Down-regulation of CDCA8 mRNA in Huh-1 and Huh-7 cells 48hr after transfection with 15nmol/L CDCA8 siRNA. The data expressed relative to GAPDH and normalized to NC siRNA treatment. All statistical analysis was performed using Bootstrap *t*-test. *P<0.05; **P<0.01.

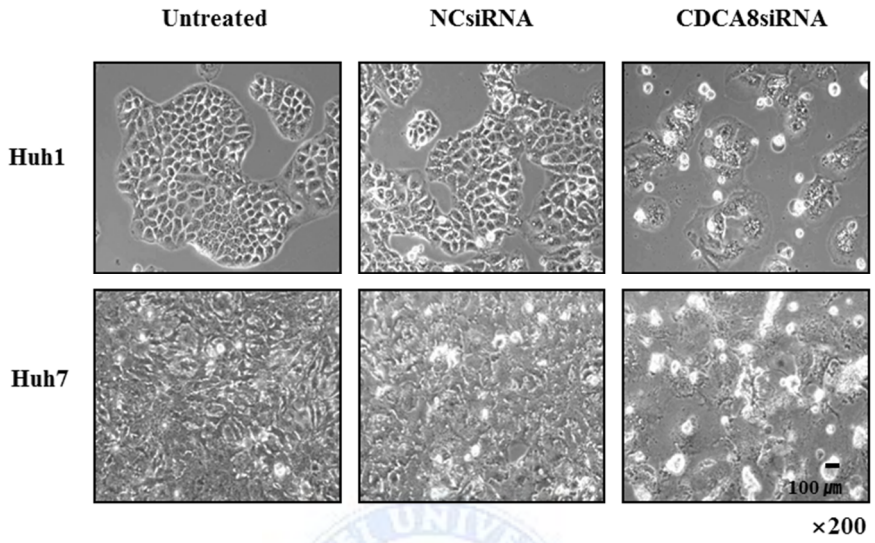


Figure 2. siRNA knockdown of CDCA8 inhibits growth of HCC cells. Representative light microscopy images of Huh-1 and Huh-7 cells 4 days after transfection with NC siRNA and CDCA8 siRNA. Scale bar, 100μm. NC siRNA, negative control siRNA.

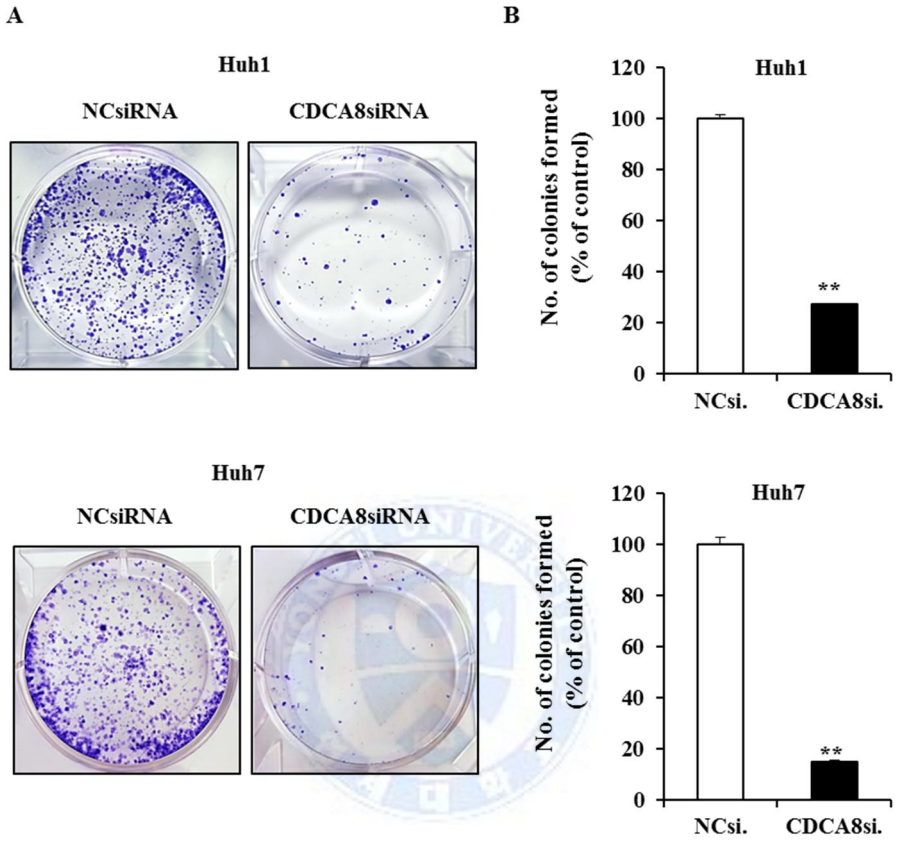


Figure 3. siRNA knockdown of CDCA8 inhibits long-term colony formation in HCC cells. (A) Observation of colony formation in Huh-1 and Huh-7 cells, using a clonogenic assay 12 days after transfection with 15nmol/L NC siRNA and CDCA8 siRNA. (B) Measurement of number of colonies formed in Huh-1 and Huh-7 cells. The data represent three independent experiments. **P<0.01 using the Poisson generalized linear model.

2. CDCA8 knockdown influences cell cycle progression and induces apoptosis in HCC cells

To evaluate the effect of CDCA8 knockdown on the cell cycle, flow cytometry was performed to detect the cell cycle distribution of Huh-1 and Huh-7 cells (Figure 4). Fluorescence-activated cell sorting (FACS) analysis revealed accumulation of G2/M-phase cells and a concomitant decrease in the G0/G1-phase cells in both examined HCC cell lines 2 days after treatment with CDCA8-siRNA when compared with the NC siRNA, which was used as control (Figure 5). These results supported the theory that CDCA8 knockdown may contribute to the cell division via promoting the progression of the G2/M phase in the cell cycle.[12] More significantly, the growth inhibition and cell cycle arrest might reflect the strong induction of apoptosis as measured through the detection of the apoptotic marker Annexin V using FACS (Figure 6). In other words, at 48hr after transfection with CDCA8 siRNA, the percentage of early-apoptotic cell populations (Q3 region) plus late-apoptotic cell populations (Q2 region) in Huh-1 and Huh-7 cells increased by about 29% and 90%, respectively. To confirm the apoptotic induction through CDCA8 knockdown, we conducted an ELISA assay to detect the cleaved K18 in apoptotic cells but not in the necrotic cells. Namely, HCC cells with silenced CDCA8 showed a

strong induction of apoptosis as measured by an accumulation of apoptosis-specific caspase-cleaved K18 (ccK18) fragments (Figure 7B). The results showed that CDCA8 knockdown significantly increased the cleaved K18 fragments in Huh-1 and Huh-7 cells approximately 1.5- and 1.3- fold, respectively, at 48hr after transfection with CDCA8 siRNA. Therefore, these data demonstrated that siRNA-mediated knockdown of CDCA8 in HCC cells induces cell cycle arrest and increases propensity to undergo apoptotic cell death.

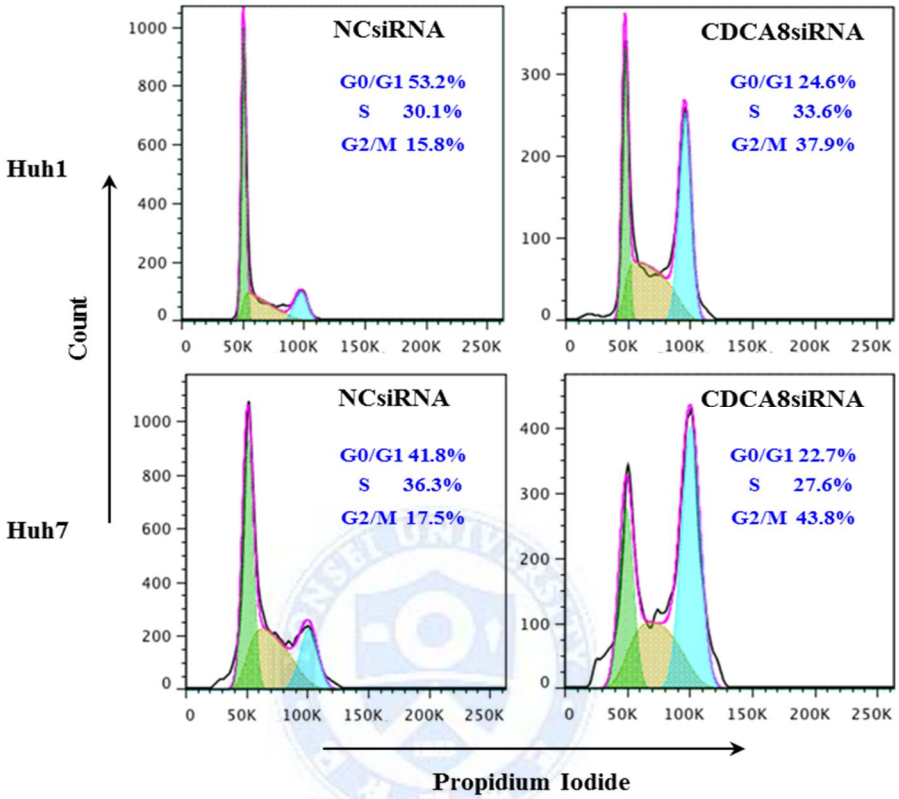


Figure 4. CDCA8 silencing delays cell-cycle progression in HCC cells. FACS analysis of cell-cycle progression at 48hr after transfection with NC siRNA and CDCA8 siRNA. The distribution of cell cycle was analyzed using PI staining and the proportion of DNA in the different phase was calculated using FlowJo software. Representative histograms of cell cycle distribution from three independent experiments are shown.

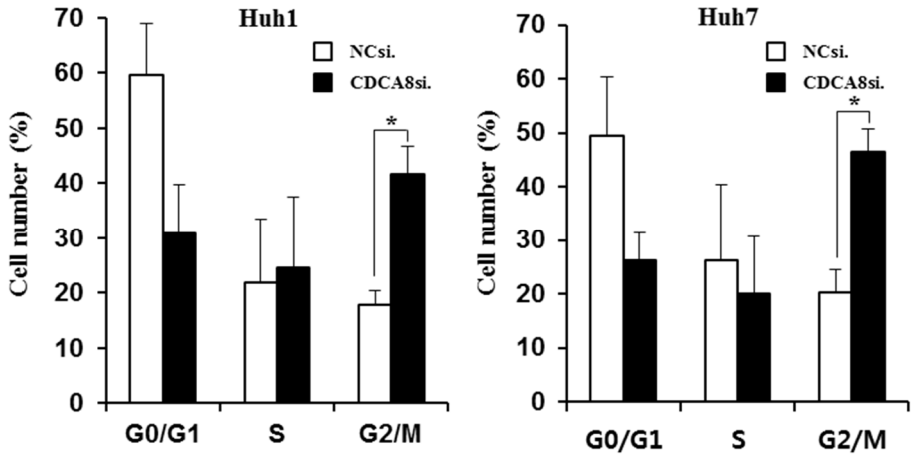


Figure 5. CDCA8 silencing induces cell-cycle arrest in HCC cells. (A) Cell cycle distribution (G0/G1, S, and G2/M) of Huh-1 and Huh-7 cells. HCC cells were treated with siRNA and cells were processed for flow cytometric analysis. Flow cytometry analysis of HCC cells treated with siRNA confirmed cell cycle arrest in G2/M. The data represent three independent experiments. *P<0.05 by Student's *t*-test with equal variance.

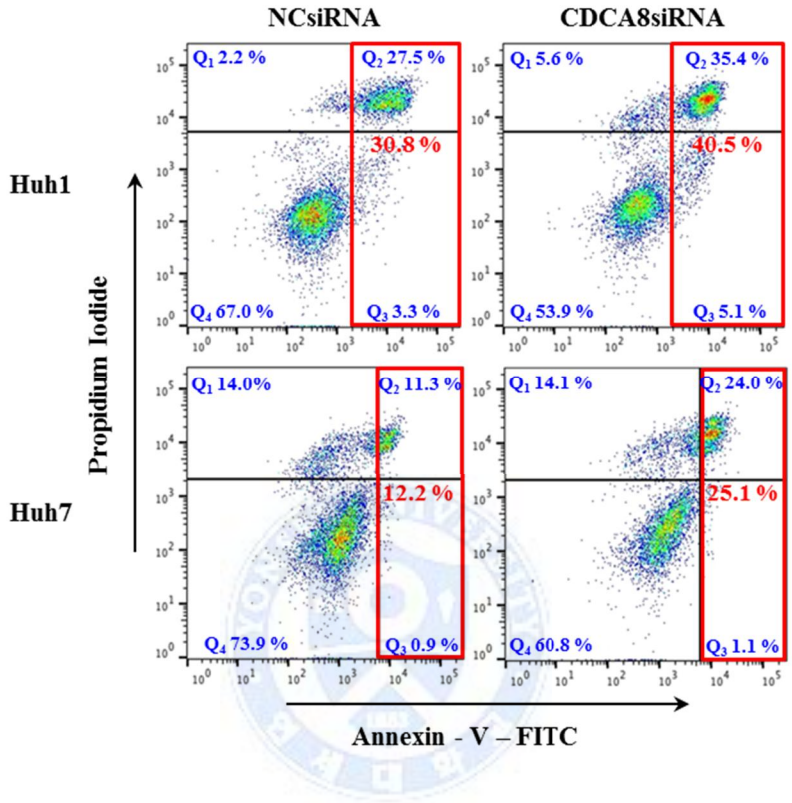


Figure 6. CDCA8 silencing induces apoptosis in HCC cells. Detection of the apoptotic cell population through FACS analysis with the intensity of apoptotic marker Annexin V at 48hr after transfection with 15nmol/L NC siRNA and CDCA8 siRNA. Representative results from three independent experiments are shown.

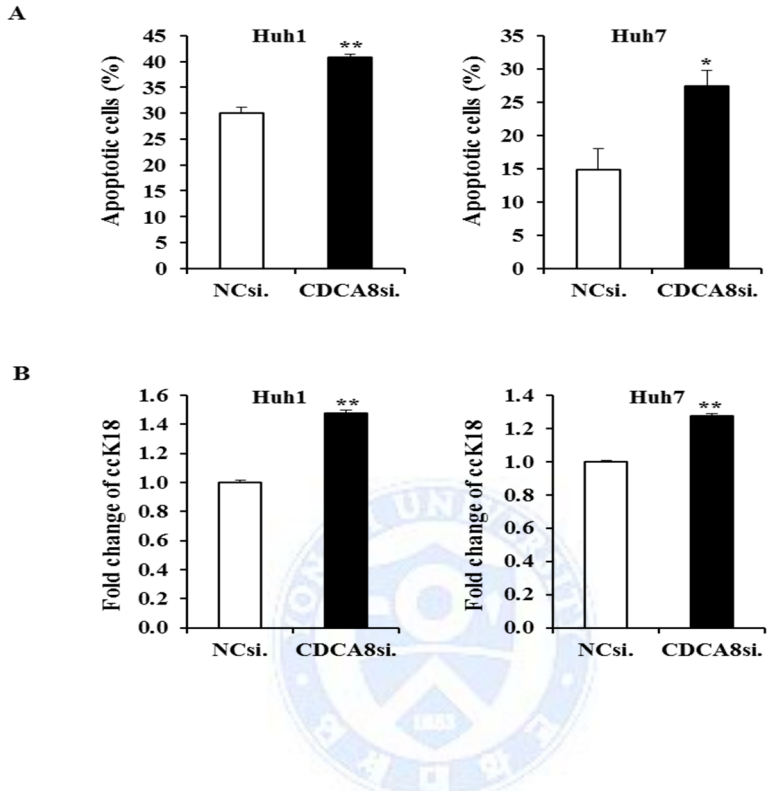
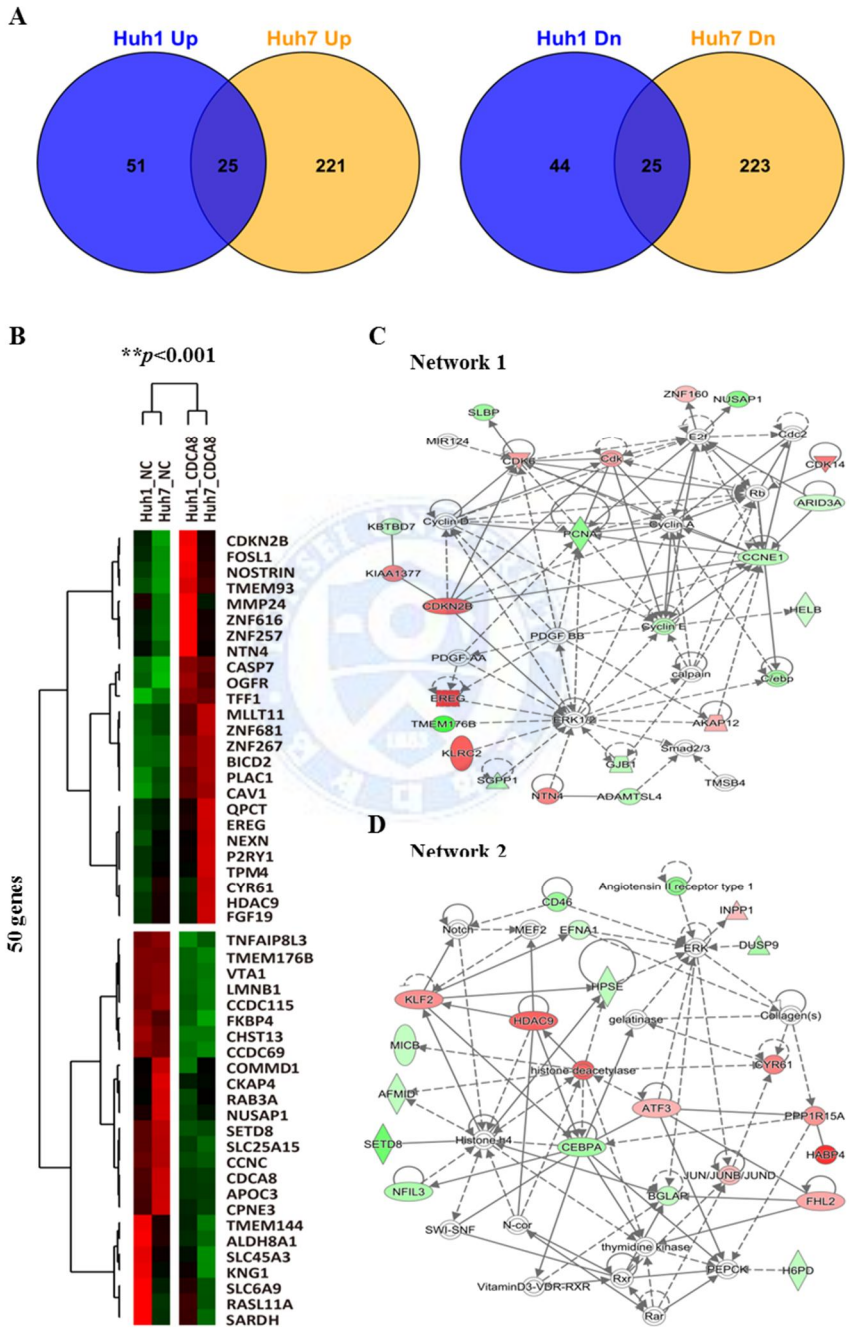


Figure 7. siRNA knockdown of CDCA8 induces apoptotic cell death in HCC cells. (A) Detection of the apoptotic cell population through FACS analysis based on the intensity of the apoptotic marker Annexin V in Huh-1 and Huh-7 cells at 48hr after transfection with 15nmol/L NC siRNA and CDCA8 siRNA. (B) Detection of caspase-cleaved keratin 18 (ccK18) fragments by M30 Apoptosense ELISA 2 days after transfection experiments. The data represent three independent experiments. All statistical analysis was performed using Bootstrap *t*-test. *P<0.05; **P<0.01.

3. The molecular mechanisms underlying the growth inhibitory effects of CDCA8 knockdown in HCC cell

To study the molecular basis of the observed growth inhibition caused by CDCA8 silencing, we compared the global gene expression profiles of CDCA8-deficient Huh-1 and Huh-7 cells to those of sham-treated control cells using next-generation RNA sequencing. The global gene expression analysis revealed that CDCA8-specific knockdown resulted in the up- and down-regulation of 145 RNA transcripts in Huh-1 and 494 transcripts in Huh-7 cells (Figure 8A). Comparing these two gene sets to make a distinction between common anti-cancer mechanisms revealed a significant overlap of 50 genes (25 up- and 25 down-regulated genes) defined using a Bootstrap ANOVA with 10,000 repetitions and at least a 2-fold change ($P<0.001$) (Figure 8B). In addition, this effect was considered to be a common CDCA8 knockdown signature in HCC cells. Ingenuity pathway analysis (IPA) showed that the 50 genes were functionally enriched in the top two networks strongly connected with proliferating cell nuclear antigen (PNCA, network 1) and ATF3 (network 2), respectively (Figure 8C and 8D). Coincide with IPA analysis, CDCA8 knockdown impacted molecular alterations in restricted number of defined oncogenic pathways that played a crucial role in inhibiting cell growth and

induce apoptosis. Specifically, the expression levels of ATF3, which functionally acts as a tumor suppressor,[13] and PPP1R15A, also known as growth arrest and DNA damage-inducible protein GADD34, which is involved in growth arrest,[14] were induced. Subsequent Western blot analysis proved the common increase of the ATF3 and GADD34 tumor suppressor proteins in addition to a concomitant decrease of pro-caspase 3, activating apoptotic signaling[15] and PARP-1, a nuclear enzyme essential for genomic stability[16] in HCC cells (Figure 9). Therefore, this finding suggests that the up-regulation of the tumor suppressive ATF3 and GADD34 genes is an essential and common mechanism of growth inhibition in CDCA8 deficient HCC cells.


A
Huh1 Up
Huh7 Up
51
25
221
Huh1 Dn
Huh7 Dn
44
25
223
B
**p<0.001
Huh1_NC
Huh7_NC
Huh1_CDCA8
Huh7_CDCA8
50 genes
CDKN2B
FOSL1
NOSTRIN
TMEM93
MMP24
ZNF616
ZNF257
NTN4
CASP7
OGFR
TFF1
MLLT11
ZNF681
ZNF267
BICD2
PLAC1
CAV1
QPCT
EREG
NEXN
P2RY1
TPM4
CYR61
HDAC9
FGF19
TNFAIP8L3
TMEM176B
VTA1
LMNB1
CCDC115
FKBP4
CHST13
CCDC69
COMMD1
CKAP4
RAB3A
NUSAP1
SETD8
SLC25A15
CCNC
CDCA8
APOC3
CPNE3
TMEM144
ALDH8A1
SLC45A3
KNG1
SLC6A9
RASL11A
SARDH
C
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SLBP
MIR124
E2f
Cdc2
CDK14
CDK6
Cdk
Rb
ARID3A
KBTBD7
Cyclin B
Cyclin A
PCNA
CCNE1
KIAA1377
CDKN2B
HELB
Cyclin E
PDGF BB
PDGF AA
Calpain
Cebp
EREG
TMEM176B
ERK1/2
AKAP12
KLRC2
Smad2/3
GJB1
SGPP1
TMSB4
NTN4
ADAMTSL4
D
Network 2
Angiotensin II receptor type 1
CD46
INPP1
Notch
MEF2
EFNA1
ERK
DUSP9
KLF2
HPSE
HDAC9
gelatinase
Collagen(s)
MICB
histone deacetylase
CYR61
AFMID
ATF3
PPP1R15A
SETD8
Histone h4
CEBPA
HABP4
JUN/JUNB/JUND
NFIL3
BGLAP
FHL2
SWI-SNF
N-cor
thymidine kinase
PEPCK
H6PD
Rxr
VitaminD3-VDR-RXR
Rar

Figure 8. Transcriptomic analysis of gene expression by RNA sequencing following CDCA8 siRNA treatment. (A) Venn diagrams representing the number of mRNA transcripts up- or down-regulated in Huh-1 and Huh-7 cells at 48hr after transfection with CDCA8 siRNA. (B) A heat map of the 50 commonly deregulated genes in Huh-1 and Huh-7 cells after normalization to the corresponding sham-treated cells (10,000 repetitions in bootstrap ANOVA with contrast tests and a threshold cut-off of twofold change, **P<0.001, red (induced) and green (repressed), log_2-based scale). (C, D) Two putative top networks with high scores (>19) strongly associated with PCNA (C) and ATF3 (D) based on Fisher's exact test (p value < 10^{-19}), indicating a high probability of biological interactions. Up- and down-regulated genes are shown in red and green, respectively. The genes shown in grey are associated with the regulated genes.

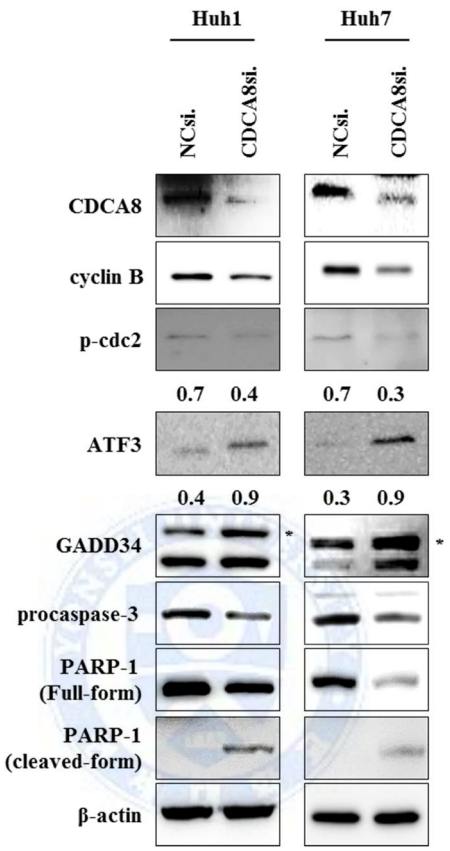

Figure 9. Molecular mechanisms of therapeutic response to CDCA8 targeting. Western blot analysis of CDCA8, ATF3, GADD34, procaspase-3, PARP-1, and cleaved PARP-1 functionally involved in apoptotic progression and p-cdc2 and cyclin B involved in cell cycle regulation respectively. Whole-cell lysates were prepared at 48hr after transfection with CDCA8 siRNA. β-actin was included as a loading control.

IV. DISCUSSIONS

HCC is the fifth most common malignancy and the third highest cause of cancer-related death worldwide and its emergence is increasing.[17] Cell cycle deregulation is one of the hallmarks in diverse types of cancers.[7] CDCA8 overexpression has been found in various types of tumors, including lung, gastric, colorectal, and liver cancer,[11,19,20] implying that CDCA8 targeting may be an effective therapeutic strategy against various cancer types. However, the significance of CDCA8 up-regulation in HCC has remained undetermined. In this study, we demonstrate that siRNA-mediated knockdown of CDCA8 caused a consistent and strong induction of apoptotic cell death and delayed cell cycle progression in the examined human HCC cells. These results suggest that overexpression of CDCA8 may contribute to both cell survival and thus represent a prognostic marker for malignant conversion in HCC.

In a previous study, CDCA8 is associated with in cell cycle progression.[9] However, the molecular mechanism underlying the induction of cancer cell death through CDCA8 knockdown remains unclear. In this study, siRNA-mediated knockdown of CDCA8 induced extensive apoptosis in both the Huh-1 and Huh-7 HCC cell lines, as evidenced by the detection of ccK18 fragments (Figure 6 and Figure 7). The phenotypic changes induced by CDCA8

knockdown were associated with the coordinated and common dysregulation of 50 genes (Figure 8B), as assessed through a global transcriptomic analysis using RNA sequencing, including up-regulation of ATF3 (Figure 8D), tumor suppressor that plays a pivotal role in inhibiting cell proliferations.[21] We also observed that the up-regulation of ATF3 parallels the increase of GADD34 tumor suppressor,[22] a downstream effector of ATF3,[23] and decreases PARP-1, Caspase 3, p-cdc2, and cyclin B (Figure 9), providing mechanisms for CDCA8 silencing mediated inhibition of cell cycle progression and induction of apoptosis in HCC cells. Moreover, CDCA8 silencing increased the expression of apoptosis-related (CYR61[24,25] and Caspase 7[26]) and tumor suppressor (KLF2[27] and CDKN2B[28]), which was paralleled by the down-regulation of key molecules involved in a wide range of cellular response to growth (BGLAP[29] and ADAMTSL4[30]) and cell cycle progression (SLBP[31] and cyclin E[32]), and lipid metabolism (C/ebp[33] and SGPP1[34]). However, the complexity of CDCA8 knockdown relation to lipid metabolism requires further investigation. In future studies, further investigations into the primary effect of CDCA8 knockdown that can elicit the growth inhibition of HCC cells via lipid metabolism are warranted.

In summary, in the present study using human hepatocellular carcinoma cell

lines, we found out that CDCA8 knockdown caused the inhibition of cell proliferation and clonogenicity, induced apoptosis, and led to cell cycle arrest. We also provided evidence for the molecular mechanisms by which CDCA8 silencing induces apoptosis in HCC cells. The anti-tumorigenic effects of CDCA8 knockdown might reflect the up-regulation of the ATF3 tumor suppressor. In conclusion, this study is the first report that targeting of CDCA8 might be used as an effective approach to HCC therapy.

V. CONCLUSION

These data suggest that CDCA8 is an important regulator of HCC cell growth and survival and represent a promising molecular target for systemic therapy of human HCC treatment.

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ABSTRACT (IN KOREAN)

간암 치료 표적을 위한 Cell Division Cycle Associated 8 유전자의 신규 기능 규명

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전 태 원

전 세계적으로, 치사율이 높은 간암은 때늦은 진단과 효과적인 치료법의 부재 등으로 인해 예후가 좋지 않은 양상을 보이는 편이다. 한편, Cell Division Cycle Associated 9 (CDCA8) 유전자는 chromosomal passenger complex 의 구성요소로서, 염색체 분리에 관여하는 양 극성 방추제 안정에 필요한 물질이다. CDCA8 유전자는 간암에서 과발현된다고 알려져 있지만 그 역할에 대해서는 아직 잘 알려져 있지 않다. 그래서 본 연구에서는 CDCA8 유전자를 억제하면 간암 세포의 성장이 억제될 것이라는 가설을 확인해 보고자 하였다.

그 결과, RNA 간섭현상을 이용하여 CDCA8을 억제시켰더니, 세포주기를 억제하고 세포사멸을 유도하여 간암 세포의 증식이 억제된다는 것을 확인할 수 있었고, CDCA8 유전자의 발현 억제에 대한 Next Generation Sequencing (NGS) 발현 분석을 하였더니 CDCA8 유전자의 억제는 세포의 항 증식 효과를 유발한다는 걸 확인할 수 있었으며, 이러한 효과들은 CDCA8에 의해 조절받는 유전자들의 조절장애와 연관이 있다는 알 수 있었다. 항 증식 효과는 일련의 분자적인 변화들에 의해 유발되었으며, 그 중 ATF3 와 GADD34는 상향 조절되었으나, 세포증식 및 세포전이를 조절하는 BGLAP의 발현은 억제됨을 확인하였다. 또한, CDCA8 유전자의 억제는 세포사멸 신호를 증가시키는 caspase-3 과 PARP-1의 활성을 증가시킴을 확인하였다. 이와 같은 결과들로 보아, 본 연구의 결과는 CDCA8 유전자가 간암의 표적치료로 사용 될 수 있는 분자적 타겟이 될 수 있을 것이라 생각된다.

핵심되는 말: CDCA8, 간암, 표적치료, 세포사멸, 세포주기