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HMGCS2 functions as a tumor suppressor and has a prognostic impact in prostate cancer

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ABSTRACT

Background: Accumulating studies reported that 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) may function as either an oncogene or a tumor suppressor in various human cancers. However, its involvement in prostate cancer (PCa) remains unknown. Therefore, the aim of this study was to investigate the clinical significance of HMGCS2 expression and its functions in PCa.

Methods: Expression levels of HMGCS2 mRNA and protein were detected by quantitative Polymerase Chain Reaction (qPCR), Western blot and immunohistochemistry, respectively. Associations of HMGCS2 expression with various clinicopathological features and patients' prognosis of PCa were statistically evaluated. Roles of HMGCS2 dysregulation in cell proliferation, invasion and migration of PCa cell lines were also determined.

Results: HMGCS2 protein expression was significantly reduced in PCa tissues compared to adjacent benign prostate tissues at protein levels ($P < 0.05$). Clinically, low HMGCS2 mRNA expression was dramatically associated with high Gleason score (GS) and pathological grade, as well as the presence of distant metastasis of PCa patients. In addition, PCa patients with low HMGCS2 mRNA expression more frequently had shorter disease-free survival and biochemical recurrence-free survival (all $P < 0.05$). HMGCS2 expression was identified as an independent factor to predict both disease-free and biochemical recurrence-free survivals of PCa patients. Moreover, loss-of-function experiments demonstrated that HMGCS2 knockdown-expression promotes cell proliferation, colony formation, invasion and migration of PCa cells in vitro and lower the apoptotic rate of PCa cells in vitro.

Conclusions: Our data indicate that HMGCS2 may be capable of predicting the risk of biochemical recurrence in PCa patients after radical prostatectomy and functions as a tumor suppressor in PCa cancer, implying its related pathway potential as a drug candidate in anti-PCa therapy.

1. Background

Prostate cancer (PCa) represents one of the most diagnosed genitourinary malignancy in males worldwide and ranks as the second leading cause of cancer-related death in men in the USA [1,2]

Although radical prostatectomy (RP) is the primary treatment for localized PCa, about 25–50 % of patients with organ-confined PCa fail local therapy and develop biochemical recurrence (BCR), which is considered to be the earliest indicator of recurrent disease [3]. The five-year survival rate for patients with localized PCa is nearly 100%, in contrast, this rate for patients with BCR is approximately 20–25% [4]. Growing clinical evidence suggest that PCa patients with an equivalent prostate-specific antigen (PSA) level, Gleason score, and pathological stage often have different outcomes, which may be caused by molecular heterogeneous subtypes [5]. Therefore, it is a major clinical challenge to understand the molecular pathology underlying carcinogenesis and cancer progression of PCa for identifying effective indicators of prognosis and establishing efficient therapeutic strategies.

Emerging evidence indicates that fundamental differences exist between the metabolic pathways of normal and malignant cells [6]. In contrast to normal cells, which derive most of their usable energy through oxidative phosphorylation, cancer cells depend heavily on

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substrate phosphorylation pathways to meet energy demands [7]. Ketone bodies was a product of substrate phosphorylation pathways which may be vital fuel in ketogenesis for tumor initiation or metastasis [8]. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS2) a part of the HMG-CoA family of proteins, is a potential regulatory point in the pathway that converts acetyl-CoA to ketone bodies [9–11]. The expression of HMGCS2 has been found in liver, pancreas and skeletal muscle [12]. It mainly localizes in colonic epithelium to regulate cell differentiation [13]. In human cancers, HMGCS2 is differently expressed in estrogen receptor-negative breast cancer [14]and apocrine carcinoma of the breast [15]. HMGCS2 mRNA was increased in colorectal cancer [16], rectal cancer [17], and correlated with poor prognosis and chemoradiotherapy. On the other hand, HMGCS2 expression was decreased in esophageal squamous cell carcinoma and associated with poor 5-year overall survival and relapse-free survival [18]. In particular, Saraon et al. [19], reported that the enzymes of the ketogenic pathway, including HMGCS2, were dysregulated in high-grade PCa, implying a possibility to be candidate biomarkers for the diagnosis and prognosis of high-grade disease. However, the involvement of HMGCS2 in PCa has not been fully elucidated.

To investigate the clinical significance of HMGCS2 expression and its functions in PCa, herein, we detected the expression levels of HMGCS2 mRNA and protein by quantitative Polymerase Chain Reaction (qPCR), Western blot and immunohistochemistry, respectively. Then, the associations of HMGCS2 expression with various clinicopathological features and patients' prognosis of PCa were statistically evaluated. Roles of HMGCS2 dysregulation in cell proliferation, colony formation, invasion and migration of PCa cell lines were also determined.

2. Methods

2.1. Patients and tissue samples

For immunohistochemistry analysis, a tissue microarray (TMA) of 80 cases containing 50 cases of prostate cancer, 20 case of hyperplasia prostate tissue, 7 cases of adjacent benign tissue and 3 normal prostate tissue was purchased from Biomax company (Cat No. PR807c, USA). Detailed clinical information is included in this TMA. Patients who had been administered chemotherapy or radiotherapy prior to surgery were all excluded from this study.

For further evaluation of the clinical relevance and further survival analysis of HMGCS2, tow publicly available dataset of prostate cancer was collected and analysis respectively, The first data is The Cancer Genome Atlas (TCGA) (http://www.cbioportal.org/study?id=prad_tcga#summary) including 498 primary PCa patients with mRNA sequencing expression data and relevant clinical information, the second is Taylor's Data from GEO (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE21032) including 150 primary PCa patients with microarray expression data.

2.2. Cell culture

the human PCa cell lines, LNCaP, DU145 were purchased from the American Type Culture Collection (Manassas, VA, USA) and were cultured in RPMI 1640 medium (Hyclone, USA) supplemented with 10% fetal bovine serum (Gibico, USA), 2 mM L-glutamine, and antibiotics. All cell lines were maintained at 37 ℃ in a humidified chamber supplemented with 5% CO_2.

2.3. Cell transfection

To inhibit the expression of HMGCS2 mRNA and protein in PCa cells, the lentiviral short hairpin RNA (shRNA) plasmid of targeting HMGCS2 gene was construct by HYYMed company (China). The LNCaP, DU145 cells lines were transfected by the lentivirus of shRNA and finally got the stable cell line through puromecin treatment.

2.4. Western blot analysis

Expression levels of HMGCS2 protein in PCa cell lines and clinical PCa tissues were detected by Western blot analysis according to the protocol of our previous studies [20]. The used antibodies detail are: anti-HMGCS2 rabbit monoclonal antibody (Cat No. ab59235, Abcam, USA), anti-GAPDH rabbit monoclonal antibody (Cat No. M30008Abmart, China).

2.5. Immunohistochemistry

Cellular localization and expression levels of HMGCS2 protein in clinical PCa tissues were examined by immunohistochemistry. The immunohistochemistry staining was conducted using the UltraSensitive™ SP (Mouse/Rabbit) IHC kit (catalog no. KIT-0305; MX Biotechnologies, Fuzhou, China) which contained endogenous peroxidase blocking solution, serum, secondary antibody, streptavidin-perosidase and DAB substrate-chromogen. The tissues sections were incubated overnight at 4 ˚C with a rabbit anti-human HMGCS2 Polyclonal antibody (rabbit monoclonal antibody, ab59235, abcam Co. Ltd., UK), at a dilution of 1:100. Following washing, the sections were incubated with an avidin-conjugated secondary antibody which contained in UltraSensitive™ SP (Mouse/Rabbit) IHC kit (catalog no. KIT-0305; MX Biotechnologies, Fuzhou, China) for 30 min at room temperature. Streptavidin-perosidase Peroxidase-labeled polymer (50 μl for 15 min at room temperature) and substrate-chromogen (100 μl for 2 min at room temperature) were used to observe the staining of the target protein. For negative controls, the primary antibody was omitted in each IHC run [20].

The intensity of dyeing score was according to the protocol in reference [21]: Given the heterogeneity of the staining of the proteins, tumor specimens were scored in a semiquantitative manner. The percentage was grouped as follows: 0 (0%), 1 (1–10%), 2 (11–50%) and 3 (> 50%). The staining intensity was categorized as follows: 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). A final score was obtained for each case by the multiply of the percentages and the intensity scores. Finally, according to HMGCS2 protein expression samples were divided into two groups with high (IRS ≥ 4) and low (IRS < 4).

2.6. RT-qPCR

Total RNA was extracted from cultured prostate cells (˜5 × 106 cells) with the RNeasy mini kit (Qiagen, Germany). The Invitrogen SuperScript III First-Strand System (Invitrogen; Thermo Fisher Scientific, Inc.) was used for reverse transcription (RT) with random primers [Hexadeoxyribonucleotide mixture; pd (N)6; cat. no. 3801; Takara Biotechnology Co., Ltd., Dalian, China]. Real-time PCR primers were as follows: HMGCS2 forward, 5′- TCG ACC CAA CAA TAA CAG ATG C -3′ and reverse, 5′- TCT CGT ATC TTT CTT GGC GAC T -3′; GAPDH forward, 5′- CAT GGG TGT GAA CCA TGA GAA GTA -3′ and reverse, 5′- CAG TAG AGG CAG GGA TGA TGT TCT -3′. Amplification was achieved using the following protocol: 48 ˚C for 30 min, 95 ˚C for 1 min followed by 40 cycles at 95 ˚C for 15 s, 52 ˚C for 30 s and 72 ˚C for 30 s. The relative mRNA expression levels of HMGCS2 were normalized to GAPDH mRNA. The results were calculated using the $2^{-\Delta\Delta}$Cq method [22].

2.7. Cell proliferation, colony formation, invasion and migration assay and apoptotic rate test

Functions of HMGCS2 in cell proliferation, colony formation, invasion and migration of PCa cells were evaluated by CCK-8, colony formation assay, transwell and the scratch wound-healing motility assays, apoptotic rate test by cytometer, respectively, according to our

previous description [20,21].

2.8. Statistical analyses

SPSS software for Windows (version 17.0, SPSS Inc, IL, USA) was used to perform all statistical analyses in the current study. Data of continuous variables were expressed as mean ± S.D. Western blot and qPCR data were statistically analyzed using Wilcoxon signed-rank test. Associations of HMGCS2 mRNA and protein expression with various clinicopathological parameters were evaluated by Pearson $\chi 2$ test or Fisher's exact test. Survival analysis was performed by Kaplan-Meier method and Cox regression model. For in vitro functional analyses, the differences between groups were analyzed using a Student *t* test when comparing only two groups or one-way analysis of variance when comparing more than two groups. Differences were considered statistically significant when the *P* value was less than 0.05.

3. Results

3.1. HMGCS2 protein expression levels were decreased in PCa tissues

Immunohistochemistry analysis was performed to examine the expression patterns of HMGCS2 protein in human PCa tissues and benign prostate tissues. The HMGCS2 protein were mainly expressed in the cytoplasm and cytomembrane of prostate cancer. Consistently, the positive immunostainings of HMGCS2 protein in PCa tissues were distinctly weaker than that in benign prostate tissues as shown in Fig. 1**C**((IRS of prostate cancer patients tissue: 2.73 ± 2.33; IRS of normal prostate tissue: 4.10 ± 1.66, IRS of hyperplasia prostate tissue: 3.95 ± 1.55, Fig. 1**B, C**).). Statistically and more importantly, the immunoreactive scores of HMGCS2 protein in PCa tissues with high Gleason score were also decreased compared to those with low Gleason score (IRS of prostate cancer patients with GS < 7: 4.67 ± 2.50; IRS of prostate cancer patients with GS ≥ 7: 2.47 ± 2.21, P = 0.029, Fig. 1**B, C**).

3.2. Reduced expression of HMGCS2 associates with aggressive clinicopathological parameters of PCa patients

To evaluate the associations of HMGCS2 expression with various clinicopathological parameters of PCa, all PCa patients in three groups were separatively divided into low HMGCS2 expression and high HMGCS2 expression groups by using the median HMGCS2 expression level as a cut-off value. As shown in Table 1, in our TMA cohort, the PCa patients with low HMGCS2 protein expression more frequently had high Gleason score than those with high HMGCS2 protein expression (P = 0.044). Of the 44 prostate cancer tissue with GS ≥ 7 samples, 34 (77.3%) demonstrated low levels, while 10 (22.7%) high levels of HMGCS2. Furthermore, the protein expression levels of HMGCS2 in tumor tissues were significantly increased compared with normal prostate tissues (2.73 ± 2.33 and 4.10 ± 1.66, respectively; P = 0.041; Fig. 1).

In addition, we also analyzed the clinical relevance of HMGCS2 mRNA in PCa based on TCGA and Taylor data. Similarly, reduced expression of HMGCS2 mRNA was significantly associated with high Gleason score (for TCGA data: P < 0.001; for Taylor data: P < 0.001, Table 1). More importantly, PCa patients with advanced pathological grade (for Taylor data: P = 0.019, Table 1) and tumor stage (for TCGA data: P = 0.013, Table 1), as well as with positive lymph node metastasis and distant metastasis (for Taylor data: both P < 0.001 both, Table 1) often had lower HMGCS2 mRNA expression levels than those with early pathological grade, tumor stage, lymph node metastasis, and

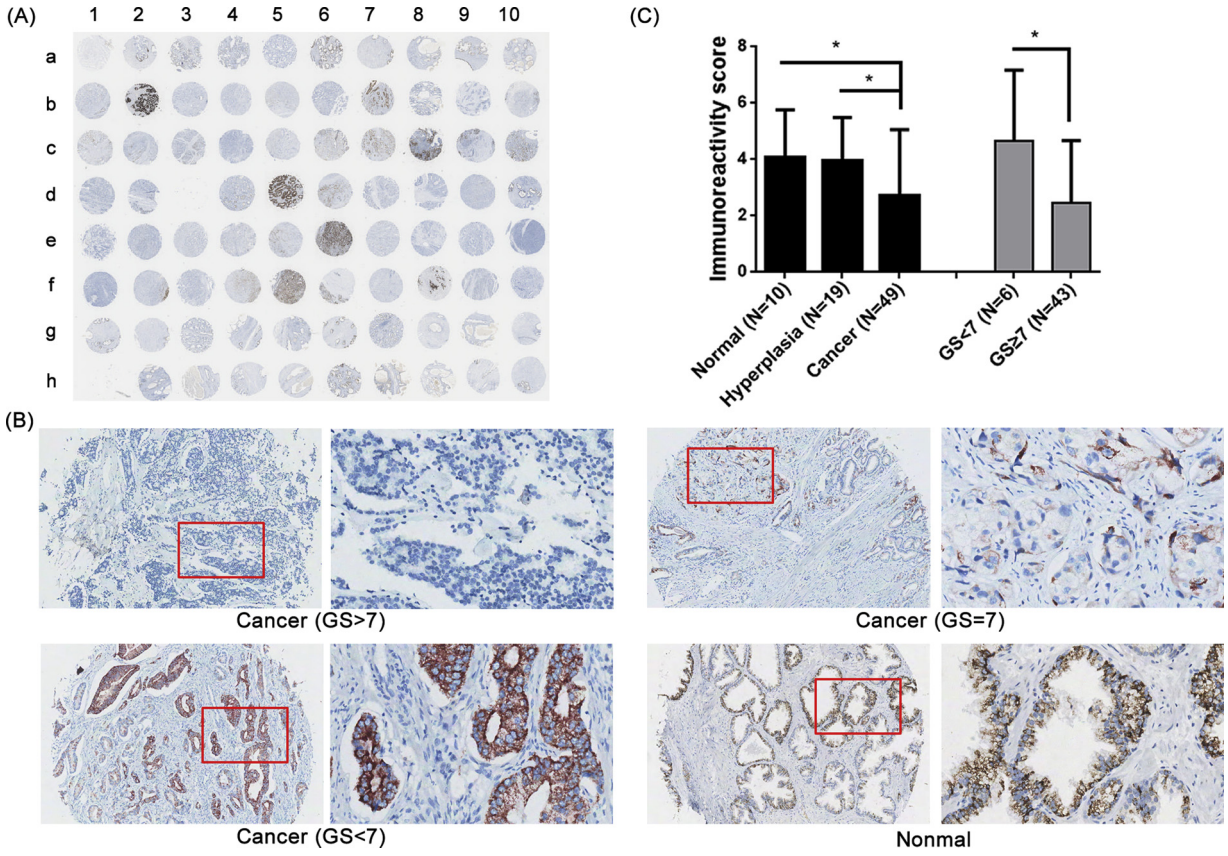

Fig. 1. HMGCS2 protein expression were decreased in PCa tissues. (A and B) Immunohistochemical stainings of HMGCS2 protein in benign prostate tissues and PCa tissues; (C) Statistically, the immunoreactive scores of HMGCS2 protein in PCa, hyperplasia and normal prostate tissues, PCa tissues with high Gleason score were also decreased compared to those with low Gleason score (both P < 0.05).

Table 1
Correlation of HMGCS2 expression with clinicopathologic characteristics in patients with prostate cancer.

Clinical features		TMA			TCGA				Taylor			
	Case	Low, n (%)	High, n (%)	*P*	Case	Low, n (%)	High, n (%)	*P*	Case	Low, n (%)	High, n (%)	*P*
Age												
< 60	2	1(50.0)	1(50.0)	0.486	202	93(46.0)	109(54.0)	0.085	93	35(37.6)	58(62.4)	0.594
≥60	48	35(72.9)	13(27.1)		296	156(52.7)	140(47.3)		57	19(33.3)	38(66.7)	
Serum PSA levels (ng/ml)												
< 4	—		—	—	414	206(49.8)	208(50.2)	0.225	24	8(33.3)	16(66.7)	0.122
≥4	—		—		27	16(59.3)	11(40.7)		123	60(48.9)	53(43.1)	
Gleasonscore												
< 7	6	2(33.3)	4(66.7)	0.044*	292	126(43.2)	166(56.8)	< 0.001**	117	34(29.1)	83(70.9)	0.001**
≥7	44	34(77.3)	10(22.7)		206	123(59.7)	83(40.3)		22	15(68.2)	7(31.8)	
Pathological grade												
< 2	1	1(100.0)	0(0.0)	0.720	—	—	—	—	86	24(27.9)	62(72.1)	0.019*
≥2	49	35(71.4)	14(28.6)		—	—	—		55	26(47.3)	29(52.7)	
Tumor stage												
T1	1	0(0.0)	1(100.0)	0.280	178	78(43.8)	100(56.2)	0.013*	80	34(42.5)	46(57.5)	0.204
T2-T4	49	36(73.5)	13(26.5)		229	127(55.5)	102(44.5)		65	33(50.8)	32(49.2)	
Lymph node metastasis												
N1	7	4(57.1)	3(42.9)		80	47(58.8)	33(41.2)		16	12(75.0)	4(25.0)	
N1	7	4(57.1)	3(42.9)		80	47(58.8)	33(41.2)		16	12(75.0)	4(25.0)	
Distant metastasis												
M0	44	32(72.7))	12(27.3)	0.545	456	228(50.0)	228(50.0)	0.502	122	36(29.5)	86(70.5)	0.001**
M1	6	4(66.7)	2(33.3)		3	2(66.7)	1(33.3)		28	18(64.3)	10(35.7)	

Note: The "–" indicates that there is no information of patients in our cohort. * P < 0.05, ** P < 0.01.

negative distant metastasis.

3.3. Reduced expression of HMGCS2 mRNA predicts poor prognosis in PCa patients

On the basis of the TCGA and Taylor datasets, we evaluated the prognostic impact of HMGCS2 mRNA expression in PCa patients using the median value of its expression levels as a cutoff point for grouping. As shown in Fig. 2, pairwise comparisons showed significant differences in the disease-free survival (for TCGA dataset: P < 0.001, Fig. 2A) and BCR-free survival (for TCGA dataset: P < 0.001, Fig. 2**B**; for Taylor dataset: P = 0.014, Fig. 2C) between PCa patients in low and high HMGCS2 mRNA expression groups. Further univariate and multivariate analyses using a COX regression model revealed that HMGCS2 mRNA expression was significantly associated with disease-free and BCR-free survivals of PCa patients. In TCGA dataset, the HR of HMGCS2 mRNA expression for the disease-free survival and BCR-free survival was 0.385 (0.248-0.598), P < 0.001 and 0.411(0.231-0.730), P = 0.002, respectively. In Taylor dataset, the HR of HMGCS2 mRNA expression for the BCR-free survival was 0.346 (0.177-0.678), P = 0.002. It was demonstrated that HMGCS2 mRNA act as a suppressor gene in prostate cancer. PSA, Gleason score and Tumor stage were the gold standard of prostate cancer. In order to evaluate whether or not the HMGCS2 can be an independent prognostic factor for prostate cancer, we selected PSA, Gleason score, Tumor stage and HMGCS2 mRNA expression for the multivariate analysis. In TCGA dataset group, HMGCS2 mRNA expression together with the factor of PSA, Gleason score and tumor stage can be an independent prognostic factor. But in the Taylor dataset group, the result of multivariate analysis is negative which selected PSA, Gleason score, Tumor stage and HMGCS2 mRNA expression four factors, so we performed the multivariate analysis included tumor stage (HR: 0.447(0.229-0.871), P < 0.001) and HMGCS2 mRNA expression (HR: 0.447(0.229-0.871), P = 0.018) (Table 2).

3.4. HMGCS2 inhibits cell proliferation, colony formation, invasion and migration of PCa cells in vitro

To assess the effects of HMGCS2 in aggressive features of PCa cells, we established stable cell lines LNCaP and DU145 with the reduced expression of HMGCS2 following lentivectors transfection of the specific shRNA. Both qPCR and Western blot analyses confirmed that the cell lines were successfully established (Fig. 3A). CCK-8 assay and colony formation assay showed that the knockdown of HMGCS2 distinctly promote the cell proliferation and colony formation of PCa cell lines (CCK8 for DU145 and LNCaP cells: both P < 0.05, Fig. 3**B**; colony formation for DU145 and LNCaP cells: both P < 0.01, Fig. 3C).

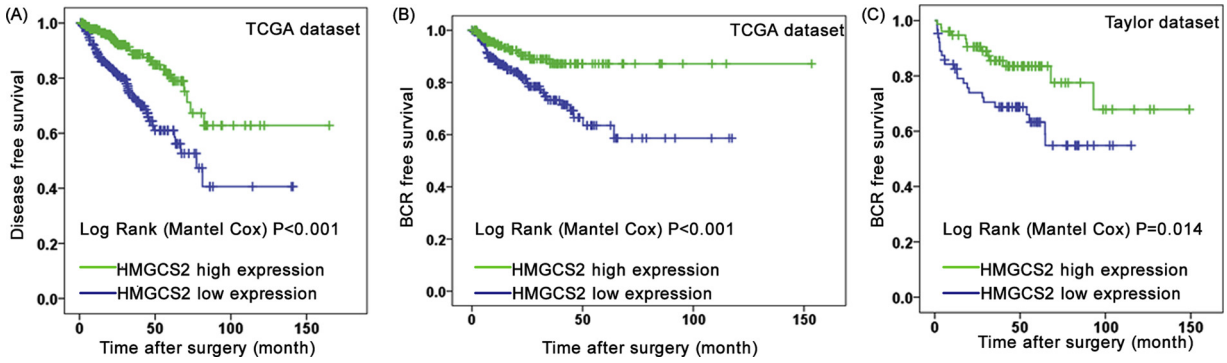

Fig. 2. Kaplan-Meier analyses of disease-free (A) and biochemical recurrence (BCR)-free (B and C) survivals of prostate cancer (PCa) patients based on HMGCS2 expression in TCGA and Taylor datasets. We dichotomized its expression level into high and low groups based on median cutoff of their expression data.

Table 2
Prognostic value of HMGCS2 expression for the survival by Cox proportional hazards model.

Variable	TCGA dataset (Disease free survival)		TCGA dataset (BCR free survival)		Taylor dataset (BCR free survival)	
	HR (95%CI)	*P*	HR (95%CI)	P	HR (95%CI)	*P*
Univariate analysis						
Age(≥60 vs. < 60)	1.488(0.968-2.287)	0.070	1.386(0.806-2.385)	0.238	1.092(0.577-2.067)	0.786
Gleason score(> 7 vs. ≤ 7)	9.464(1.319-67.921)	0.025*	3.110(1.841-5.255)	< 0.001**	9.167(2.417-34.767)	0.001**
Tumor stage (T2-T4 vs. T1)	2.972(1.732-5.102)	< 0.001**	3.340(1.673-6.669)	0.001**	5.808(2.954-11.422)	< 0.001**
Lymph Node Stage (N1 vs. N0)	1.801(1.111-2.920)	0.017*	1.879(1.049-3.365)	0.034*	13.341(3.518-50.590)	< 0.001**
Distant metastasis (M1 vs. M0)	20.22(0.000-10172941)	0.740	20.271(0.000-340180034)	0.723	19.578(10.038-38.187)	< 0.001**
PSA(≥4 vs. < 4)	2.750(1.376-5.494)	0.004**	6.279(3.131-12.592)	< 0.001**	0.679(0.232-1.933)	0.481
HMGCS2 expression(high vs. low)	0.385(0.248-0.598)	< 0.001**	0.411(0.231-0.730)	0.002**	0.346(0.177-0.678)	0.002**
Multivariate analysis						
Gleason score(> 7 vs. ≤ 7)	3.103(1.916-5.025)	< 0.001**	2.172(1.228-3.842)	0.008**	—	—
Tumor stage (T2-T4 vs. T1)	2.139(1.231-3.715)	0.007**	2.321(1.145-4.705)	0.001**	4.636(2.256-9.525	< 0.001**
PSA(≥4 vs. < 4)	2.172(1.053-4.481)	0.036*	3.270(1.523-7.021)	0.002**	—	—
HMGCS2 expression(high vs. low)	0.468(0.283-0.774)	0.003**	0.488(0.261-0.914)	0.025*	0.447(0.229-0.871)	0.018*

Note: The "–" indicates that there is no information of patients in our cohort. * P < 0.05, ** P < 0.01.

Transwell assay and Wound-healing assay clearly revealed that the knockdown of HMGCS2 significantly enhance the invasive activities and the migratory abilities of both DU145 and LNCaP cells compared with those of control cells (all P < 0.05, Fig. 3**D,3E**). In contrast, the knockdown of HMGCS2 expression with the specific shRNA in DU145 and LNCaP cell lines could dramatically increase the apoptotic rate (both P < 0.01, Fig. 3F).

4. Discussion

Cancer cell metabolism has been indicated to be closely related to carcinogenesis and cancer progression by offering enough energy through enhancing lipid biosynthesis and glycolysis [8**Error! Bookmark not defined.**]. In particular, lipid metabolism may be an essential process to influence various aggressive phenotypes of cancer cells, such as cell growth, proliferation, invasion, migration, dissemination under oxidative or energy stress [23]. During this process, the ketogenic pathway is a critical part which produces ketone bodies via decomposing acetyl-CoA to provide energy for cancer cells in the form of ATP [24]. HMGCS2, as a mitochondrial enzyme involved in the ketogenic pathway, has been reported to be correlated with the development, progression and patients' survival of several cancers [20,21], but its involvement into PCa remains unknown, thus, HMGCS2 draws our attention. In the current study, we firstly confirmed the downregulation of HMGCS2 protein in clinical prostate cancer tissues. Statistically, low HMGCS2 expression was dramatically associated with high Gleason score and pathological grade, as well as the presence of distant metastasis of PCa patients. Notably, PCa patients with low HMGCS2 expression more frequently had shorter disease-free and biochemical recurrence-free survivals. HMGCS2 expression was identified as an independent factor to predict both disease-free and biochemical recurrence-free survivals of PCa patients. These findings support the evidence that HMGCS2 downregulation may contribute to prostate carcinogenesis, aggressive progression and unfavorable clinical outcome of patients.

As a rate-limiting enzyme, HMGCS2 controls generation and reutilization of ketone bodies which drive neoplastic growth accompanied by starvation of all components of tumor environment [25]. The *HMGCS2* gene has been reported to be expressed in liver, colon, pancreas, heart, testis and skeletal muscle of the human body [26]. Functionally, it condenses acetyl-CoA with acetoacetyl-CoA to form HMG-CoA, catalyzing the first reaction in ketogenesis. The aberrant expression of HMGCS2 has been observed in several human cancers. In a recent report of Tang et al. [18], HMGCS2 downregulation more frequently occurred in primary esophageal squamous cell carcinoma tissues than their non-tumor counterparts, and also was significantly associated with advanced clinicopathological features, as well as the shorter five-year overall survival and relapse-free survival; Camarero et al. [27] indicated that HMGCS2 protein expression was down-regulated preferentially in moderately and poorly differentiated colon carcinomas; In contrast, Wang [28] and Gromov [15] groups respectively found that HMGCS2 was overexpressed in estrogen receptor-negative breast cancer and apocrine carcinoma of the breast, respectively; Chen et al. [16] observed that HMGCS2 gene expression was increased in colorectal cancer, and correlated with poor prognosis; Lee et al. [17] reported HMGCS2 overexpression in non-responders to neoadjuvant concurrent chemoradiotherapy in rectal cancer. The above data suggest that HMGCS2 may be differentially expressed and exert different functions in various human cancers. To the best of our knowledge, no data concerning HMGCS2 expression in PCa are available. Herein, following our confirmation of HMGCS2 downregulation in PCa cells and tissues, as well as its associations with aggressive cancer progression and prognosis, we hypothesized that HMGCS2 might function as a tumor suppressor in this cancer. To verify this hypothesis, we performed loss-of-function experiments to demonstrate that HMGCS2 knockdown expression promote cell proliferation, colony formant, invasion and migration of PCa cells in vitro, and lower the apoptotic rate of PCa cells in vitro. The molecular mechanisms underlying the tumor suppressive role of HMGCS2 in PCa remain uncovered and required further investigation.

5. Conclusions

Our data indicate that HMGCS2 may be capable of predicting the risk of biochemical recurrence in PCa patients after radical prostatectomy and function as a tumor suppressor in this cancer, implying its related pathway potential as a drug candidate in anti-PCa therapy.

Competing interests

The authors declare that they have no competing interests.

Compliance with ethical standards

This study was approved by the human study ethics committees at Guangzhou First People's Hospital. All procedures performed in these studies involving human participants were in accordance with the ethical standards of the institutional research committee. Informed consent was also obtained from all patients. All samples were handled and made anonymous according to the ethical and legal standards.

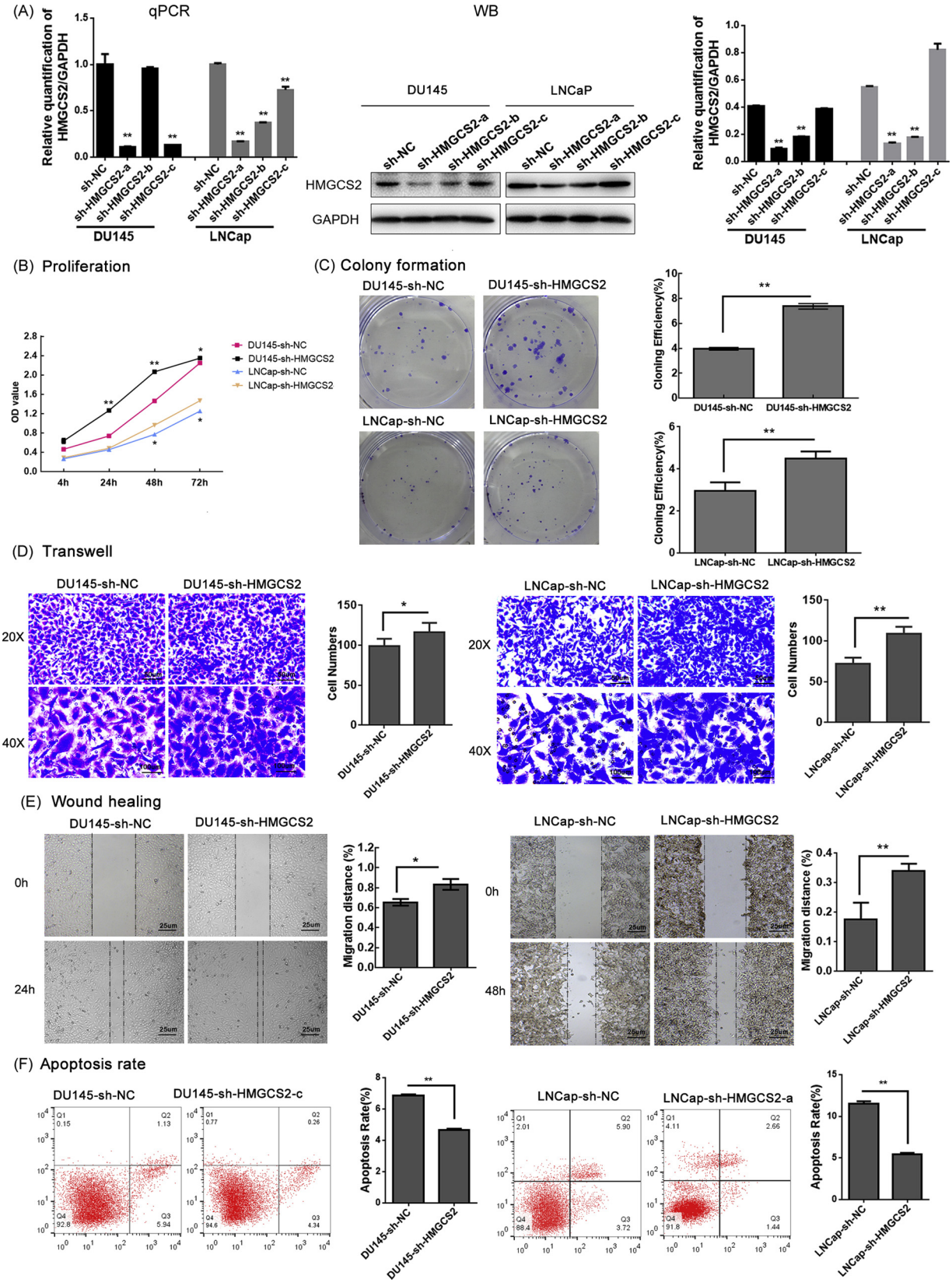

Fig. 3. Knockdown of HMGCS2 expression enhances cell proliferation, colony formantion, invasion and migration of LNCaP and DU145 cells. (A). The inhibition of HMGCS2 in both LNCaP and DU145 cells was verified by qRT–PCR and Western blot after transfection. (B¯E). CCK-8, Colony formation, Transwell and Wound healing analyses showed that the downregulation of HMGCS2 enhanced cell proliferation, invasive ability and motility of LNCaP and DU145 cells, respectively. (F) Apoptosis analyses about LNCaP and DU145 cells which knockdown the HMGCS2. Statistical analysis was performed with three independent experiments. Data were presented as Mean ± SD. *P < 0.05, **P < 0.01 compared with negative control.

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Authors' contributions

WZ and YW: participated in study design and coordination, analysis and interpretation of data, material support for obtained funding, and supervised study. SW, MX and HZ: performed most of the experiments and statistical analysis and drafted the manuscript. Other authors: carry out the experiments and sample collection. All authors read and approved the final manuscript.

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Not applicable.

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