Int J Clin Exp Pathol 2018;11(12):5682-5693
www.ijcep.com /ISSN:1936-2625/IJCEP0080604

Original Article

WDR1 predicts poor prognosis and promotes cancer progression in hepatocellular carcinoma

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Received May 30, 2018; Accepted August 23, 2018; Epub December 1, 2018; Published December 15, 2018

Abstract: WDR1, an activator of cofilin-mediated actin depolymerization, is involved in various actin-dependent processes of living cells including cell migration and cytokinesis. Recently, several studies have found that WDR1 is dysregulated in several types of cancer and is associated with cancer metastasis. However, its role in hepatocellular carcinoma (HCC) remains unknown. In this study, we found that WDR1 expression was aberrantly upregulated at the mRNA and protein levels in HCC cell lines and HCC tissues. WDR1 overexpression was highly correlated with tumor aggressive phenotypes such as capsulation formation, microvascular invasion (MVI), tumor node metastasis (TNM) stage, and was an independent poor prognostic factor for overall survival (OS) and disease-free survival (DFS) for HCC patients after curative surgery. Furthermore, WDR1 overexpression significantly promoted HCC cell migration, invasion and proliferation. In contrast, WDR1 downregulation inhibited HCC cell migration, invasion and proliferation. Conclusion: This study indicates that WDR1 could be used as a new useful prognostic marker and may be a potential therapeutic target for HCC.

Keywords: WDR1, hepatocellular carcinoma, progression, prognosis

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide [1]. Liver resection is still the mainstay treatment of choice for HCC [2]. Though lots of improvement have been achieved in hepatic surgery techniques during recent decades, the 5-year overall survival rate of HCC patients still hovers around 30% [3]. It is mainly due to the high rate of tumor metastasis and recurrence after liver resection. Therefore, a better understanding of the molecular features involved in metastasis is urgently needed.

WD-repeat protein (WDR) 1, also named AIP1, is an activator of cofilin-mediated actin depolymerization [4, 5]. It is well documented that WDR1 is involved in regulating the dynamics of the cytoskeleton and various actin-dependent biological processes of living cells, especially in cell migration and cytokinesis [6-8]. Recently, the role of WDR1 in tumors has gotten increased attention, and aberrant WDR1 expression has been observed in several tumors including breast cancer, thyroid neoplasia, ovarian carcinoma, and glioblastoma [9-12]. Especially in breast cancer, the data showed that WDR1 could remarkably promote cancer cell migration and be closely associated with tumor metastasis [13]. In primary glioblastoma, high WDR1 expression was significantly correlated with tumor invasive phenotypes [12]. These data indicate that WDR1 plays a critical role in tumor progression, which prompts us to hypothesize that WDR1 may also play a role in the progression of HCC. Yet, its significance in HCC still remains unknown.

In this study, the expression of WDR1 mRNA and protein in HCC cell lines and HCC tissues was detected. The relationship of WDR1 expression with clinicopathological characteristics and postoperative survival in 106 HCC patients were analyzed. Furthermore, we investigated the function of WDR1 in HCC by a series of *in vitro* experiments.

Materials and methods

HCC samples

The study was approved by the Ethics Committee of The First Affiliated Hospital of Nanchang University and Jiangxi Pingxiang People's Hospital. 30 pairs of frozen fresh HCC tissues and corresponding adjacent nontumorous liver tissues (ANLTs) were collected at the Department of General Surgery, The First Affiliated Hospital of Nanchang University and Jiangxi Pingxiang People's Hospital from January 2015 to April 2015. 106 pairs of paraffin-embedded HCC tissues and matched ANLTs were randomly selected from HCC patients undergoing surgical resection at the Department of General Surgery, The First Affiliated Hospital of Nanchang University (Nanchang, China) from January 2010 to January 2012. All patients enrolled in this study were histopathologically diagnosed and had follow-up data recorded.

Prognostic study

All HCC patients were regularly followed-up by experienced and well-trained researchers. The follow-up period was defined as the interval between the date of the operation and the patient's death or the last follow-up. The median follow-up was 39.0 months (range 3.0-96.0 months). Deaths from other causes were treated as censored cases. The recurrence and metastasis was surveillance by clinical examination, serial monitoring of alpha-fetoprotein (AFP) levels and ultrasonography (US) or computed tomography (CT), or a magnetic resonance imaging (MRI) scan at a 3 month interval. Recurrence and metastasis were diagnosed by clinical examination, serial AFP level, and US, or CT, or MRI scan. Disease-free survival (DFS) was defined as the length of time after liver resection during which a patient survived without signs of HCC recurrence or metastasis. Overall survival (OS) was defined as the interval between the operation and death or between the operation and the last observation for the surviving patients. Data of conventional clinical and pathological variables were also collected for analysis, including gender, age, serum AFP level, HBsAg, liver cirrhosis, Child-Pugh classification, tumor number, tumor size, capsular formation, microvascular invasion (MVI), Edmondson-Steiner grade, TNM stage, and BCLC stage. MVI was defined as tumor cells forming a thrombus in the peritumoral vessels, and can only be assessed after careful histological assessment of the whole surgical specimen [14, 15]. The follow-up data were regularly updated in the database for each patient. Patients alive at the end of follow up or dead from causes without any sign of recurrence or metastasis were censored.

Cell lines and cell culture

PLC/PRF5, Hep3B and HepG2 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA). The HCCLM3 was a gift from the Liver Cancer Institute of Fudan University (Shanghai, China). The L02 was bought from the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's Modified Eagle medium (DMEM, Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Life Technologies), and maintained in 5% CO_2 humidified incubator at 37°C.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cell lines and fresh frozen tissue specimens using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. qRT-PCR was carried out using a SYBR® Green Real-time PCR Master Mix assay kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. The fold inductions of mRNA expression were calculated using the formula $2^{-\Delta\Delta Ct}$. The primers of WDR1 were as follows: forward, 5'-TGGGATTTACGCAATTAGTTGGA-3', reverse, 5'-CCAGATAGTTGATGTACCCGGAC-3'. GAPDH was taken as a control using the following primers: forward, 5'-GTCTCCTCTGACTTCAACAGCG-3', reverse, 5'-ACCACCCTGTTGCTGTAGCCAA-3'.

Western blot (WB)

Total protein was extracted using a RIPA lysis buffer, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). The blotted membranes were incubated with 1:1000 rabbit anti-WDR1 polyclonal antibody (Protientech, Wuhan, China) at 4°C overnight. After washing with PBST, the membrane was incubated with a 1:4000 HRP-

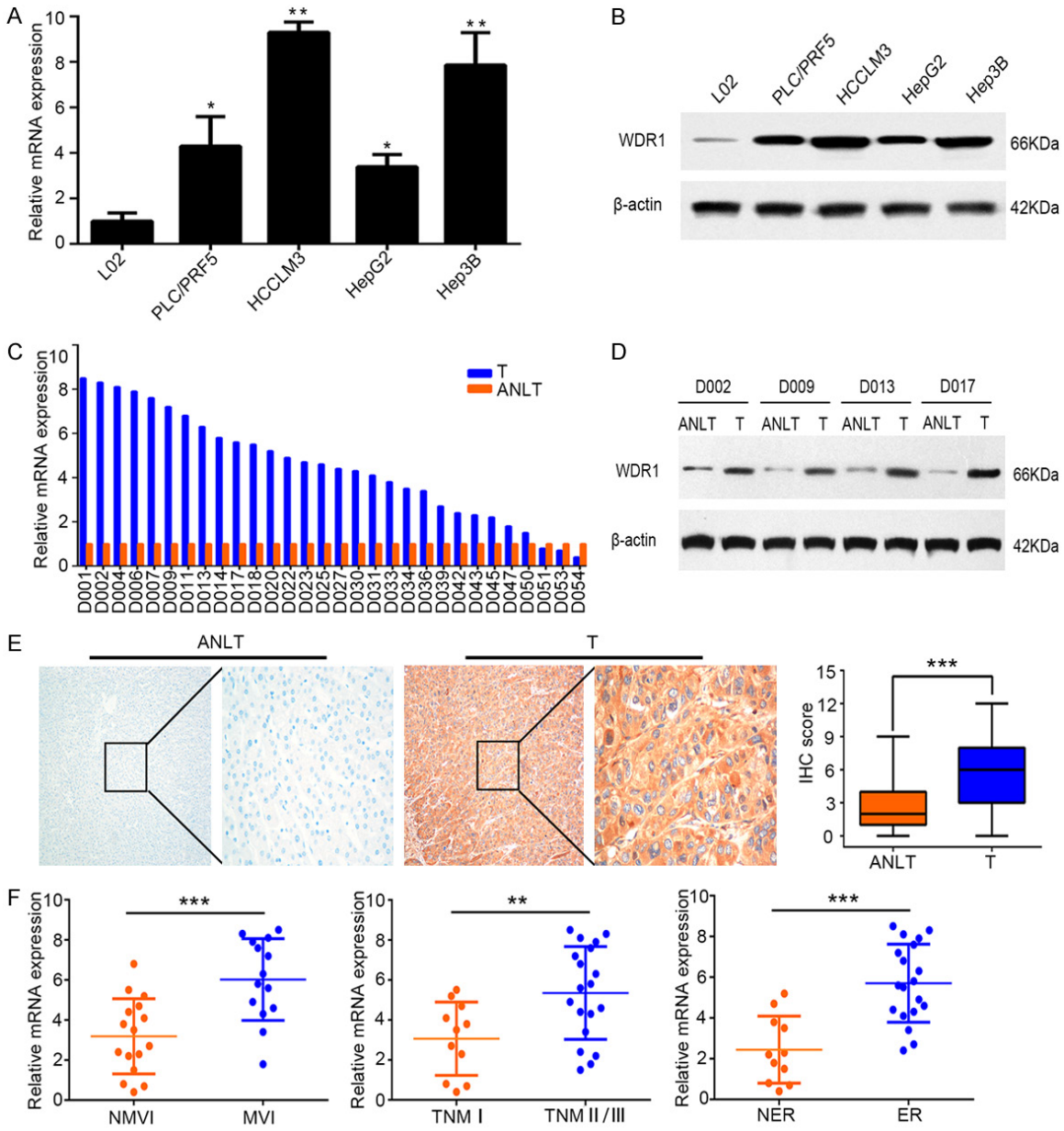

Figure 1. WDR1 was significantly upregulated and associated with metastasis in HCC. (A, B) mRNA (A) and protein (B) expression of WDR1 were upregulated in HCC cell lines compared with normal liver cell line L02, as detected by qRT-PCR and WB. (C) WDR1 mRNA expression was higher in 30 snap-frozen HCC tissues than in matched adjacent nontumorous liver tissues (ANLTs). (D) The representative Western-blotting pictures of protein expression of WDR1 in four paired snap-frozen HCC tissues and matched ANLTs. (E) HCC exhibited a higher WDR1 expression than corresponding ANLTs by IHC. Left panel: Representative IHC staining of WDR1 in ANLT and HCC, Magnification: left: 100 ×, right: 400 ×.; Right panel: Box-plot analyzing the immunohistochemical scores in HCC tissues and ANLTs. (F) WDR1 mRNA expression was significantly higher in tumors with microvascular invasion (MVI), advanced stage (TNM II/III) and early recurrence (ER, within 2 years) than those without MVI (NMVI), with early stage (TNM I), and without early recurrence (NER) respectively. **P* < .05, ***P* < .01, ****P* < .001.

conjugated secondary antibody (KPL, Gaithersburg, MD) for 30 min at 37°C. The band was detected with enhanced chemiluminescence regents (Termo Scientifc, Rockford, IL). Beta-actin protein was used as a loading control.

Immunohistochemistry (IHC)

Paraffin-embedded tissues were sectioned into 4 μm and then dewaxed and rehydrated. Antigen retrieval was performed using a microwave-pretreated EDTA buffer (1 mM, pH 8.0) for

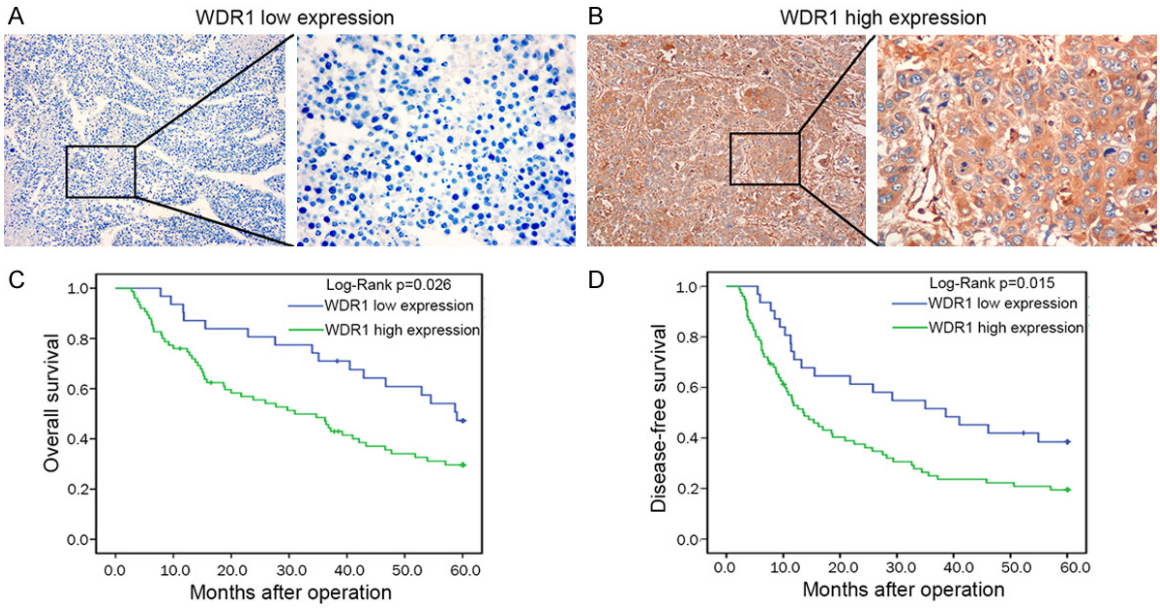

Figure 2. WDR1 predicted a poor prognosis of HCC patients. (A, B) Representative IHC staining of WDR1 low expression (A) and high expression (B) in HCC. Magnification: left: 100 ×, right: 400 ×. (C, D) Survival analysis of disease-free survival (DFS; C) and overall survival (OS; D) in the high WDR1 expression group (75 cases) and low WDR1 expression group (31 cases). According to the IHC staining, 106 cases of HCC patients were divided into two groups: the high WDR1 expression group (75 cases) and low WDR1 expression group (31 cases). We analyzed the survival of two groups using a Kaplan-Meier curve and a log-rank test, and HCC patients with high WDR1 expression showed poorer OS (*P*=.026; C) and DFS (*P*=.015; D) than HCC patients with low WDR1 expression.

10 minutes. 0.3% hydrogen peroxide was used to block endogenous peroxidase activity for 15 mins at room temperature. After being blocked with normal goat serum (Zhongshan Goldenbridge Biotechnology, Beijing, China), the slides were incubated with 1:100 rabbit anti-WDR1 polyclonal antibody (Protientech, Wuhan, China) at 4°C overnight. This was followed by the incubation of a secondary antibody (Zhongshan Goldenbridge Biotechnology, Beijing, China) for 30 min at room temperature. The negative controls were without primary antibody incubation during this procedure. Finally, the slides were incubated with 3,3'-diaminobenzidine (DAB; Zhongshan Goldenbridge Biotechnology) and counterstained with hematoxylin before being examined by microscopy.

Regarding the final immunostaining score (IS), we adopted the scoring criteria we used before [16]. The staining was examined in a blinded fashion by two independent pathologists. The IS was defined by the consistency of the grading by the two pathologists. The expression levels of WDR1 were scored based on the staining intensity (SI) and the percentage of positive cells (PP). SI was classified into four grades: 0, negative; 1, weak; 2, moderate; 3, strong. PP was defined into five categories: 0, 0% positive cells; 1, 0-25% positive cells; 2, 25-50% positive cells; 3, 50-75% positive cells, and 4, 75-100% positive cells. IS=SI × PP. To classify the continuous IS values into the low expression and high expression groups, we chose a commonly used cutoff point for the analysis (range 0-12, cut point ≤ 3 versus > 3) as previously described.

Cell transfection

Two HCC cell lines (HepG2 and HCCLM3) were selected to modify gene expression to conduct *in vitro* experiments. Lentiviral vectors containing the human WDR1 gene and lentiviral vectors encoding short hairpin RNAs were purchased from OriGene Technologies Incorporated (Rockville, MD). HepG2 cells were transfected with lentiviral vectors containing the WDR1 gene and the HCCLM3 cells were transfected with lentiviral vectors encoding the WDR1-shRNA-Sequence, named as HepG2-WDR1 and HCCLM3-shWDR1 respectively. Empty vectors were used as the control groups, named as HepG2-Ctrl and HCCLM3-

Table 1. Correlation between WDR1 and clinicopathologic characteristics of HCC

Characteristics	n	WDR1 expression		*P*-Value
		High (75)	Low (31)	
Gender				
Female	28	21	7	.565
Male	78	54	24	
Age (years)				
≤ 60	55	40	15	.643
> 60	51	35	16	
AFP (ng/ml)				
≤ 20	41	31	10	.383
> 20	65	44	21	
HBsAg				
Negative	36	23	13	.265
Positive	70	52	18	
Liver cirrhosis				
Absence	42	27	15	.236
Presence	64	48	16	
Tumor size (cm)				
≤ 5	45	36	9	.072
> 5	61	39	22	
Tumor number				
Single	52	40	12	.171
Multiple (≥ 2)	54	35	19	
Capsulation formation				
Presence	35	30	5	.017
Absence	71	45	26	
Microvascular invasion				
Absence	59	36	23	.014
Presence	47	39	8	
Edmondson-Steiner grade				
I/II	46	35	11	.291
III/IV	60	40	20	
TNM stage				
I	39	22	17	.013
II/III	67	53	14	

AFP, alpha-fetoprotein; TNM, tumor node metastasis.

shCtrl respectively. After transfection, the cells were selected using 2.5 μg/ml of puromycin (OriGene) for 2 weeks to select stable clones. qRT-PCR and WB were used to confirm the WDR1 expression in the above cells. The inhibitory efficiency of the three WDR1-shRNA sequences was validated and the WDR1-shRNA-Sequence3 (shWDR1-3) was adopted for subsequent study due to its highly effective inhibition of WDR1 expression in the HCCLM3 cells.

Wound-healing assay

A wound-healing assay was used to measure HCC cell migration. The cells were seeded into a 6-well plate at a density of 5×10^5 cells per well in a culture medium containing DMEM with 10% FBS. After they reached 100% confluence, the cells were pre-incubated with mitomycin (10 ug/ml; Sigma, St. Louis, MO) to inhibit cell proliferation for 1 h at 37°C. Then a scratch was made using a 10 μl pipette tip. The medium was changed and the cells were cultured for 24 hours. The percentage of wound closure was calculated for three randomly chosen fields.

Transwell assay

About 1×10^5 cells in a serum-free medium were placed into the upper chamber of the insert (BD Biosciences, MA, USA). After 24 hours of incubation in 5% CO_2 at 37°C, the cells in upper chamber were removed with cotton swabs and then were fixed by 20% methanol, and then stained with a solution containing 0.1% crystal violet (Beyotime Institute of Biotechnology, Beijing, China). These experiments were performed in triplicate and five random fields were chosen for the cell count.

MTT assay

The cells were seeded into 96-well plates at 1×10^3 per well. Every 24 h, 20 μl of MTT (5 mg/ml) was added and incubated for 4 h, and then the medium was abandoned and 150 μl of DMSO was added and rocked for 15 min at room temperature. Finally, the absorbance was detected by a microplate reader at 490 nm using the ELX-800-type ELISA reader (Bio-Tek, VT, USA). Each experiment was conducted three times.

Colony formation assay

Cells were added into 6-well culture dishes with 500 cells per well. After being cultured for 2 weeks, colonies were gently washed with PBS

Table 2. Univariable and multivariable analysis of factors associated with overall survival (OS) by Cox proportional hazards regression mode

Characteristics	OS			
	Univariable Analysis		Multivariable Analysis	
	HR (95% CI)	*P*-Value	HR (95% CI)	*P*-Value
Gender				
Female *vs.* Male	1.155 (0.650-2.051)	.624		NA
Age, years				
≤ 60 *vs.* > 60	1.229 (0.761-1.984)	.400		NA
AFP, ng/ml				
≤ 20 *vs.* > 20	1.346 (0.808-2.242)	.253		NA
HBsAg				
Negative *vs.* Positive	1.629 (0.948-2.798)	.077		NA
Liver cirrhosis				
Absence *vs.* Presence	1.480 (0.893-2.452)	.128		NA
Tumor size, cm				
≤ 5 vs. > 5	2.195 (1.314-3.669)	.042		NS
Tumor number				
Single *vs.* Multiple	1.581 (0.971-2.574)	.066		NA
Capsulation formation				
Absence *vs.* Presence	2.468 (1.367-4.458)	.030		NS
Microvascular invasion				
Absence *vs.* Presence	3.536 (2.150-5.817)	< .001	2.771 (1.593-4.821)	< .001
Edmondson-Steine grade				
I/II *vs. III*/IV	3.034 (1.790-5.145)	< .001	2.033 (1.080-3.824)	.014
TNM stage				
I *vs. II*/III	3.369 (1.885-6.023)	< .001	2.249 (1.178-4.291)	.010
WDR1 expression				
Low *vs.* High	2.323 (1.219-4.427)	.026	1.876 (1.068-3.296)	.038

HR: hazard rate; CI: confidence interval; NA: no application; NS: no significance.

and stained with 0.1% crystal violet stain. Only positive colonies (diameter > 40 um) in the dishes were counted and compared [17]. These experiments were performed in triplicate.

Statistical analysis

SPSS 18.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. The differences between two groups were analyzed by Student's *t* test when the variance was homogeneous. If the variance was not homogeneous, the differences between two groups were analyzed using the Mann-Whitney *U* test. X^2 analysis was used to analyze the correlation between WDR1 expression and clinicopathologic features. Survival curves were constructed using the the Kaplan-Meier method and compared using the the log-rank test. The Cox proportional hazards regression model was established to identify independent factors for overall survival (OS) and disease-free survival (DFS) of HCC patients. All the tests were two-tailed and $P < 0.05$ was considered statistically significant.

Result

WDR1 expression is significantly upregulated and associated with metastasis in HCC

Firstly, the expressions of WDR1 in HCC cell lines and the normal liver cell line L02 were examined. Compared with L02, the expressions of WDR1 mRNA (**Figure 1A**) and the protein (**Figure 1B**) were significantly higher in the HCC cell lines. Subsequently, the expression of WDR1 was analyzed in 30 pairs of fresh frozen tissues from HCC patients. qRT-PCR showed that WDR1 mRNA expression in HCC tissues

Table 3. Univariable and multivariable analysis of factors associated with Disease-free survival (DFS) by Cox proportional hazards regression mode

Characteristics	DFS			
	Univariable Analysis		Multivariable Analysis	
	HR (95% CI)	*P*-Value	HR (95% CI)	*P*-Value
Gender				
Female *vs.* Male	1.076 (0.641-1.805)	.782		NA
Age, years				
≤ 60 *vs.* > 60	1.130 (0.725-1.762)	.589		NA
AFP, ng/ml				
≤ 20 *vs.* > 20	1.284 (0.805-2.048)	.294		NA
HBsAg				
Negative *vs.* Positive	1.546 (0.943-2.533)	.084		NA
Liver cirrhosis				
Absence *vs.* Presence	1.340 (0.846-2.123)	.212		NA
Tumor size, cm				
≤ 5 vs. > 5	2.057 (1.288-3.284)	.003		NS
Tumor number				
Single *vs.* Multiple	1.547 (0.986-2.427)	.057		NA
Capsulation formation				
Absence *vs.* Presence	2.345 (1.380-3.986)	.002		NS
Microvascular invasion				
Absence *vs.* Presence	3.377 (2.135-5.342)	< .001	2.824 (1.703-4.684)	< .001
Edmondson-Steine grade				
I/II *vs.* III/IV	2.298 (1.444-3.655)	< .001	1.884 (1.060-3.350)	.021
TNM stage				
I *vs.* II/III	2.875 (1.733-4.769)	< .001	2.226 (1.231-4.024)	.007
WDR1 expression				
Low *vs.* High	2.036 (1.218-3.403)	.015	1.880 (1.120-3.163)	.034

was significantly higher than that in matched ANLTs (**Figure 1C**). The similar upregulation of the WDR1 protein in HCC tissues was further confirmed by WB (**Figure 1D**). Further, IHC assays were performed in matched paraffin-embedded tissues from 106 HCC patients, and the data showed that WDR1 expression was highly located in the cytoplasms, and the expression level of WDR1 was significantly higher in the HCC tumors than it was in ANLT (*P* < .001, **Figure 1E**).

In addition, the WDR1 mRNA expression was assessed in different HCC subgroups among 30 pairs of frozen fresh tissues. WDR1 mRNA expression was significantly higher in tumors with microvascular invasion (MVI) than in tumors without MVI (NMVI; *P* < .001, **Figure 1F**). Advanced stage patients (TNM II/III) showed higher WDR1 mRNA expression than early stage patients (TNM I; *P*=.009, **Figure 1F**). Remarkably, patients with early recurrence (ER) also exhibited a higher level of WDR1 mRNA expression than those without early recurrence (NER; *P* < .001, **Figure 1F**). Above all, these data showed that WDR1 expression was markedly upregulated and may be associated with tumor metastasis in HCC.

Correlation of WDR1 expression with clinicopathological characteristics of HCC

Based on the IHC score in HCC tissues from a set of 106 HCC patients, the HCC patients were divided into two groups: the low WDR1 expression group (**Figure 2A**) and the high WDR1 expression group (**Figure 2B**). We analyzed the correlation between the expression of WDR1 and the clinicopathological characteristics of HCC patients. We found that WDR1 expression was significantly associated with capsulation formation (*P*=0.017), microvascular invasion (MVI; *P*=0.014), and the tumor node metastasis (TNM) stage (*P*=0.013, **Table 1**).

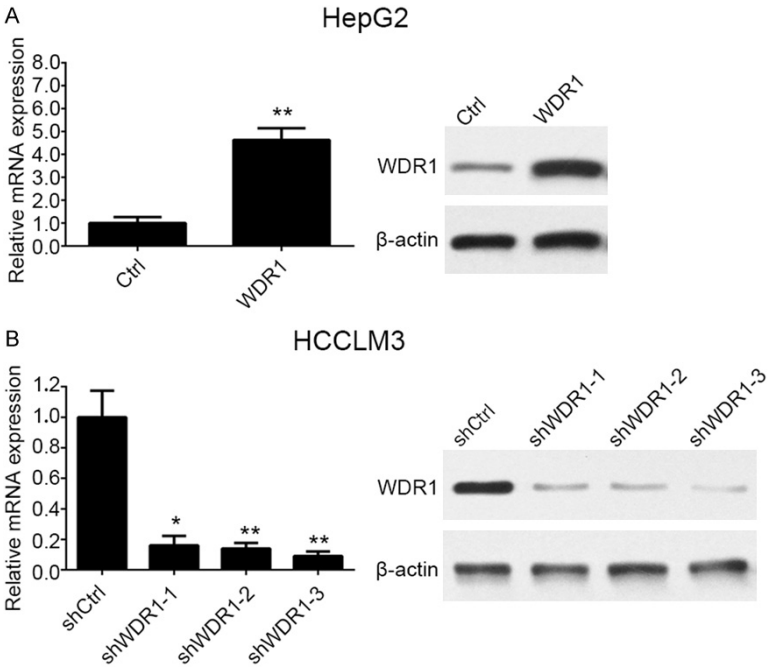

Figure 3. WDR1 was overexpressed and knocked down in HCC cell lines by lentivirus transfection. A. WDR1 expression was upregulated by WDR1-expression lentivirus particles in HepG2 cells. qRT-PCR and WB were used to verify the upregulation of exogenous WDR1 expression. B. WDR1 expression was downregulated by WDR1-shRNA lentivirus particles in HCCLM3 cells. qRT-PCR and WB were applied to check the inhibitory efficiency of three designed WDR1 shRNAs lentivirus particles. WDR1-shRNA-Sequence3 (shWDR1-3) was adopted for subsequent study due to its highly effective inhibition of WDR1 expression in HCCLM3, which was named shWDR1 for simplicity. *$P < .05$, **$P < .01$.

High WDR1 expression indicates poor prognosis in HCC

To evaluate the significance of WDR1 expression on HCC patients' survival, a Kaplan-Meier analysis and a Cox regression analysis were applied. The Kaplan-Meier survival analysis revealed that patients with high WDR1 expression had a worse OS (P=.026, **Figure 2C**) and DFS (P=.015, **Figure 2D**) than those with a low WDR1 expression. Univariate Cox regression analysis showed that WDR1 expression, tumor size, capsulation formation, MVI, Edmondson-Steiner grade, and TNM stage were significantly associated with OS (**Table 2**) and DFS (**Table 3**) in patients with HCC. Additionally, a multivariate Cox regression analysis indicated that WDR1 expression, MVI, Edmondson-Steiner grade and TNM stage were independent predictors of OS (**Table 2**) and DFS (**Table 3**) in HCC. Above all, the data showed that not only could WDR1 be useful as a new prognostic marker, but it could also be associated with HCC progression.

WDR1 promotes HCC cells migration, invasion and proliferation in vitro

Finally, to investigate the role of WDR1 in HCC progression, we manipulated WDR1 expression in HepG2 cells using ectopic expression and in HCCLM3 using shRNA knockdown. The expression levels of WDR1 in the two HCC cell lines were confirmed by qRT-PCR and WB after WDR1 gene expression manipulation. As shown in **Figure 3**, both the level of mRNA and the protein were remarkably elevated in HepG2-WDR1 (**Figure 3A**) and downregulated in the HCCLM3-shWDR1-3 cells (**Figure 3B**). Wound healing and transwell assays were performed to examine cell migration and invasion capabilities respectively. As demonstrated in **Figure 4A**, **4C**, compared to the control groups, the overexpression of WDR1 significantly promoted the migration and invasion of the HepG2 cells. On the other hand, the downregulation of WDR1 prominently impaired the migration and invasion capability of HCCLM3 cells compared to the control group (**Figure 4B**, **4D**). We further explored the role of WDR1 in the regulation of cell proliferation. MTT assays showed that the overexpression of WDR1 markedly promoted the proliferation of HepG2 cells (**Figure 5A**). Moreover, the knockdown of WDR1 also led to a significant decrease in the proliferation capability of HCCLM3 cells (**Figure 5B**). A similar effect of WDR1 on proliferation was observed by colony formation assays in HepG2 (**Figure 5C**) and HCCLM3 cells (**Figure 5D**). These results demonstrated that WDR1 could promote HCC progression by enhancing the migration, invasion and proliferation of cells.

Discussion

Hepatocellular carcinoma, as one of the most common cancers in the world, is characterized by a high rate of metastasis and recurrence after curative resection [18-20]. Tumor metas-

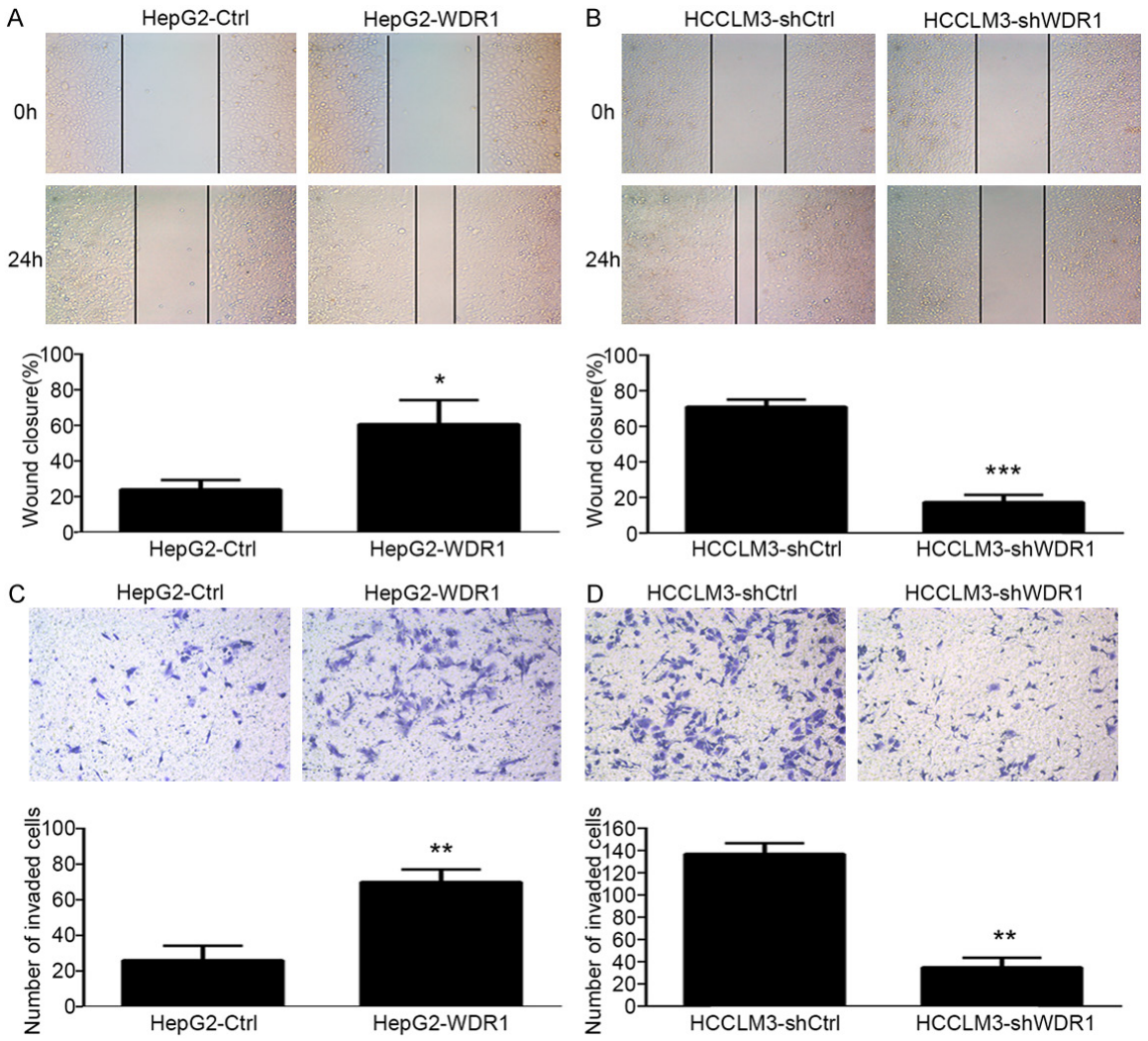

Figure 4. WDR1 promotes HCC cell migration and invasion of HCC cells in vitro. (A, B) Wound healing assays were performed in HepG2 cells with WDR1 overexpression or control (A) and HCCLM3 cells with WDR1 knockdown or control (B). (C, D) Transwell assays were performed in HepG2 cells with WDR1 overexpression or control (C) and HCCLM3 cells with WDR1 knockdown or control (D). $*P < .05$, $**P < .01$, $***P < .001$.

tasis is a complex process and is associated with numerous molecular changes. Though many biomarkers that correlate with HCC metastasis have been investigated, an effective biomarker still urgently needs to be identified and applied to clinical practice.

WDR1, as an important regulator of actin filament dynamics, promotes cytoskeleton remodeling and therefore facilitates cell migration and invasion [6-8]. It is well documented that cell migration and invasion are necessary for tumor metastasis [21]. Thus, these studies imply a role of WDR1 in promoting tumor progression. Interestingly, it has been demonstrated that WDR1 was upregulated in the highly metastatic gallbladder cancer cell line (GBC-SD18H) [22]. In breast cancer, upregulated WDR1 was observed in the invading front margin of the tumor and can promote cancer cell migration significantly [13, 23, 24]. In this study, we found WDR1 was upregulated both in HCC cell lines and tumor tissues. Notably, among those results, we found that high metastatic potential cell lines (HCCLM3 and Hep3B) exhibited a higher expression of WDR1 both in mRNA and protein levels than in low metastatic potential cell lines (PLC/PRF5 and HepG2). Additionally, through further stratification analysis on WDR1 mRNA expression in 30 pairs matched HCC snap-frozen tissues, it was striking to find that HCC with MVI or/and advanced

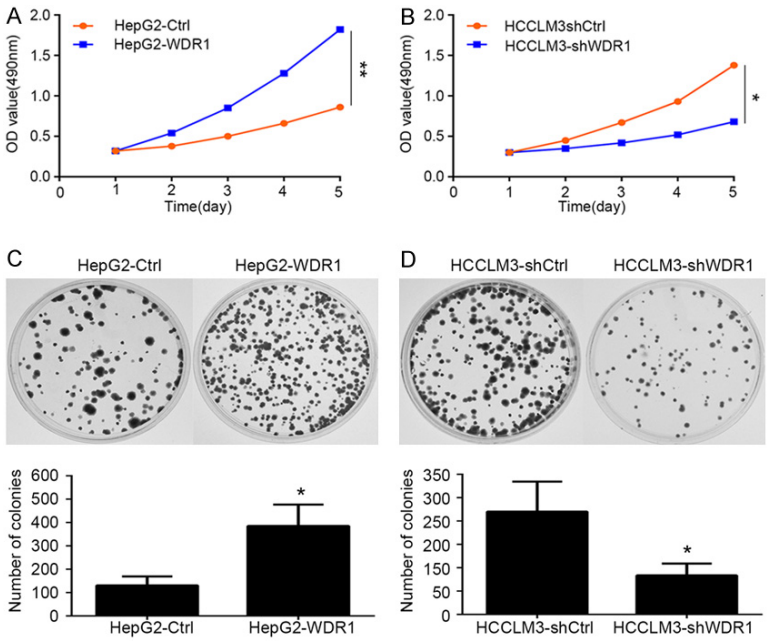

Figure 5. WDR1 contributes to HCC cell proliferation. (A, B) MTT assays were used to analyze the proliferation in HepG2^{WDR1} or HepG2^{Ctrl} (A) and HCCLM3^{shWDR1} or HCCLM3^{shCtrl} (B). (C, D) Colony formation assays were used to analyze the proliferation in HepG2^{WDR1} or HepG2^{Ctrl} (C) and HCCLM3^{shWDR1} or HCCLM3^{shCtrl} (D). *$P < 0.05$, **$P < 0.01$.

tumor stage exhibited a higher WDR1 mRNA expression than those without MVI or/and with an early tumor stage. Importantly, patients with early recurrence, which is typically caused by the dissemination of metastatic HCC cells, [25] also showed a higher level of WDR1 mRNA expression than those without early metastasis or recurrence. In all, these data indicate a positive role of WDR1 in HCC progression.

As cancer progression is the main causative factor for HCC mortality, we further interrogated whether WDR1 could predict the prognosis of HCC patients. Based on the above results, we further analyzed the relationship between WDR1 expression and HCC clinicopathological features and prognosis. A high expression of WDR1 was found to be significantly correlated with aggressive pathological characteristics including capsulation formation absence, MVI presence, and advanced TNM stage. Subsequently, by univariate and multivariate analysis, we demonstrated that high WDR1 expression was an independent risk factor of prognosis for HCC patients. It suggested that HCC patients with a high expression of WDR1 were more likely to suffer from tumor recurrence and had a worse survival time. Similarly, Lee et al. [13] reported that WDR1 overexpression was a poor prognostic factor for breast cancer patients. Additionally, WDR1 was reported to be correlated with the invasive clinicopathological characteristics of glioblastoma and bad OS and PFS in glioblastoma patients [12]. Thus, WDR1 might be a tumor-specific promoting factor and greatly associated with HCC progression.

To further elucidate the role of WDR1 in HCC progression, we explored the biological effect of WDR1 on HCC cells. In the present study, we showed that WDR1 overexpression in HepG2 markedly promoted cell migration and invasion, whereas WDR1 downregulation in HCCLM3 showed the opposite results. What's more, we investigated the influence of WDR1 on HCC cell proliferation capability. We found that overexpression of WDR1 significantly promoted the proliferation of HepG2 cells and the proliferation capability of HCCLM3 cells was markedly inhibited by the knockdown of WDR1. These results were consistent with the involvement of WDR1 in cancer progression in other malignancies [13, 24]. Above all, these data provided evidence about the function of WDR1 in HCC's aggressive progression.

Overall, our study identified that WDR1 predicts a poor prognosis and promotes cell migration, invasion, and proliferation in HCC. WDR1 may be a critical molecule in HCC progression and serve as a useful prognostic factor for HCC.

Acknowledgements

This work was supported by the Natural Science Foundation of Jiangxi, China (20142BAB205055) and also partly supported by the National Natural Science Foundation of China (81702922).

Disclosure of conflict of interest

None.

Abbreviations

WDR1, WD-repeat protein 1; HCC, hepatocellular carcinoma; ANLTs, adjacent nontumorous liver tissues; OS, overall survival; DFS, disease-free survival; AFP, alpha-fetoprotein; TNM, tumor-node-metastasis; MVI, microvascular invasion; ER, early recurrence.

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