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RESEARCH ARTICLE

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Long noncoding RNA DLX6-AS1 promotes liver cancer by increasing the expression of WEE1 via targeting miR-424-5p

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Abstract

Long noncoding RNAs (lncRNAs) played an important role in tumorigenesis and development of hepatocellular carcinoma (HCC). In this study, we first demonstrated that lncRNA DLX6 antisense RNA 1 (DLX6-AS1) was upregulated in cancer tissues and cells lines compared with normal adjacent and cell line. Knock-down DLX6-AS1 by transfection with small interfering RNA (siRNA) suppressed cell proliferation, migration, and invasion of HCC cells. Cell cycle analysis showed that cells transfected with siRNA were arrested in G0/G1 phase. Then, we performed dual-luciferase reporter assay and RNA immunoprecipitation (RIP) assay to show that DLX6-AS1 could bind with miR-424-5p. And cotransfection inhibitor of miR-424-5p with siRNA of DLX6-AS1 could abolish the inhibitory effect of siRNA of DLX6-AS1 on cell proliferation, migration, and invasion. Moreover, we further demonstrated that the oncogene WEE1 G2 checkpoint kinase (WEE1) was the target of miR-424-5p and expression levels of WEE1 were positive correlation with that of DLX6-AS1. Taken together, these results suggested that upregulated DLX6-AS1 promoted cell proliferation, migration, and invasion of HCC through increasing expression of WEE1 via targeting miR-424-5p.

KEYWORDS

DLX6-AS1, hepatocellular carcinoma, long noncoding RNA, miR-424-5p, WEE1

1 | INTRODUCTION

Hepatocellular carcinoma (HCC), a highly aggressive primary liver cancer, is one of the most commonly diagnosed cancers in the world and contributes to the second significant cause of cancer-related death with poor 5-year survival rate.[1,2] Improved knowledge in hepatocarcinogenesis and immuno-oncology has enabled the development of novel therapy methods, such as targeted drug therapy, immunotherapy, small molecule inhibitors or miRNA-based therapy.[3] In addition, a new drug Opdivo (nivolumab) has been approved by Food and Drug Administration to treat patients who do not respond to Nexavar (sorafenib).[4] However, owing to the continually increased incidence and mortality of HCC, there is an urgent need to develop effective treatment. Thus, elucidation of the molecular mechanism underlying the HCC tumorigenesis will assist in achieving this goal.

Long noncoding RNAs (lncRNAs) are noncoding RNAs (ncRNAs) which are defined as 200 nt-100 kb transcripts without open reading frame (ORF).[5] Since their discovery in 1990s, numerous studies have shown the roles of lncRNAs in various cellular processes, including cell proliferation, cell cycle, cell differentiation, and apoptosis.[6] Although the regulatory mechanisms of lncRNA are not fully understand, they are known to control gene expression and cellular activity via chromatin remodelling,[7] transcriptional coactivation and corepression,[8] alternative splicing and translational

regulation of mRNA,[9] scaffolds,[9] and miRNA sponge.[10] Dysregulation of lncRNAs has been widely identified in HCC and was found to be associated with tumorigenesis and progression of HCC, suggesting their potential as therapeutic targets and/or bio-markers.[11,12] For example, upregulation of small nucleolar RNA host gene 20 (*SNHG20*) predicts poor prognosis in HCC.[13] lncRNAs activated by transforming growth factor-β, lncRNA-ATB, promotes epithelial-mesenchymal transition and colonization of HCC by upregulating zinc finger e-box binding homeobox 1/2 (ZEB1/2) and binding with interleukin 11 (IL-11 mRNA).[14]

microRNAs (miRNAs) are another type of ncRNAs that can downregulate gene expression by binding to the 3′-untranslated region (3′-UTR) of the targets.[15] Using microarray analysis, a subset of miRNAs were found dysregulated in the development of HCC.[16] Several reviews have shown that a large number of miRNAs, including let-7a, miR-34a, and miR-10, play an important role in apoptosis, proliferation, metastasis in HCC by targeting various genes.[17] Furthermore, recent studies have shown that lncRNA could function as competing endogenous RNAs (ceRNA) to regulate gene expression by competing with miRNAs in HCC.[18] Thus, targeting ceRNA mechanism by using artificial lncRNA to block multiple miRNAs provides a new therapeutic strategy.[19]

In this study, we focused on the lncRNA DLX6 antisense RNA 1 (DLX6-AS1). DLX6-AS1 is upregulated in lung adenocarcinoma and associated with both histological differentiation and TNM stage.[20] In renal cell carcinoma, DLX6-AS1 is also upregulated and promotes RCC cell growth and tumorigenesis via targeting miR-26a.[21] However, the function of DLX6-AS1 in HCC has not been studied. Thus, in this study, we compared the expression levels of DLX6-AS1 in cancer tissues to the adjacent tissues and determined the cellular function of DLX-AS1 in HCC. Moreover, we found DLX-AS1 might function by increasing the expression of the WEE1 oncogene via targeting miRNA-424-5p.

2 | MATERIALS AND METHODS

2.1 | Tissue specimens and clinical data

The present study was approved by the ethics committee of Union Hospital, Tongji Medical College, and all patients provided written informed consent. Patients included 17 males and 13 females with a mean age of 59.5 years (ranged from 42 to 73 years) between March 2016 and March 2018. A total of 60 tissue samples (30 HCC tumor tissue samples and their adjacent normal tissue samples) were obtained and stored in liquid nitrogen. No patients underwent radiation or chemotherapy before surgery.

2.2 | Cell culture and transfection

Human HCC cell lines (MHCC97L, HCCLM3, SK-HEP-1, Hep3B, and Huh7), normal human liver cell line (LO2) and HEK293t cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY), 100 units of penicillin/mL and 100 ng of streptomycin/mL (Gibco, Grand Island, NY) at 37°C in 5% CO_2.

Small interfering RNAs (siRNAs) of DLX6-AS1 and si-NC, mimics and inhibitor of miR-424-5p and scramble RNA (NC) were transfected into cells with Lipofectamine RNAiMAX Transfection Reagent (Invitrogen, Carlsbad, CA) and plasmids were transfected with Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocols.

2.3 | siRNA, mimics and inhibitor of miRNA and plasmids

The full length of DLX6-AS1 (NCBI Gene ID: 285987) was amplified using KOD DNA polymerase and cloned into pEX-3 vector. Wild-type and mutant copies of 3′-UTR of DLX6-AS1 and WEE1 were amplified and cloned into pmirGLO vector. DLX6-AS1 siRNAs and si-NC, mimics and inhibitor of miR-424-5p and scrambles were purchased from GenePharma Co, Ltd (Shanghai, China). Sequences of DLX6-AS1 siRNAs were:

siRNAs	Sequences (5′ to 3′)
si-DLX6-AS1#1	AAGGCCACTGCATATGAGTTG
si-DLX6-AS1#2	GATTTCTAAACCCTGATCATT
si-DLX6-AS1#3	GGAAAGAAGAGATTAGAAGAA

2.4 | Quantitative reverse-transcription polymerase chain reaction

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed as described in previous study.[20] In brief, total RNA was extracted using TRIzol following the manufacturer's instructions (Invitrogen, Carlsbad, CA). For DLX6-AS1 qRT-PCR, total RNA was reverse-transcribed into cDNA using PrimeScript RT reagent kit with gDNA Eraser (RR047Q, Takara, Takara Biomedical Technology (Beijing) Co, Ltd, China) and glyceraldehyde 3-phosphate dehydrogenase was used as reference control. For miRNA qRT-PCR, cDNA was reverse-transcribed with One Step PrimeScript miRNA cDNA Synthesis Kit (Takara Biotechnology Co, Ltd, Dalian, China) and U6 was used as reference control. The reverse primers for qRT-PCR were provided in the kit.

The other primers were listed below. qRT-PCR was performed on an ABI 7500 System (Applied Biosystems, Carlsbad, CA) with SYBR Premix Ex Taq (Takara Biotechnology Co, Ltd).

Primers	Sequences (5′ to 3′)
DLX6-AS1-forward	AGTTTCTCTCTAGATTGCCTT
DLX6-AS1- reverse	ATTGACATGTTAGTGCCCTT
GAPDH-forward	AGAGGCAGGGATGATGTTCTG
GAPDH- reverse	GACTCATGACCACAGTCCATGC
miR-424-5p-forward	CAGCAGCAATTCATGTTTTGAA
U6-forward	ACAGTAGTCTGCACATTGGTTA

2.5 | 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay and colony formation assay

To determine cell proliferation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and colony formation assay were performed.[22] Cells were seeded at 10^4 cells per well in 96-well plates. After culturing for indicated time, MTT reagent (Sigma, Shanghai, China) was added into each well and cultured for another 4 hours. Subsequently, the medium was discarded and dimethyl sulfoxide was added into each well to dissolve the crystals. Finally, OD_{490} was measured using a microplate reader.

For colony formation assay, 500 cells were seeded into six-well plate and cultured for 14 days. Colonies were fixed with 4% paraformaldehyde for 10 minutes and stained with crystal violet for 30 minutes, then photographed. Colonies with more than 50 cells were counted.

2.6 | Flow cytometric analysis

To determine cell cycle distribution, flow cytometric analysis was performed. Cells were subjected to flow cytometry after fixation and permeabilization with propidium iodide (PI) staining solution.

2.7 | Wound healing assay

Cell migration was determined by wound healing assays. Briefly, after cell monolayer reached confluence, cells were scraped by a 200 μL tip and gently washed with phosphate-buffered saline. Images were captured at 0 and 24 hours and the width of wounds at 0 and 24 hours was measured. Cell mobility was calculated based on the healing distance.

2.8 | Matrigel transwell assay

Matrigel transwell assays were performed to assess cell invasion. Cells were seeded into upper chambers (membranes with 8 μm pore size; Millipore pore, Costar) coated with Matrigel (BD Biosciences, San Jose, CA). A total of 600 μL of complete medium was added to the bottom chamber. After 24 hours incubation at 37°C, cells on the top surface of the membrane were removed using a cotton swab. Cells on the bottom surface (invading cells) were fixed with 4% paraformaldehyde, stained with crystal violet and washed with distilled water. Cells were then counted using an inverted microscope.

2.9 | Dual-luciferase reporter assay

HEK293t cells were seeded in 24-well plates and cotransfected with mimics of miR-424-5p or NC with pmirGLO plasmids containing wild-type or mutant copy of 3′-UTR of DLX6-AS1 (for miR-424-5p and DLX6-AS1 binding assay) or that of *WEE1* (for miR-424-5p and *WEE1* binding assay) using Lipofectamine 3000. After 48 hours, luciferase activity assay was performed following the dual-luciferase reporter assay system (E1910; Promega, Beijing, China).

2.10 | RNA immunoprecipitation

To determine the enrichment of miR-424-5p by DLX6-AS1, RNA immunoprecipitation (RIP) was performed in cells transfected with DLX6-AS1 plasmids using Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Bedford, MA). Briefly, cells were lysed in the lysis buffer containing protease inhibitor cocktail and RNase inhibitor on ice, and 10% of the whole cell lysates were kept as input (positive control). After immunoprecipitation, the RNA was purified and relative amount of RNAs and proteins were quantified by qRT-PCR and Western blot analysis, respectively. Normal IgG controls were assayed as negative control.

2.11 | RNA pull-down

RNA pull-down analysis was modified based on the previously described method.[23] Briefly, biotinylated *miR-424-5p* mimics, mutant *miR-424-5p* mimics or NC control were transfected into cells for 24 hours. Then, cells were lysed with lysis buffer containing protease inhibitor cocktail and RNase inhibitor on ice. The extracts containing complexes of biotinylated RISC and mRNA were incubated with streptavidin-coated magnetic beads. After washing, RNA was purified and quantified by qRT-PCR.

2.12 | Statistical analysis

All experiments were performed in triplicate, and data are presented as means and standard deviations. Statistical analyses were performed using the SPSS 20.0 software (Chicago, IL). One way analysis of variance and Student *t* test were used in the data obtained from qRT-PCR, MTT assay, colony formation assays, would healing assay, matrigel transwell assay and dual-luciferase reporter assay. Correlations among DLX6-AS1, miR-424-5p, and WEE1 were analyzed with a Spearman rank correlation. $P < 0.05$ was considered to indicate a significant difference.

3 | RESULTS

3.1 | DLX6-AS1 is upregulated in liver cancer and HCC cell lines

To determine the function of DLX6-AS1 in HCC, we first measured the expression levels of DLX6-AS1 in liver cancer tissues and HCC cell lines by qRT-PCR. The results showed that the expression levels of DLX6-AS1 were upregulated in tumors compared with the adjacent normal tissues (Figure 1A). In HCC cell lines, the expression levels of DLX6-AS1 were also upregulated compared with normal human liver cell line LO2 and SK-HEP-1. Hep3B had the highest expression levels among the six cell lines tested (Figure 1B). These results indicated that the expression levels of DLX6-AS1 were upregulated in HCC. SK-HEP-1 and Hep3B cells were chosen for further in vitro studies.

3.2 | DLX6-AS1 promotes cell proliferation, migration, and invasion of SK-HEP-1 and Hep3B cells

Since DLX6-AS1 was found to be upregulated in HCC, we then investigated the cellular function of DLX6-AS1 in SK-HEP-1 and Hep3B by downregulating the expression of DLX6-AS1 using siRNA. Three DLX6-AS1 siRNAs and siNC (control) were synthesized and transfected into SK-HEP-1 and Hep3B cells. The qRT-PCR results showed that siDLX-AS1#3 had the best knock-down efficiency (Figure 2A). Thus, si-DLX-AS1#3 was chosen for subsequent experiments investigating the cellular function of DLX6-AS1. We first determined cell proliferation in DLX6-AS1 knockdown cells. Growth curves obtained by the MTT assay showed that DLX6-AS1 knock-down decreased the cell growth rate (Figure 2B). Similarly, the colony formation assay showed less colony numbers in DLX6-AS1 knock-down cells compared with control (Figure 2C). Considering cell proliferation is tightly controlled by cell cycle, we then measured cell cycle distribution. SK-HEP-1 and Hep3B cells transfected with siDLX-AS1#3 had larger number of cells in G1 phase and less cells in G2/M phase (Figure 2D). These results indicated that DLX-AS1 knockdown arrested cells in G1 phase, thereby decreasing cell proliferation.

Then, we determined the effect of DLX-AS1 knock-down on cell migration and invasion by wound scratch assay and matrigel transwell assay, respectively. The results demonstrated that SK-HEP-1 and Hep3B cells transfected with si-DLX-AS1#3 migrated much slower (Figure 2E), and less invasive (Figure 2F) compared with control. These findings suggest that upregulated expression of DLX-AS1 in HCC may promote cell proliferation, migration and invasion.

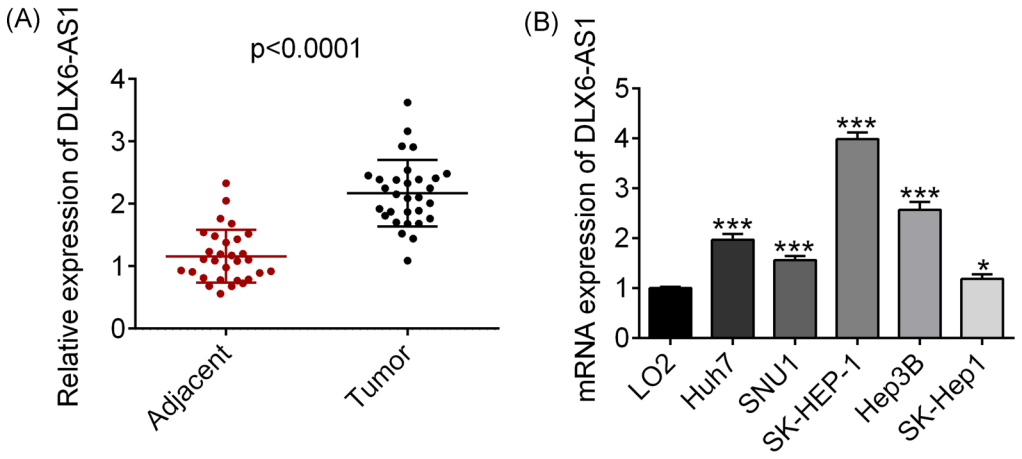

FIGURE 1 The expression of DLX6-AS1 in the HCC tissues and cell lines as determined by qRT-PCR. A, The expression of DLX6-AS1 is upregulated in the HCC cancer tissues compared with the adjacent normal tissues. B, Upregulated DLX6-AS1 in the HCC cell lines compared with normal human liver cell line (LO2). *$P < 0.05$; ***$P < 0.001$. HCC, hepatocellular carcinoma; qRT-PCR, quantitative reverse-transcription polymerase chain reaction

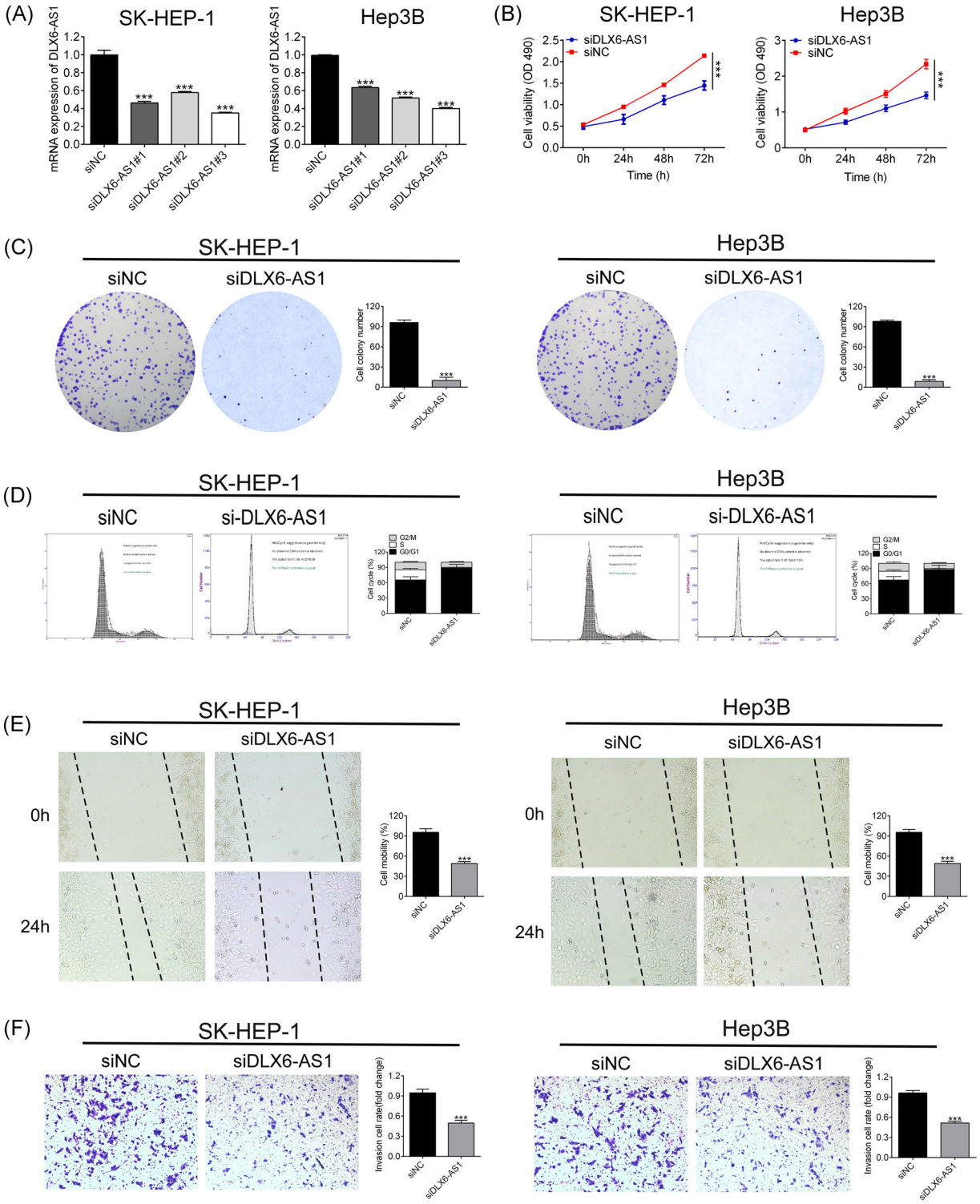

FIGURE 2 Cellular function of DLX6-AS1 in SK-HEP-1 and Hep3B. A, DLX6-AS1 is knocked-down in cells transfected with siRNAs. B, Growth curves were obtained by MTT assay. Decreased growth rate in cells transfected with siDLX6-AS1#3. C, Representative images of colony formation assay and the number of colonies were counted. D, Cell cycles were analyzed by flow cytometry. Transfection with siDLX6-AS1#3 changes the cell cycle distribution. E, Representative images of wound healing assay and cell mobility are shown. Transfection with siDLX6-AS1 decreases cell migration. F, Representative images of the matrigel transwell assay and cells invaded through matrigel were counted. ***$P < 0.001$. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; siRNA, small interfering RNA

3.3 | DLX6-AS1 binds directly to miR-424-5p

One of the regulatory mechanisms of lncRNAs is through their function as miRNA sponge. We investigated the potential miRNA binding partner of DLX6-AS1 through bioinformatic prediction on the starbase (http://starbase.sysu.edu.cn/), which revealed that DLX6-AS1 could potentially bind to the 3′-UTR of miR-424-5p (Figure 3A). To validate this, we then performed dual-luciferase reporter assays, which showed that mutations in the 3′-UTR of miR-424-5p abolished the binding of DLX6-AS1 to miR-424-5p (Figure 3B). Moreover, we performed RIP assay and RNA pulldown assay to show direct binding between DLX6-AS1 and miR-424-5p. Previous studies have demonstrated that miRNAs are present in the form of miRNA ribonucleoprotein complexes (miRNPs) containing Ago2.[5] Thus, we first determined the enrichment of miR-424-5p in Ago2-immunoprecipitated complex from cells transfected with DLX6-AS1 plasmids. The results showed enrichment of miR-424-5p in the complex compared with input (10% of the whole cell lysate. Figure 3C). Then, RNA pulldown assay showed that the relative amount of DLX6-AS1 was much higher in cells transfected with biotinylated miR-424-5p mimics than those transfected with biotinylated miR-424-5p mutant mimics (Figure 3D). These findings suggest that DLX6-AS1 binds to miR-424-5p in a sequence-specific manner.

3.4 | DLX6-AS1 regulates cell proliferation, migration, and invasion through *miR-424-5p*

Considering DLX6-AS1 could directly bind to miR-424-5p (Figure 3), we further explored whether DLX6-AS1 may regulate cell proliferation, migration, and invasion through miR-424-5p. A "rescue" experiment was performed by co-transfecting miR-424-5p inhibitor and siDLX-AS1#3. The MTT assay and colony formation assay results showed that cell proliferation inhibition caused by siDLX-AS1#3 was abolished by cotransfection with miR-424-5p inhibitor (Figure 4A and 4B). Cell cycle analysis also showed that cells cotransfected with siDLX-AS1#3 and miR-424-5p inhibitor had similar cell cycle distribution compared with cells cotransfected with NCs, whereas transfection with siDLX-AS1#3 or miR-424-5p inhibitor alone led to cell arrest in G0/G1 or the S phase (Figure 4C). Similarly, wound scratch assay and matrigel transwell assay results showed that cotransfection with miR-424-5p inhibitor rescued cell migration and invasion inhibition caused by siDLX-AS1#3

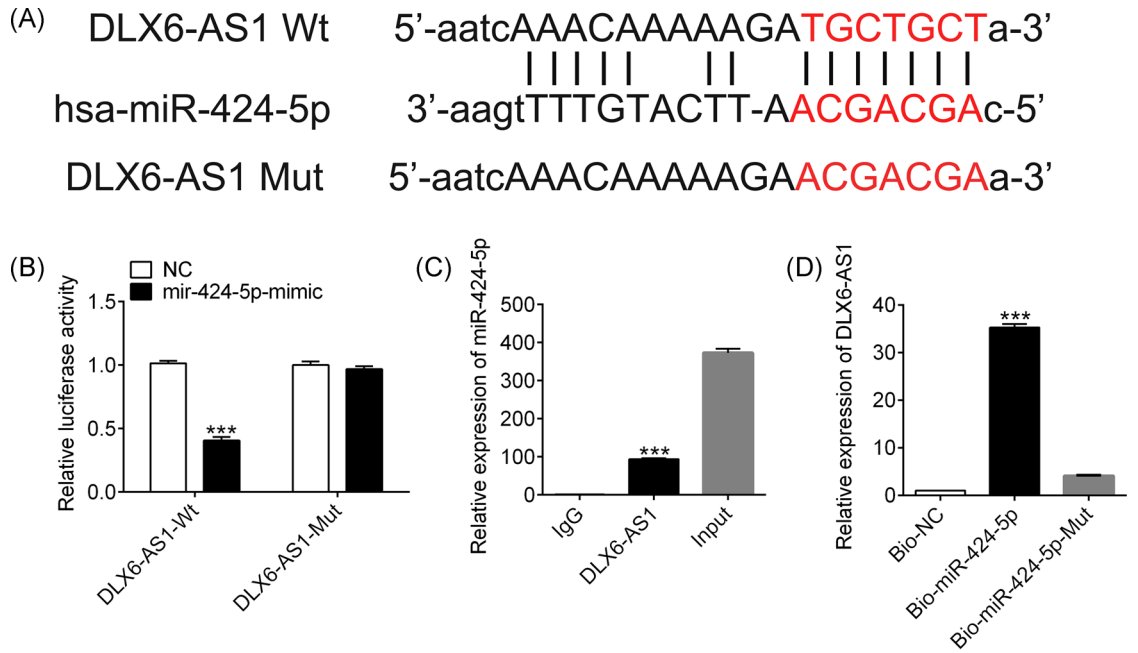

FIGURE 3 DLX6-AS1 targets miR-424-5p. A, The predicted miR-424-5p binding sites on the 3′-UTR of DLX6-AS1 are shown. B, Dual-luciferase reporter assay were performed and mutations in the DLX6-AS1 binding site abolish the inhibitory effect of miR-424-5p mimics on the luciferase activity. C, The expression levels of miR-424-5p and DLX6-AS1 were determined by qRT-PCR in Ago2-immunoprecipitated complex, and miR-424-5p and DLX6-AS1 are enriched compared with positive control (input) and negative control (anti-IgG). The protein levels of Ago were determined to show efficient immunoprecipitation of anti-Ago from cell extracts. D, The expression levels of DLX6-AS1 were determined by qRT-PCR in the complex which were pulled down by biotinylated miR-424-5p mimics, mutant miR-424-5p mimics or NC. ***$P < 0.001$. 3′-UTR, 3′-untranslated region; Mut, mutant; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; Wt, wild-type

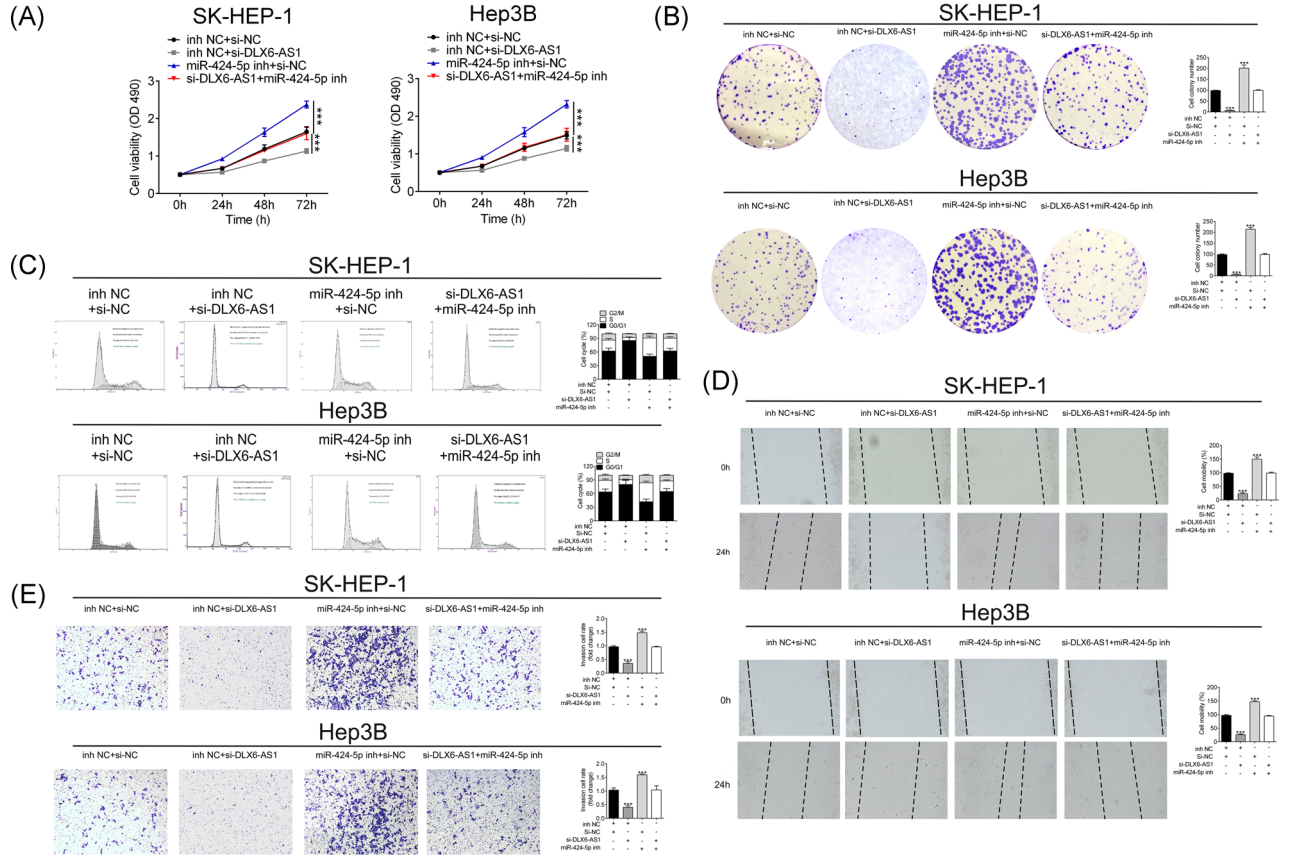

FIGURE 4 Transfection with miR-424-5p inhibitor abolishes the effect of siDLX6-AS1. A, Growth curves were obtained by MTT assay. B, Representative images of colony formation assay are shown and the numbers of colonies were counted. C, Cell cycle distribution was determined by flow cytometry. D, Representative images of wound healing assay are shown and cell mobility was calculated. Transfection with siDLX6-AS1 decreases cell migration. E, Representative images of matrigel transwell assay and cells invaded through matrigel were counted. ***$P < 0.001$. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

(Figure 4D and 4E). Taken together, these results indicated that DLX6-AS1 confers its functions in HCC via miR-424-5p.

3.5 | WEE1 is the target of miR-424-5p

miRNAs play an important role in cancer by decreasing the expression of their targets. Using targetscan (http://www.targetscan.org/), *WEE1* was predicted to be the target of miR-424-5P (Figure 5A). Dual-luciferase reporter assay results showed that transfection of miR-424-5p mimics decreased the luciferase activity in cells transfected with wild type 3′-UTR of *WEE1,* whereas mutations in the miR-424-5P binding sites in the 3′-UTR of *WEE1* abolished the inhibitory effect on the luciferase activity (Figure 5B). Moreover, the expression levels of WEE1 were decreased/increased in SK-HEP-1 and Hep3B cells transfected with mimics/inhibitor of miR-424-5p, respectively, as shown by qRT-PCR and Western blot analysis (Figure 5C and 5D). These results indicated that *WEE1* was a direct target of miR-424-5p. To confirm whether DLX6-AS1 upregulation may induce the expression of *WEE1*, we determined the expression levels of *WEE1* in SK-HEP-1 and Hep3B cells transfected with DLX6-AS1 overexpression plasmids or siDLX-AS1#3 by qRT-PCR and Western blot analysis. The results showed that *WEE1* was upregulated/decreased in cells transfected with DLX6-AS1 overexpression plasmids or siDLX-AS1#3, respectively (Figure 5E and 5F). Finally, we performed correlation analysis in the clinical specimens, which showed that the expression levels of DLX6-AS1/miR-424-5p were negatively correlated with that of miR-424-5p/*WEE1*, whereas the DLX6-AS1 levels were positively correlated with that of *WEE1* (Figure 5G). These results indicated that DLX6-AS1 increased the expression of the WEE1 by downregulating miR-424-5p.

4 | DISCUSSION

WEE1 is a nuclear kinase regulating G2–M transition, and overexpression of WEE1 has been found in various cancers including malignant melanoma, breast cancer, osteosarcoma, glioma, and gastric cancer.[24] Previous

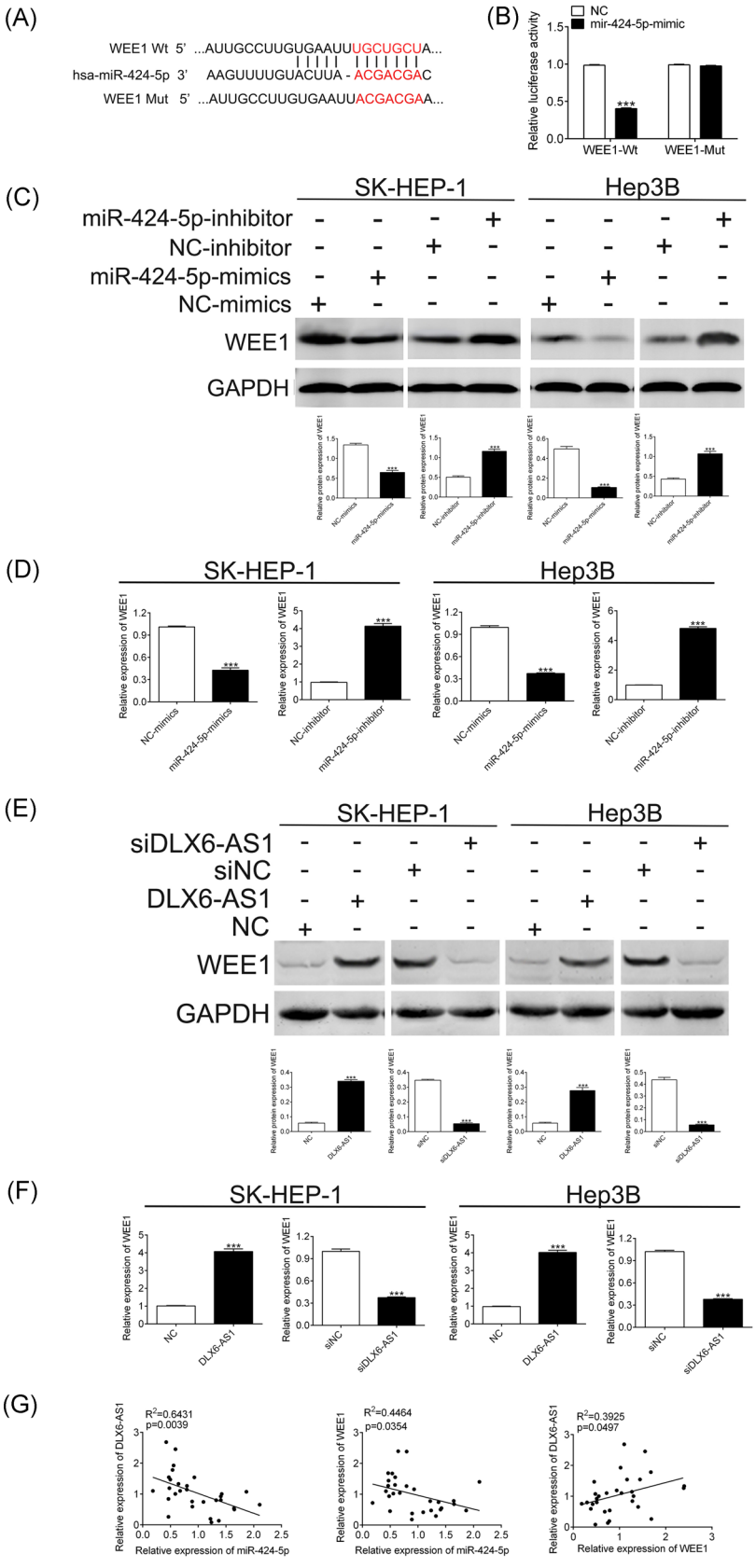

FIGURE 5 miR-424-5p targets the 3′-UTR of *WEE1*. A, The predicted miR-424-5p binding sites in the 3′-UTR of *WEE1* are shown. B, Dual-luciferase reporter assay was performed and mutations in the 3′-UTR of *WEE1* abolish the inhibitory effect of miR-424-5p mimics on the luciferase activity. C, D, The expression levels of WEE1 are decreased/increased in SK-HEP-1 and Hep3B cells transfected with mimics/inhibitor of miR-424-5p as determined by Western blot analysis and qRT-PCR. E, F, The expression levels of WEE1 are increased/decreased in SK-HEP-1 and Hep3B cells transfected with DLX6-AS1 overexpression plasmid/siRNA as determined by Western blot analysis and qRT-PCR. G, Correlations among the expression of DLX6-AS1, miR-424-5p, and WEE1 were analyzed with a Spearman's rank correlation. ***$P < 0.001$. 3′-UTR, 3′-untranslated region; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; siRNA, small interfering RNA

studies have shown that the inhibition of WEE1 could be a potential target therapy for cancers.[24,25] A highly specific small molecule inhibitor of WEE1, named AZD1775, has shown potential therapy effect in various cancers, including glioblastoma, refractory myeloid malignancies, metastatic colorectal cancer, ovarian cancer, head and neck squamous cell carcinoma, and small cell lung cancer.[26] These results suggest that WEE1 could be a general therapy target for cancers. Upregulated expression of WEE1 is associated with poor prognosis of HCC, and applying AZD1775 to HCC cells could sensitize these cells to radiation.[27] In this study, we proposed another potential mechanism to inhibit WEE1 by downregulating DLX6-AS1.

Among the 87.3% actively transcribed human genome, only less than 3% are protein coding and the remaining are ncRNAs.[28] The huge amount of actively transcribed ncRNAs indicates their important role in physiological and pathological processes. lncRNAs are regulatory ncRNAs which can bind to DNA, RNA, and protein.[29] Recent studies on lncRNA have demonstrated that lncRNA could function as tumor promoters or tumor suppressors in HCC.[30] For example, aberrantly upregulated lncRNA urothelial carcinoma–associated 1 (UCA1) reverses the inhibitory effect of miR-216b on the growth and metastasis of HCC cells by directly binding to miR-216b.[31] LncRNA CDKN2B antisense RNA 1(CDKN2B-AS1) suppresses metastasis, and invasion of HCC by decreasing the expression of miR-122-5p.[32] In this study, we found that DLX6-AS1 was upregulated in HCC and the upregulation of DLX6-AS1 promoted cell proliferation, migration, and invasion, suggesting the potential function of DLX6-AS1 as an oncogene. Consistently, other studies on DLX6-AS1 have shown the overexpression of DLX6-AS1 in lung adenocarcinoma and renal cell carcinoma and its ability in promoting tumorigenesis.[20,21] Moreover, we found that DLX6-AS1 could function as a sponge for miR-424-5p. By decreasing the expression levels of miR-424-5p in HCC cells using miR-424-5p inhibitor abolished the effect of si-DLX6-AS1. In line with our results, previous study revealed the downregulation of miR-424-5p in HCC and the reduction of miR-424-5p expression promotes the tumorigenicity of HCC cells.[33] Furthermore, we identified the oncogene *WEE1* as the target of miR-424-5p. Finally, we found that the expression levels of *WEE1* was in positive correlation with that of DLX6-AS1 in HCC cells and specimens. Thus, these findings have uncovered another mechanism in suppressing *WEE1* by downregulation of DLX6-AS1.

Taken together, we first determine the cellular function of DLX6-AS1 in HCC and reveal the mechanism through which DLX6-AS1 promotes the tumorigenicity of HCC cells. DLX6-AS1 positively regulates the expression of WEE1 by endogenous competing with miR-424-5p. These findings contribute to hepatocarcinogenesis and provide a potential therapeutic strategy for HCC treatment.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

We declare that this study was done by the researchers listed in this study. All liabilities related with the content of this study will be borne by the authors. YZ designed all the experiments and XT revised the paper. ML and DL formed the experiments, DL wrote the paper.

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