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LncRNA-ZDHHC8P1 promotes the progression and metastasis of colorectal cancer by targeting miR-34a

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Abstract. – OBJECTIVE: Growing evidence has shown that long non-coding RNAs (lncRNAs) can serve as prospective markers for survival in patients with colorectal adenocarcinoma. In this study, we mainly focused on the potential roles of lncRNA- ZDHHC8P1 in the development process of colorectal cancer (CRC) *via* miR-34a.

PATIENTS AND METHODS: Quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) was used to detect the expression of lncRNA ZDHHC8P1 in CRC patients and cell lines. The relationships between the expression level of ZDHHC8P1, patient clinicopathological parameters and overall survival (OS) were analyzed using the univariate Kaplan-Meier method. Proliferation ability was tested by Cell Counting Kit-8 (CCK-8) and cyclin D1 assay, through loss- and gain-of-function approaches. Moreover, the cycle distribution and wound-healing assay were used to measure the function of progression and metastasis in CRC cell lines. Furthermore, Luciferase activity proved the relationship between miR-34a and ZDHHC8P1.

RESULTS: We found that lncRNA-ZDHHC8P1 was highly expressed in heatmap of colon cancer with lncRNAtor. Additionally, the lncRNA ZDHHC8P1, an independent prognostic factor for overall survival, was increased in CRC and correlated positively with CRC progression. Most importantly, through loss- and gain-of-function approaches, we showed that ZDHHC8P1 promotes progression and metastasis *in vitro*. What's more, the ZDHHC8P1 expression level was reversely correlated with miR-34a expression in patients with CRC. Mechanistically, miR-34a was identified as an important ZDHHC8P1 functional target.

CONCLUSIONS: According to the results, this work uncovers a previously unappreciated ZDHHC8P1/miR-34a regulatory axis in controlling progression and metastasis of CRC and suggests that interfering with lncRNA-ZDHHC8P1 and miR-34a could be a viable approach to treat late-stage metastatic CRC patients.

Key Words:
LncRNA-ZDHHC8P1, MiRNA-34a, Progression and metastasis, Colorectal cancer.

Abbreviations

AJCC= American Joint Committee on Cancer, BSA= Bovine Serum Albumin, DMEM= Dulbecco's Modified Eagle Medium, CRC= Colorectal Cancer, ECL= enhancedchemiluminescence, FBS= Fetal Bovine Serum, FHC= Normal Human Colon Epithelial Cells, GAPDH= Glyceraldehyde 3-Phosphate Dehydrogenase, RT-PCR= Reverse Transcriptase-Polymerase Chain Reaction, PBS= Phosphate-Buffered Saline, PCR= Polymerase Chain Reaction.

Introduction

Colorectal cancer (CRC) is one of the most common and aggressive human malignancies, the third most commonly diagnosed cancer in men and the second in women worldwide[1,2]. Approximately 1.2 million new cases are diagnosed, causing 0.6 million deaths per year all over the world. Cancer metastasis remains the key cause of CRC death. Consequently, the assessment of prognostic factors is pivotal for the colorectal

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cancer patients[3-6]. The improvements in colorectal cancer prevention and early detection and patients' survival still require a better understanding of the underlying molecular mechanisms. Long non-coding RNAs (lncRNAs) are a new class of non-coding transcripts longer than 200 nucleotides and stably exist in the plasma and urine, with disease and tissue specificity and without protein-coding potential. They regulate the gene expression through a series of mechanisms, including transcription, post-transcription processing, genomic imprinting and chromatin modification[7-9]. Recent evidence has shown that lncRNAs have been implied to be frequently expressed aberrantly in lots of cancers and involved in a large range of biological processes[10-12]. However, the biological functions and mechanisms of lncRNAs in colorectal cancer are largely unclear. Zinc finger DHHC-type containing 8 pseudogene 1 (ZDHHC8P1) is located in the 22q11 micro-deletion region and may contribute to the behavioral deficit associated with 22q11 deletion syndrome[13]. Previous reports[14-16] showed that ZDHHC8P1 participated in the development of mental disorder diseases, such as schizophrenia and Alzheimer's disease. However, it is remarkable that the function and mechanism of ZDHHC8P1 in CRC patients has not been addressed yet.

In our study, we aimed to examine the functional role of lncRNA ZDHHC8P1 in the pathogenesis of colorectal cancer, as well as to disclose the molecular mechanisms. Firstly, we measured the expression level of ZDHHC8P1 in human CRC tissues and discovered ZDHHC8P1 was a poor prognostic factor for CRC patients. Then, proliferation ability was tested by Cell Counting Kit-8 (CCK-8) and cyclin D1 assay, through loss- and gain-of function approaches. In addition, we assessed the regulatory relationship between ZDHHC8P1 and miR-34a and the miR-34a -mediated roles of ZDHHC8P1. Our work, therefore, uncovered a critical function of lncRNA ZDHHC8P1 in CRC.

Patients and Methods

Patients

CRC tissue samples and their corresponding paracancerous tissue samples were collected by surgical resection at the Third Affiliated Hospital of Kunming Medical University from August 2015 to September 2017 (Kunming, China). Fresh primary and metastatic CRC tissue samples were also collected at the Third Affiliated Hospital of Kunming Medical University from June 2017 to June 2018 (Kunming, China). All tissue samples were frozen at -80°C. This study was approved by the Institutional Review Board of the Kunming Medical University Ethics Committee, and informed consent was obtained from each patient.

Cell Culture

Normal human colon epithelial cells (FHC) and LOVO cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). SW480, SW620, HCT116, and HT29 cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). FHC cells were cultured in Dulbecco's Modified Eagle Medium (DMEM)/F12 medium (Invitrogen, Carlsbad, CA, USA); LOVO cells were cultured in F12-K medium (Life Technologies, Inc., Gaithersburg, MD, USA). SW480 and SW620 cells were maintained in Leibovitz's L-15 medium (Gibco, Grand Island, NY, USA). HT-29 and HCT-116 cells were cultured in McCoy's 5A medium (HyClone, South Logan, UT, USA). All media were supplemented 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), penicillin (100 U/ml), and streptomycin (100 μg/ml), and all cells were cultured in an appropriate incubator.

RNA Extraction and Real Time-Quantitative Polymerase Chain Reaction Assays

Total RNA from clinical samples and cells was extracted using RNAiso Plus (TaKaRa, Otsu, Shiga, Japan), following the manufacturer's protocol. Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed using the PrimeScript™ RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's protocol. The levels of mRNA expression were quantified by standard Real Time-PCR protocol with SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan). GAPDH was used as a reference gene. The gene specific primers were reported in Table I.

Construction of Plasmid, siRNA and Cell Transfection

The full length of human ZDHHC8P1 cDNA was synthesized and subcloned into a pCDNA3.1 (Invitrogen, Carlsbad, CA, USA) vector, resulting in ZDHHC8P1-pcDNA for its overexpression. For small interfering RNAs (siRNAs) analysis, three ZDHHC8P1 siRNAs and negative control siRNA (si-NC) were provided by Invitrogen (Carlsbad,

Table I. Primers for selected genes.

Gene name	Primers	
	Sense	Antisense
ZDHHC8P1	GAGGTCCTACGCTGTGCTAC	CAAGAAGGACATCTGGGGCG
miRNA-34a	TGGCAGTGTCTTAGCTGGTTG	GCGAGCACAGAATTAATACGAC
GAPDH	TGACGTGCCGCCTGGAAAC	CCGGGCATCGAAGGTGGAAGAG
U6	CGCTTCGGCAGCACATATACT	CGCTTCACGAATTTGCGTGTC

CA, USA), and the siRNA sequences targeting the sequence of ZDHHC8P1 transcript were as follows: si ZDHHC8P1-1#5'-GGG GAG GTC CTA GCG TGT GCT ACG CUA-3'; si ZDHHC8P1-2#5'-GCU GAG ATC CTA CGC TGT GCT ACU CUU-3';si ZDHHC8P1-3#5'-CAG GAG GTC CTA CGC CGT GCT ACT UAA-3'. Cells were pre-incubated to 40-60% confluence on a six-well plate and then transfected by incubation with plasmids or siRNAs with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocols. At indicated time point after the transfection, cells were harvested for further analysis.

Flow Cytometric Analysis of the Cell Apoptosis

The treated SW620 and HT29 cells were washed twice, treated with trypsin and fixed. The cell pellet was stained with Annexin V-fluorescein isothiocyanate (FITC; Beyotime, Shanghai, China) and Propidium Iodide (PI), and flow cytometry was conducted within 5 min. The images apoptotic cells were obtained using a FACS Calibur system (BD Biosciences, Franklin Lakes, NJ, USA), and the data were analyzed using the FlowJo software (Tree Star Corp, Ashland, OR, USA).

CCK-8 Assays

The target cells were seeded into 96-well plates, with the density of 2000 cells in each well. Three replicate wells were set in each group. Cell Counting Kit-8 (CCK-8) assay was made as follows: 10 ul of Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) was added into 100 ul of Dulbecco's Modified Eagle Medium (DMEM) in each well, which was co-cultured in the dark for 2 hours at 37°C. The 96-well plate was placed at the absorbance of 450 nm. The data were collected for 5 days. The whole experiment was repeated three times.

Wound-Healing Assays

The cell loading parameters were 20 μl of cell suspension solutions at two inlets and 30 μl of cell suspension solutions at the outlet (5 million cells per ml). After the formation of confluent monolayers, the supplemented culture medium was replaced with culture medium without fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) for 24-hour starvation to synchronize the stages of HT29 cell. Then, the trypsin solution and the supplemented culture medium (120 μl) were applied at two channel inlets, respectively, for two minutes to generate a clear wound boundary. Images with wound generation were taken at 0, 24, and 48 hours with Olympus DP73 digital camera (Tokyo, Japan).

Luciferase Activity

Wt-ZDHHC8P1/mut-ZDHHC8P1 sequences were amplified and cloned into the downstream of the stop codon of the firefly Luciferase in basic vector (Promega, Madison, WI, USA). SW620 and HT29 cells were cultured overnight after being seeded into a 24-well plate, co-transfected with the Wt-ZDHHC8P1/mut-ZDHHC8P1 reporter gene plasmid and miR-34a mimics or miR-34a inhibitor. Renilla expression vector was transfected into each group to serve as a normalized control. 48 h after transfection, firefly and Renilla Luciferase activities were measured using Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). Data were normalized against the activity of the Renilla Luciferase gene.

Statistical Analysis

All the data were expressed as the mean ± SD. Each assay was applied at least three independent experiments or replicates. The significance between groups was analyzed by Student's *t*-test. p-value<0.05 was considered statistically significant. All statistical analyses were performed by post-hoc analysis using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). Multiple comparisons between groups were carried out by S-N-K methods.

Results

LncRNA ZDHHC8P1 Was Highly Expressed in Heatmap of Colon Cancer

To explore the differentially expressed lncRNA of colon cancer, we downloaded the "Colon adenocarcinoma: Person neoplasm status" which consisted of 36 cases of colon cancer tissues and 29 cases of normal colonic tissues from the lncRNAtor[17]. The candidate genes were selected from these differentially expressed lncRNAs based on an artificial criterion (p<0.01, fold change ≥ 2 or 0.5) and then validated by quantitative Real Time-PCR (qRT-PCR) in 60 pairs of colon cancer tissues and adjacent tissues. The verifying results displayed that ZDHHC8P1 was highly expressed in heatmap of colon cancer (Figure 1).

LncRNA ZDHHC8P1Was Upregulated in CRC Patients and Indicated a Poor Prognosis

We assessed the expressions of ZDHHC8P1 in 28 primary and 22 paracancerous CRC tissue samples by qRT-PCR analysis. The results revealed that the ZDHHC8P1 expression was significantly higher in tumor tissues compared with paracancerous tissues (Figure 2A). To confirm this change in CRC cells, we measured the PVT1 expression level in five CRC cell lines (SW480, SW620, HCT116, HT29 and LOVO) and normal human colon epithelial cells (FHC) by qRT-PCR. We found that the expression level of ZDHHC8P1 was markedly higher in CRC cell lines (SW480, SW620, HCT116, HT29 and LOVO) than FHC cells (Figure 1B). What's more, CRC patients were classified into a high group (≥ 2-fold, n = 56) and a low group (< 2-fold, n = 25) on the basis of the cutoff value of ZDHHC8P1 expression (Figure 1C). To investigate the potential roles of ZDHHC8P1 in CRC, we analyzed the correlation between the ZDHHC8P1 levels and the clinical pathological parameters in CRC patients. The results revealed that the expression of ZDHHC8P1 was markedly correlated with tumor differentiation, AJCC stage and lymph-vascular invasion of CRC patients (Table II). However, there was no significant correlation between ZDHHC8P1 expression and age, gender, tumor site or tumor histology (all p>0.05). Comparison of the survival curves of CRC patients revealed that ZDHHC8P1-positive (high expression) patients had significantly poorer survival than ZDHHC8P1-negative (low expression) patients (Figure 2D). Our findings indicated that lncRNA ZDHHC8P1 was correlated with the procession of colorectal cancer, and ZDHHC8P1 was an important prognostic factor of CRC patients.

Silencing ZDHHC8P1 Expression With SiRNA Inhibited the Progression of CRC

To further explore the role of lncRNA ZDHHC8P1 in CRC, we investigated the function of ZDHHC8P1 in cell proliferation and apoptosis. We used the SW620 and HT29 cells, both of which have been widely used to study CRC *in*

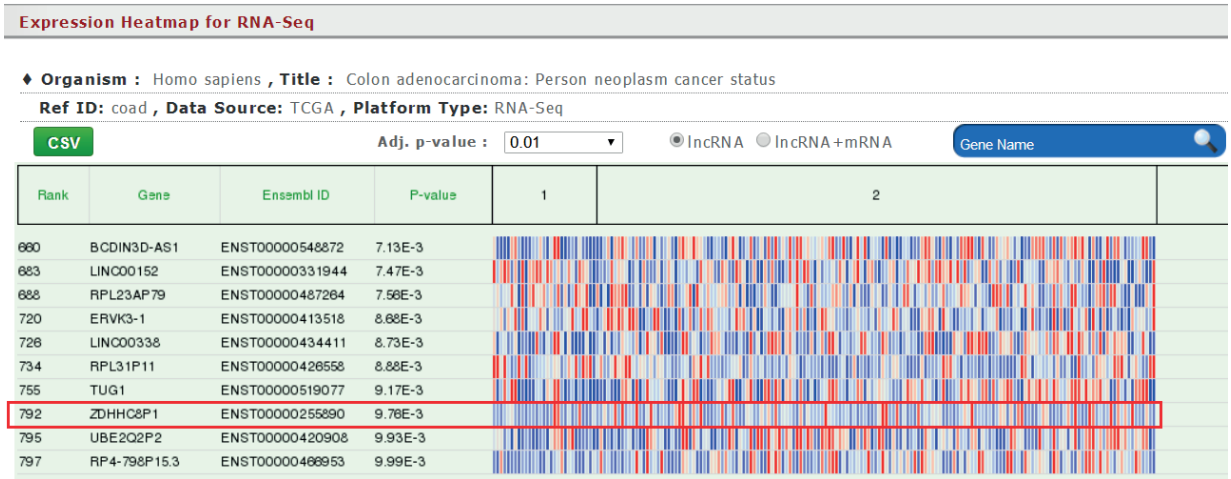

Figure 1. Differentially expressed lncRNA heatmap of colon cancer in lncRNAtor. LncRNA-ZDHHC8P1 was highly expressed in heatmap of colon cancer.

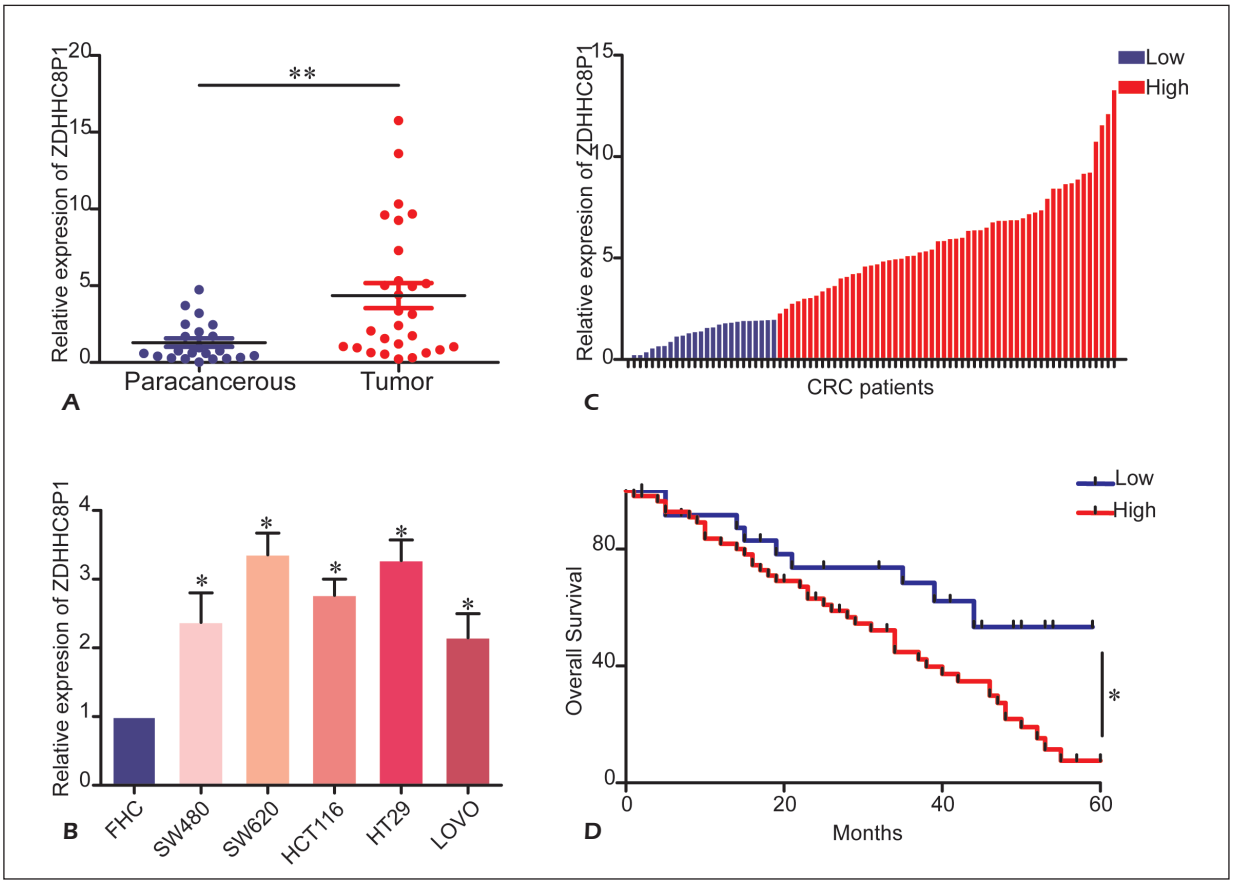

Figure 2. LncRNA-ZDHHC8P1 is a poor prognostic indicator in CRC patients. **A**, The quantitation of ZDHHC8P1 expression in human primary CRC cancer tissues (n=28) and paracancerous tissues (n=22). The relative expression levels were normalized to the mean value of all patients. $^{**}p<0.01$. **B**, Relative ZDHHC8P1 expression in cells. qRT-PCR was used to analyze ZDHHC8P1 expression in CRC cell lines (SW480, SW620, HCT116, HT29 and LOVO) and a normal human colon epithelial cell (FHC) line ($^{*}p<0.05$, vs. FHC cell). **C**, Rel-ative expression of ZDHHC8P1 expression in CRC cancer tissues (n=81). ZDHHC8P1 expression was examined by qPCR and normalized to GAPDH expression. **D**, Kaplan-Meier curves of survival differences between CRC patients. Percentage of survival for patients expressing high and low levels of ZDHHC8P1. p-values were deter-mined by the log-rank test. $^{*}p<0.05$.

vitro. Firstly, lncRNA ZDHHC8P1 expression levels in SW620 and HT29 transfected with siRNAs were significantly down-regulated compared to the control group (Figure 3A). The cell cycle distribution of the treated SW620 and HT29 cells was then assessed by flow cytometry. We discovered that silencing the ZDHHC8P1 expression with siRNAs inhibited growth by arresting cells at the G1-to-S-phase transition (Figure 3B). CCK-8 assays were used to detect the proliferation and apoptosis ability of SW620 and HT29 cells. We found that lncRNA ZDHHC8P1 knockdown could decrease the proliferation ability in both cell lines (Figure 3C-D). What's more, the knockdown of lncRNA ZDHHC8P1 decreased the expression of cyclin D1 by using the qRT-PCR (Figure 3E-F).

Overexpression of ZDHHC8P1 Promotes CRC Cells Growth and Their Metastasis in vitro

To further validate the oncogenic properties and roles of ZDHHC8P1 on CRC, we established CRC cell lines (SW620 and HT29) with ZDHHC8P1 stable over-expression with the plasmid. qRT-PCR indicated that ZDHHC8P1 was efficiently over-expressed by the over-expression vector in SW620 and HT29 cells (Figure 4A). In addition, ZDHHC8P1 over-expressed HT29 cells migrated more rapidly to close the scratched wounds, compared with cells with the control vector, as tested by using a scratched wound-healing assay (Figure 4B). CCK-8 assay was performed to assess the proliferation

Table II. Association between LncRNA-ZDHHC8P1 expression in CRC patients' characteristics.

	ZDHHC8P1			
	Total	High	Low	*p*
Age				
≥60	71	52	20	
<60	10	4	5	0.089
Sex				
Male	53	35	18	
Female	28	21	7	0.406
Tumor site				
Rectum	55	39	16	
Colon	26	17	9	0.615
Tumor histology				
Adenocarcinoma	63	46	17	
Mucinous adenocarcinoma	18	10	8	0.157
Tumor differentiation				
Well/moderate	35	19	16	
Poor	46	37	9	0.012*
AJCC stage				
II-III	57	35	22	
IV	24	21	3	0.020*
Lymph-vascular invasion				
Absence	45	26	19	
Presence	36	30	6	0.013*

The statistical significance of difference was measured by Pearson's χ^2-test. *$p<0.05$.

of SW620 and HT29 cells, which showed that overexpressing ZDHHC8P1 promoted cell proliferation (Figure 4C-D). Moreover, elevated expression of lncRNA ZDHHC8P1 increased the expression of cyclin D1 (Figure 4E-F). The results revealed that lncRNA ZDHHC8P1 could promote proliferation and inhibit apoptosis of CRC cells, promoting the progression and metastasis of CRC *in vitro*.

LncRNA ZDHHC8P1 Could Directly Target MiRNA-34a in CRC Cells

As we have found that lncRNA ZDHHC8P1 could promote oncogenesis via regulating the proliferation and apoptosis, the molecular mechanism remained unclear. Studies have demonstrated lncRNA might act as a competing endogenous RNA or a molecular sponge in regulating the biological functions of miRNA. We suspected that ZDHHC8P1 regulated proliferation and apoptosis through interaction with miR-34a. To further investigate the association of miR-34a and ZDHHC8P1, a wt-ZDHHC8P1 Luciferase reporter vector (wt-ZDHHC8P1), a mut-ZDHHC8P1 3'UTR Luciferase reporter vector (mut-ZDHHC8P1) with mutations on predicted miR-34a binding site in ZDHHC8P1 was constructed (Figure 5A). After that, we conducted a Luciferase reporter assay. Compared with other groups, co-transfection with miR-34a mimic and wt-ZDHHC8P1 significantly decreased the Luciferase activity of SW620 and HT29 cells. The data revealed that miR-34a could directly bind to ZDHHC8P1 binding sites (Figure 5B-C). Furthermore, we constructed a ZDHHC8P1 probe to mimic cellular ZDHHC8P1 in CRC cells, and we found that miR-34a was markedly enriched by the ZDHHC8P1 probe in SW620 and HT29 cells (Figure 5D). To explore the potential relationship of oncogenic functions between ZDHHC8P1 and miR-34a, we detected the expression of miR-34a in the 20 serum samples of CRC patients and analyzed the correlation. The data showed the expression of miR-34a was reversely correlated with the expression of ZDHHC8P1 (R^2=0.6553, $p<0.005$; Figure 5E). Additionally, ZDHHC8P1 knockdown suppressed and ZDHHC8P1 overexpression facilitated miR-34a expression both in SW620 and HT29 cells (Figure 5F-G). These data revealed miR-34a directly bound to ZDHHC8P1 at the recognitive sites and ZDHHC8P1 could promote proliferation of SW620 and HT29 cells by regulating miRNA-34a.

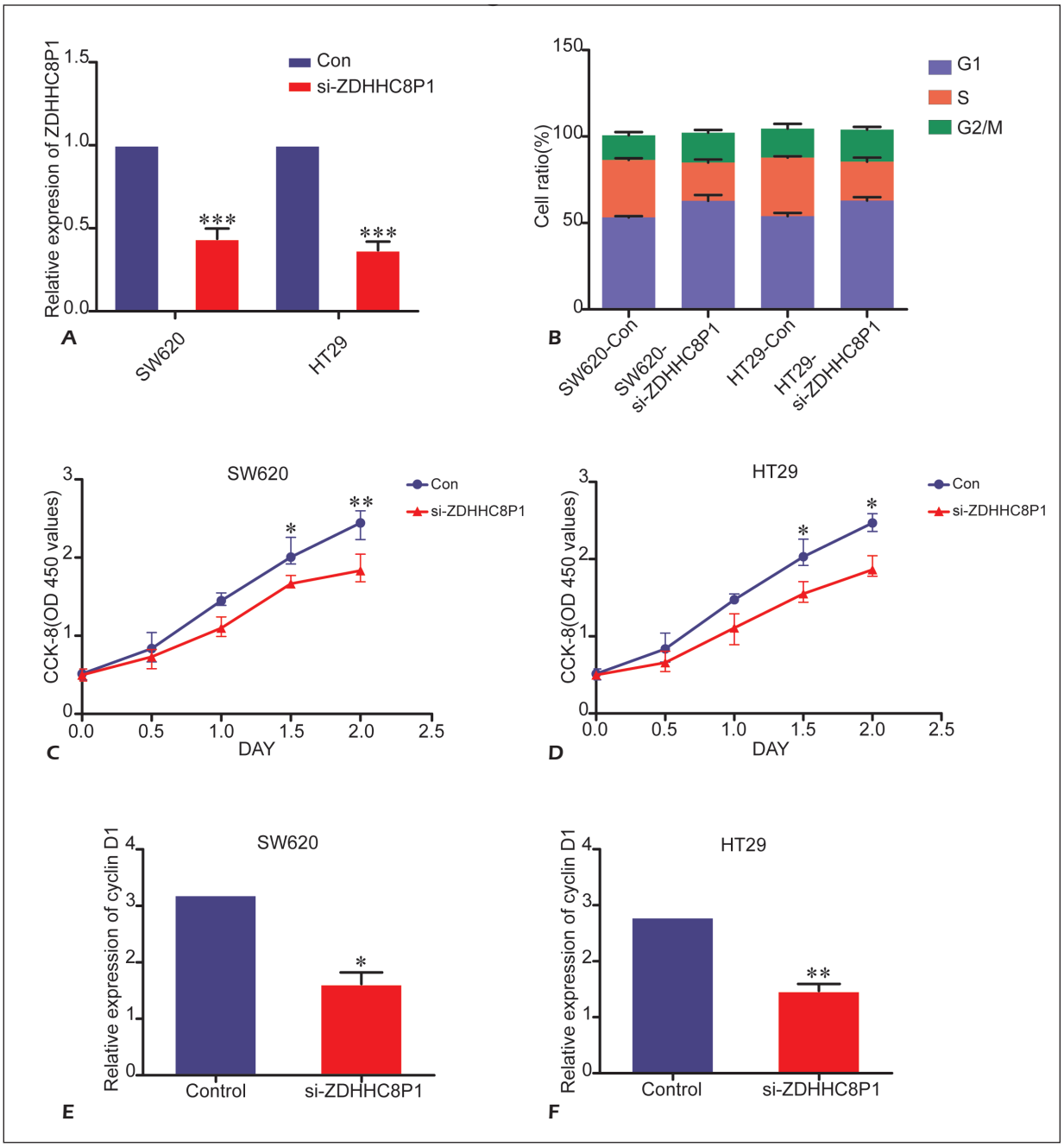

Figure 3. Silencing ZDHHC8P1 expression with siRNA inhibits the progression of CRC. **A**, qRT-PCR was used to measure the expression level of ZDHHC8P1 in the treated SW620 and HT29 cells. ***p<0.001. **B**, The cycle distribution of the treated SW620 and HT29 cells was assessed by flow cytometry. **C-D**, The proliferation and viability of SW620 and HT29 cells were measured using the Cell Counting Kit-8 (CCK-8) colorimetric assay after lncRNA ZDHHC8P1 knockdown. **p<0.01, *p<0.05. **E-F**, Low levels expression of lncRNA ZDHHC8P1 regulated the expression of cyclin D1. **p<0.01, *p<0.05.

Discussion

Colorectal cancer is one of the deadliest solid malignancies. Owing to the lack of specific biomarkers for its diagnosis, early-stage CRC patients still develop recurrence and therapeutic effect monitoring or prognosis might also be responsible for the low survival rate[1,18-20]. Hence, there is a critical need for reliable prognostic factors pinpointing a poor outcome. Knowledge is now rapidly emerging on the involvement of noncoding RNAs in the development, diagnosis, and prognosis of colorec-

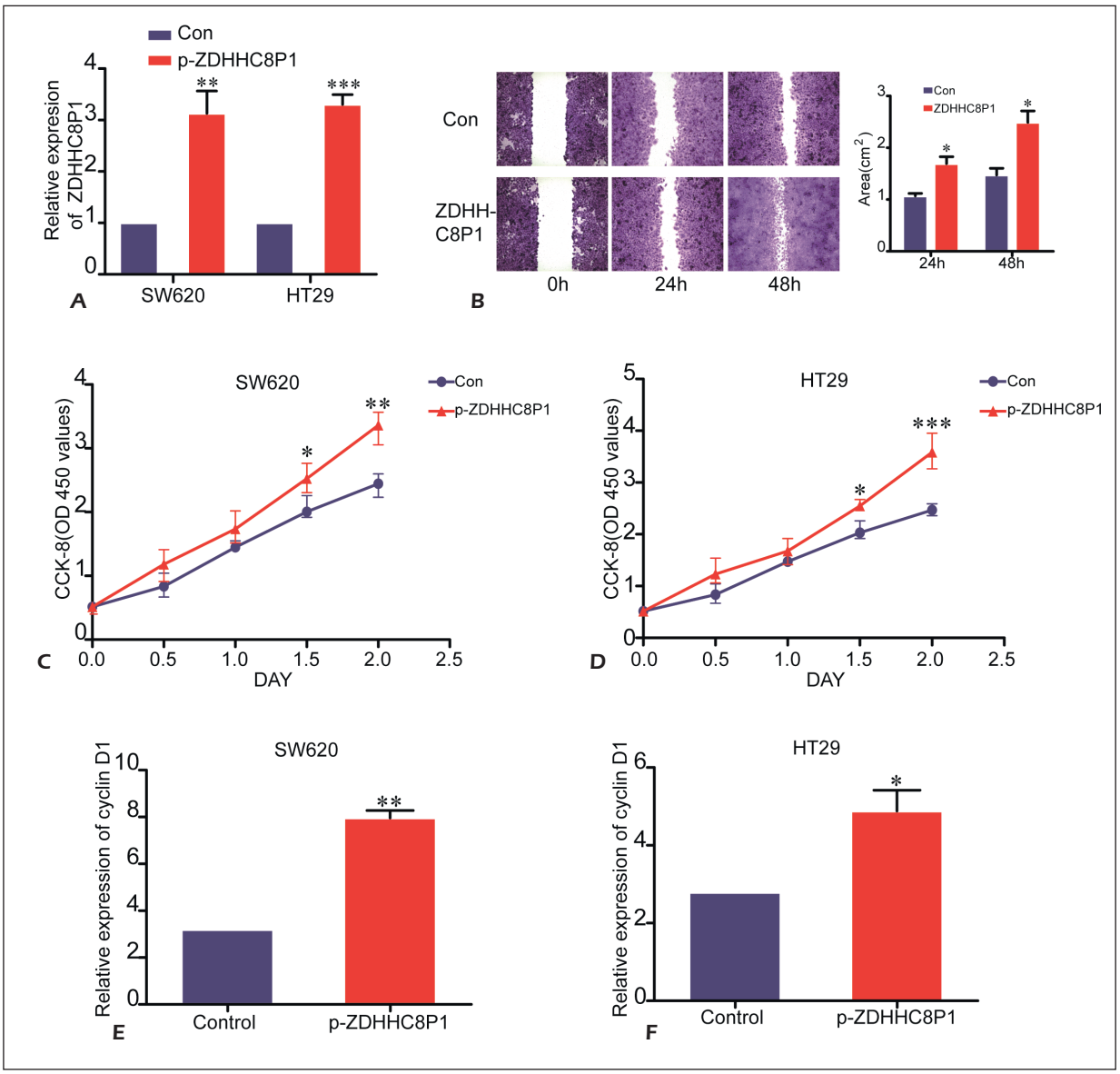

Figure 4. Overexpression of ZDHHC8P1 promotes the metastasis of CRC. **A**, qRT-PCR was used to measure the expression level of ZDHHC8P1 in the treated SW620 and HT29 cells. ***p<0.001. **B**, A wound-healing assay performed with ZDHHC8P1-over-expressed HT29 cells at 0, 24, and 48 hours, and the average wound-healing area. *p<0.05. **C-D**, The proliferation and viability of SW620 and HT29 cells were measured using the Cell Counting Kit-8 (CCK-8) colorimetric assay after lncRNA ZDHHC8P1 overexpression. ***p<0.001, **p<0.01, *p<0.05. **E-F**, High levels expression of lncRNA ZDHHC8P1 regulated the expression of cyclin D1. **p<0.01, *p<0.05.

tal cancer, which has been widely investigated[21]. Long non-coding RNAs (lncRNAs), a type of vital ncRNAs, have been verified to play important regulating roles in CRC. Iguchi et al[22] reported that lncRNA-ATB increased in colon cancer and correlated with tumor size, tumor invasion and lymph node metastasis. Li et al[23] analyzed the prognostic value of 21 lncRNAs in 30 colorectal cancer patients and reported that higher levels of AFAP1-AS1, BCAR4, H19, HOXA-AS2, MALAT1 or PVT1 could predict poor prognosis of colorectal cancer patients. Zhang et al[24] found that low expression of lncRNA LINC01133 contributed to CRC metastasis and poor prognosis or might serve as a promising biomarker. These studies indicated that lncRNAs play an important role in regulating CRC but further studies are still needed to identify detailed functions and mechanisms. ZDHHC8P1 has been known to be an attractive candidate gene for schizophrenia due to the gene's location in the

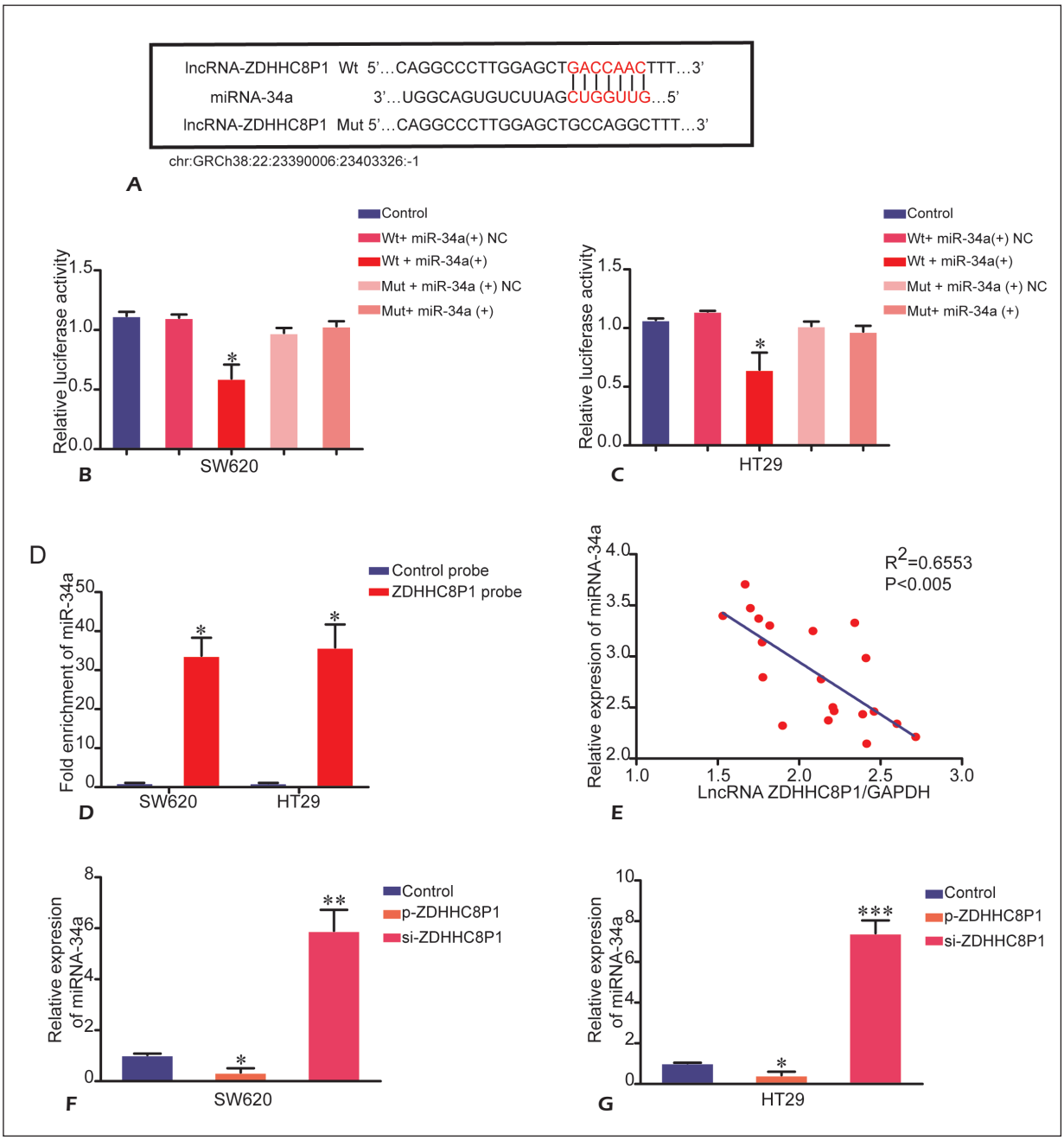

Figure 5. miR-34a is a direct target of ZDHHC8P1 in CRC cells. **A**, Bioinformatics analysis predicted binding sites between ZDHHC8P1 and miRNA-34a. **B-C**, The Luciferase reporter assay. Co-transfection with miR-34a and ZDHHC8P1 Wt significantly increased the Luciferase activity of SW620 and HT29 cells compared with others. *p<0.05. **D**, MiR-34a was remarkably enriched by ZDHHC8P1 in SW620 and HT29 cells transfected with a ZDHHC8P1 probe. *p<0.05. **E**, The LncRNA ZDHHC8P1 expression level was reversely correlated with miR-NA-34a expression in patients with CRC. R2=0.6553, **p<0.005. **F-G**, Up-regulation of LncRNA ZDHHC8P1 markedly decreased the expression of the expression of miRNA-34a, while down-regulation of LncRNA ZDHHC8P1 significantly increased the expression of the expression of miRNA-34a in SW620 and HT29 cells. ***p<0.001, **p<0.01, *p<0.05.

microdeletion region[15,16,25]. However, the role of ZDHHC8P1 in colorectal cancer is not well investigated. Recent evidence demonstrates that some clinicopathological characteristics such as tumor differentiation and vascular invasion can be used as independent prognostic factors for survival; however, to date, optimal prognostic biomarkers for CRC have not been fully established[26,27]. In our

work, lncRNA ZDHHC8P1 was found to be increased and correlated with tumor differentiation, final stage and lymph-vascular invasion in CRC patients. Moreover, CRC patients with high expression of ZDHHC8P1 have a poor survival rate. Through loss- and gain-of function approaches, we showed that ZDHHC8P1 promotes cells proliferation and induces apoptosis *in vitro*. Thus, our findings establish a previously unrecognized and important role for lncRNA ZDHHC8P1 in CRC patients and malignant progression. These findings are remarkable because they indicate that lincRNA ZDHHC8P1 may be a novel therapeutic approach to treat human colorectal cancer. MiRNAs are ncRNAs that are approximately 20 nucleotides in length and regulate gene expression by inhibiting translation or degrading mRNA transcripts. Moreover, numerous studies[28,29] revealed that miRNAs are closely related to oncogenesis. Dysregulation of miR-34a, which was identified as part of the p53 tumor suppressor network, has been implicated in the development of some forms of cancer[30]. Pu et al[31] reported that miR-34a-5p targeted oncogenic CD117 gene and functioned as a tumor suppressor in human osteosarcoma. Zhang et al[32] found that miR-34a may act as a negative regulator in colon cancer by targeting PDGFRA. Tazawa et al[33] provided evidence that miR-34a functions as a potent suppressor of cell proliferation through modulation of the E2F signaling pathway in colon cancer. In this study, we found that the expression of miR-34a was negatively correlated with the expression of lncRNA ZDHHC8P1. Furthermore, transfection with lncRNA ZDHHC8P1 inhibitor significantly increased the expression of miR-34a, while transfection with lncRNA ZDHHC8P1 promoter markedly reduced the expression of miR-34a. These data also indicate that there might be a ZDHHC8P1–miRNA-34a axis in CRC cell lines.

Conclusions

We revealed that lncRNA ZDHHC8P1 was highly expressed in patients with colorectal cancer and ZDHHC8P1 was an important prognostic factor of CRC patients. Furthermore, our findings firstly uncovered that lncRNA ZDHHC8P1 could promote progression and metastasis of colorectal cancer through miR-34a. The present results elucidate a potential mechanism underlying the tumor-oncogenic role of ZDHHC8P1 in CRC and indicate that lncRNA ZDHHC8P1 might be used as a promising prognostic marker and a potential target.

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Conflict of Interests

The authors declare that they have no conflict of interest.

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