European Review for Medical and Pharmacological Sciences 2019; 23: 8321-8331

The up-regulated lncRNA DLX6-AS1 in colorectal cancer promotes cell proliferation, invasion and migration *via* modulating PI3K/AKT/mTOR pathway

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Abstract. – OBJECTIVE: Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths around the world. Recently, using the high-throughput techniques, long non-coding RNAs (lncRNAs) have been shown to play an important role in CRC progression. In the present study, we aimed to determine lncRNA DLX6 Antisense RNA 1 (DLX6-AS1) in CRC tissues and cell lines and to investigate the molecular mechanisms of DLX6-AS1 in CRC progression.

PATIENTS AND METHODS: Quantitative real-time PCR was performed to detect gene expression; cell counting kit-8, colony formation, cell invasion, and migration assays were performed to determine cell proliferation, invasion, and migration, respectively; caspase-3 activity assay kit was used to detect caspase-3 activity; *in vivo* tumor growth was evaluated in a nude mice xenograft model.

RESULTS: DLX6-AS1 was up-regulated in 60 CRC tissues when compared to normal adjacent colorectal tissues, and high expression of DLX6-AS1 was correlated with advanced T stage and distant metastasis in CRC patients. The up-regulation of DLX6-AS1 was further confirmed in CRC cell lines. The gain-of-function assays showed that DLX6-AS1 overexpression promoted HCT116 cell proliferation, invasion, and migration, but inhibited cell apoptosis; while the loss-of-function assays showed that DLX6-AS1 knockdown exerted the opposite effects in SW480 cells. *In vivo* studies revealed that DLX6-AS1 knockdown suppressed tumor growth in the nude mice xenograft model. In addition, DLX6-AS1 overexpression caused an increase in the phosphorylated phosphoinositide 3-kinase (p-PI3K), p-AKT and p-mammalian target of rapamycin (mTOR) protein levels, and DLX6-AS1 knockdown had the opposite effects. Blockade of PI3K/AKT/mTOR signalling pathway by using mTOR inhibitor partially abolished the enhanced effects of DLX6-AS1 overexpression on CRC cell proliferation and metastasis.

CONCLUSIONS: In summary, our data indicated that DLX6-AS1 promoted CRC cell proliferation, invasion, and migration but inhibited cell apoptosis via targeting PI3K/AKT/mTOR signalling pathway, suggesting the key role of DLX6-AS1 in CRC progression.

Key Words:
Colorectal cancer, DLX6-AS1, Proliferation, Invasion and migration, PI3K/AKT/mTOR.

Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths around the world[1], and the pathophysiology of CRC has been demonstrated to involve multiple genetic factors and cellular regulatory processes[2,3]. The dysregulation of oncogenes and tumor-suppressors has been regarded as the main contributor to CRC aggressiveness[4]. Though recent advances in surgeries and chemotherapy have improved the clinical outcomes of CRC treatment, the overall survival of CRC patients remains poor due to tumor recurrence and metastasis after surgical resection. As such, exploration of novel prognostic makers and deciphering the mechanisms underlying CRC progression may provide us with more effective therapeutic strategies[5].

Long non-coding RNAs (lncRNAs) belong to a type of RNA transcripts with more than 200 nucleotides in length, and lncRNAs have no protein-cod-

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ing capacity and have been detected in cytosolic and nuclear fractions[6]. With the recent advancement of lncRNA research, many characterized lncRNAs have been demonstrated to participate in a variety of biological processes such as cell proliferation, cell migration, cell differentiation, cell apoptosis, and so on[7]. Recently, using the high-throughput techniques, a growing number of lncRNAs have been shown to play an important role in CRC progression[8,9]. For example, lncRNA special AT-rich sequence-binding protein 2 (SATB2) antisense RNA 1 was frequently down-regulated in CRC tissues and cell lines, and suppressed the aggressiveness of CRC via SATB2-dependent snail transcript and epithelial-mesenchymal transition[10]. Zhuang et al[11] identified the significant up-regulation of lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) in CRC tissues, and MALAT1 promoted CRC invasion and metastasis via SLAIN motif family member 2-enhanced microtubules mobility by acting as a sponge for miR-106b-5p. Similarly, Han et al[12] showed that colorectal cancer metastasis-suppressed lncRNA was down-regulated in CRC tissues and played a tumor-suppressive role in CRC via nucleocytoplasmic shuttling of high mobility group box 2. By using microarray analysis, Li et al[13] identified lncRNA DLX6 Antisense RNA 1 (DLX6-AS1) was significantly up-regulated in lung adenocarcinoma tissues, and high DLX6-AS1 expression was associated with both histological differentiation and TNM stage. Later on, dysregulation of DLX6-AS1 was further identified in various types of malignant tumors such as renal cell carcinoma[14], hepatocellular carcinoma[15], pancreatic cancer[16], etc. However, as far as we know, the role of DLX6-AS1 in CRC has been not documented yet.

In the present study, the up-regulation of DLX6-AS1 was first reported in CRC tissues and cell lines. Further *in vitro* functional assays showed that DLX6-AS1 overexpression could result in significant enhancement of CRC progression; while DLX6-AS1 knockdown could lead to suppression of CRC progression. More importantly, mechanistic studies showed that DLX6-AS1 exerted its oncogenic actions via regulating phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathways in CRC cell lines.

Patients and Methods

Collection of Clinical Samples

A total of 60 CRC patients were recruited in this study, and the CRC tissues, as well as the normal adjacent colorectal tissues, were collected from these patients who underwent the surgical resection at Xi'an No. 1 Hospital between July 2015 and January 2018. All the patients had no anti-cancer treatments before the surgical resection. All the clinical samples were immediately stored in –80°C freezer after resection. All the patients signed the written informed consent before the sample collection. This investigation was approved by the Ethics Committee of Xi'an No. 1 Hospital.

Cell Culture, Chemicals, Plasmids, and Small Interfering RNAs (siRNAs)

The normal human colon epithelial cell line (NCM460) and three CRC cell lines, including HCT116, HT-29, and SW480 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). These cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA) in a humidified incubator with 5% CO_2 at 37°C.

The mTOR inhibitor (CCI-799) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The DLX6-AS1-overexpressing plasmids (pcDNA3.1-DLX6-AS1) and the pcDNA3.1 were purchased from GenePharma (Shanghai, China). The two siRNAs targeting DLX6-AS1 (si-DLX6-AS1#1 and #2) and the negative control (NC) siRNA (si-NC) were designed and synthesized by RiboBio (Guangzhou, China). For the treatment with CCI-799, HCT116 cells were treated with 20 μM CCI-799 for 24 h. For the cell transfections, the CRC cells were transfected with different plasmids or siRNAs using the Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA). At 24 h after transfection, further assays were performed in these transfected cells.

RNA Extraction and Quantitative Real Time-PCR (qRT-PCR)

Total RNA from cells and tissues was extracted using RNeasy Mimi Kit (Qiagen, Hilden, Germany). The reverse transcript was performed using Superscript III First Strand Synthesis kit (Invitrogen, Carlsbad, CA, USA). The real-time PCR was performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using the SYBR Green Master Mix kit (TaKaRa, Dalian, China). The quantification of relative DLX6-AS1 expression was based on the comparative threshold cycle method, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal control for DLX6-AS1 expression.

Cell Counting Kit-8 (CCK-8) Assay and Colony Formation Assay

The CRC cell proliferative ability was assessed using the CCK-8 assay kit (Beyotime, Beijing, China). Briefly, the CRC cells after different treatments were further cultured for the indicated time periods, and cells were then incubated with CCK-8 reagent at 37°C for 2 h. The CRC proliferation was determined by measuring the optical density (OD) values at a wavelength of 450 nm. For the colony formation assay, the CRC cells after different treatments were plated onto 6-well plates at a density of 1,000 cells/well, and after a further culture for 10 days, colonies were stained with 0.1% crystal violet and counted.

Transwell Invasion and Migration Assay

Transwell chambers with inserts (8 µm pore size membrane; Millipore, Burlington, NJ, USA) coated or uncoated with Matrigel were used for transwell invasion and migration assays, respectively. Briefly, 1 x 10^5 cells with different treatments were plated onto the upper chamber with fetal bovine serum (FBS)-free DMEM, and the lower chamber was filled with DMEM supplemented with 20% FBS. After a further incubation for 24 h, the invaded or migrated CRC cells were fixed with 70% ethanol and stained with 0.1% crystal violet. The stained invaded or migrated cells were counted by selecting five random fields under a light microscope.

Caspase-3 Activity

Caspase-3 activity was evaluated using a Caspase-3 activity assay kit (Abcam, Cambridge, MA, USA) according to the manufacturer's protocol. The extracted proteins from cells and tissues were incubated with reaction buffer and substrate for 4 h at 37°C in a dark environment. The caspase-3 activity was measured using a spectrophotometer (Bio-Rad, Hercules, CA, USA) at a wavelength of 450 nm.

Tumor Xenografts in Nude Mice

The female nude mice (6-8 weeks old) were purchased from the Medical Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China), and the animals were handled in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, and the experimental procedures were approved by the Animal Ethics Committee of Xi'an No.1 Hospital. The lentivirus containing shRNA that targets DLX6-AS1 (sh_DLX6-AS1) or control shRNA (sh_NC) were produced using 293T cells and packaged using ViraPower Lentiviral Packaging mix (Invitrogen, Carlsbad, CA, USA). The SW480 cells with expressing sh_DLX6-AS1 or sh_NC were generated by lentivirus infections. For the *in vivo* tumor growth assay, the SW480 cells (5 x 10^6 cells) were subcutaneously injected into the flank of the nude mice (five animals per group). The tumor growth was monitored every 7 days for 35 days, and the tumor volume was calculated as follows: volume (mm^3) = (shortest diameter)^2 x (longest diameter) x 0.5. At 35 days after initial injection of tumor cells, mice were sacrificed by cervical dislocation, and tumors were excised for further examination.

Western Blot Assay

Proteins from cells were extracted using radio-immunoprecipitation (RIPA) assay buffer (Sigma-Aldrich; St. Louis, MO, USA). Equal amounts of proteins were resolved via electrophoresis on a 10% sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto the nitrocellulose membranes (Millipore, Billerica, MA, USA). The membranes were subjected to 5% skimmed milk incubation for 1 h at room temperature. After that, the membranes were incubated with corresponding primary antibodies at 4°C overnight followed by incubating with a horseradish peroxidase (HRP)-conjugated secondary antibody for 1 h at room temperature. The bands on the blots were visualized by using enhanced chemiluminescence kit (ECL; Thermo Fisher Scientific, Waltham, MA, USA). Primary antibodies used were the following: phosphorylated phosphoinositide 3-kinase (p-PI3K; ab182651; Abcam, Cambridge, MA, USA), total (t)-PI3K (ab32089; Abcam), p-AKT (#9271; Cell Signaling Technology, Danvers, MA, USA), t-AKT (#9772; Cell Signaling Technology, Danvers, MA, USA), p-mTOR (#2971, Cell Signaling Technology, Danvers, MA, USA) and t-mTOR (#2983, Cell Signaling Technology, Danvers, MA, USA).

Statistical Analysis

The GraphPad Prism Software (Version 5.0, GraphPad Software, La Jolla, CA, USA) was utilized for data analysis. The data are presented as mean ± standard deviation. Student's *t*-test or one-way ANOVA followed by Bonferroni's post hoc test was used to compare the continuous data for two or multiple groups, accordingly. The categorical variables were determined using Chi-square test. $p < 0.05$ indicates statistical significance.

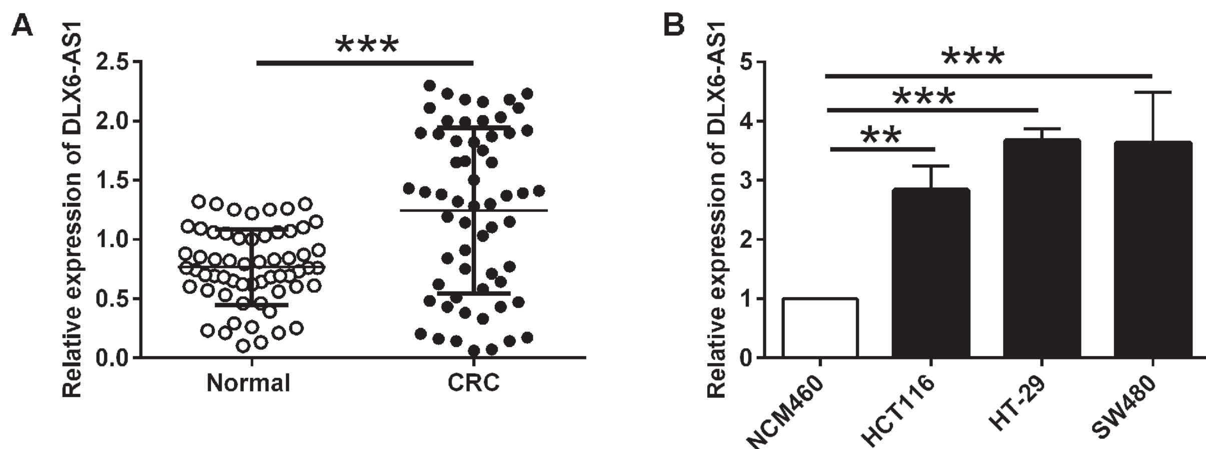

Figure 1. Up-regulation of DLX6-AS1 in CRC tissues and cell lines. **A**, QRT-PCR detection of DLX6-AS1 expression in normal adjacent colorectal tissues (n = 60) and CRC tissues (n = 60). **B**, QRT-PCR detection of DLX6-AS1 in normal human colon mucosal epithelial cell line (NCM460) and CRC cell lines (HCT116, HT-29 and SW480). N = 3. **$p < 0.01$ and ***$p < 0.001$.

Results

Upregulation of DLX6-AS1 in CRC Tissues and Cell Lines

We first collected CRC tissues, as well as the normal adjacent colorectal tissues from 60 patients, and the expression of DLX6-AS1 in these tissues was detected by qRT-PCR assay. The qRT-PCR results showed that the relative expression level of DLX6-AS1 in CRC tissues was significantly higher than that in normal adjacent colorectal tissues (Figure 1A). In addition, the expression of DLX6-AS1 in CRC tissues was classified into "low expression" and "high expression" groups based on the median values of DLX6-AS1 expression, and high expression of DLX6-AS1 was positively correlated with advanced T stage and distant metastasis, but not with age, gender, tumor size, tumor differentiation, and lymph node metastasis (Table I). To confirm this finding, we

Table I. Correlation between DLX6-AS1 expression and clinical parameters of CRC patients.

Parameters	DLX6-AS1 expression		*p*-values
	Low (n = 30)	High (n = 30)	
Age (years)			
< 60	14	11	0.601
≥ 60	16	19	
Gender			
Male	16	16	1
Female	14	14	
Tumor size			
< 5 cm	16	12	0.4379
≥ 5 cm	14	18	
T stage			
T1-T2	20	11	0.0379
T3-T4	10	19	
Tumor differentiation			
Well	18	13	0.3015
Moderate/Poor	12	17	
Distant metastasis			
No	21	12	0.037
Yes	9	18	
Lymph node metastasis			
No	23	13	0.169
Yes	7	17	

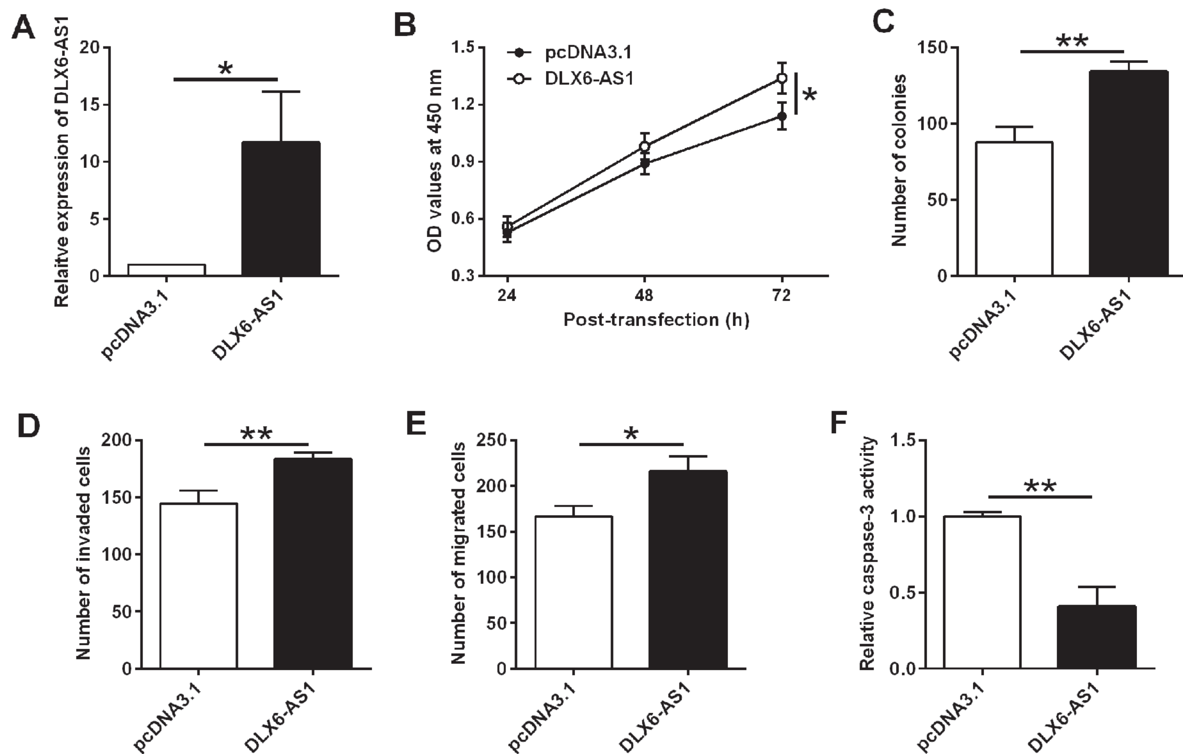

Figure 2. DLX6-AS1 overexpression promoted HCT116 cell proliferation, invasion, and migration, but inhibited HCT116 cell apoptosis. **A**, QRT-PCR detection of DLX6-AS1 in HCT116 cells following transfection with pcDNA3.1 or pcDNA3.1-DLX6-AS1. **B**, Cell proliferation, (**C**) cell growth, (**D**) cell invasion, and (**E**) cell migration in HCT116 cells following transfection with pcDNA3.1 or pcDNA3.1-DLX6-AS1 were determined by CCK-8, colony formation, transwell invasion, and migration assays, respectively. **F**, Caspase-3 activity in HCT116 cells following transfection with pcDNA3.1 or pcDNA3.1-DLX6-AS1 was determined by caspase-3 activity assay kit. N = 3. $*p < 0.05$ and $**p < 0.01$.

further determined the expression of DLX6-AS1 in normal human colon mucosal epithelial cell line (NCM460) and CRC cell lines including HCT1116, HT-29 and SW480, and the expression levels of DLX6-AS1 in these CRC cells were markedly up-regulated when compared to that in NCM460 cells (Figure 1B).

DLX6-AS1 Overexpression Promoted HCT116 Cell Proliferation, Invasion, and Migration, but Inhibited HCT116 Cell Apoptosis

To study the potential function of DLX6-AS1 in CRC progression, we performed the gain-of-function assays to evaluate the effects of DLX6-AS1 overexpression on cell proliferation, invasion, and migration, as well as apoptosis in HCT116 cells. DLX6-AS1 expression level was significantly increased in HCT116 cells following pcDNA3.1-DLX6-AS1 transfection when compared to pcDNA3.1 transfection (Figure 2A). The CCK-8 and colony formation assays showed that DLX6-AS1 overexpression potentiated the cell proliferation and cell growth of HCT116 cells when compared to control group (Figure 2B and 2C). In addition, transwell invasion and migration assays showed that the number of invaded and migrated cells following DLX6-AS1 overexpression was significantly increased when compared to control group (Figure 2D and 2E). The cell apoptosis was further assessed using caspase-3 activity assay kit, and HCT116 cells with pcDNA3.1-DLX6-AS1 transfection showed suppressed caspase-3 activity when compared to control group (Figure 2F).

DLX6-AS1 Knockdown Inhibited SW480 Cell Proliferation, Invasion, and Migration, but Induced SW480 Cell Apoptosis

Furthermore, we performed the loss-of-function assays to confirm the acting roles of DLX6-AS1 in CRC cell lines. We first designed two siRNAs for DLX6-AS1 to down-regulate DLX6-AS1 expression, and SW480 cells transfected with

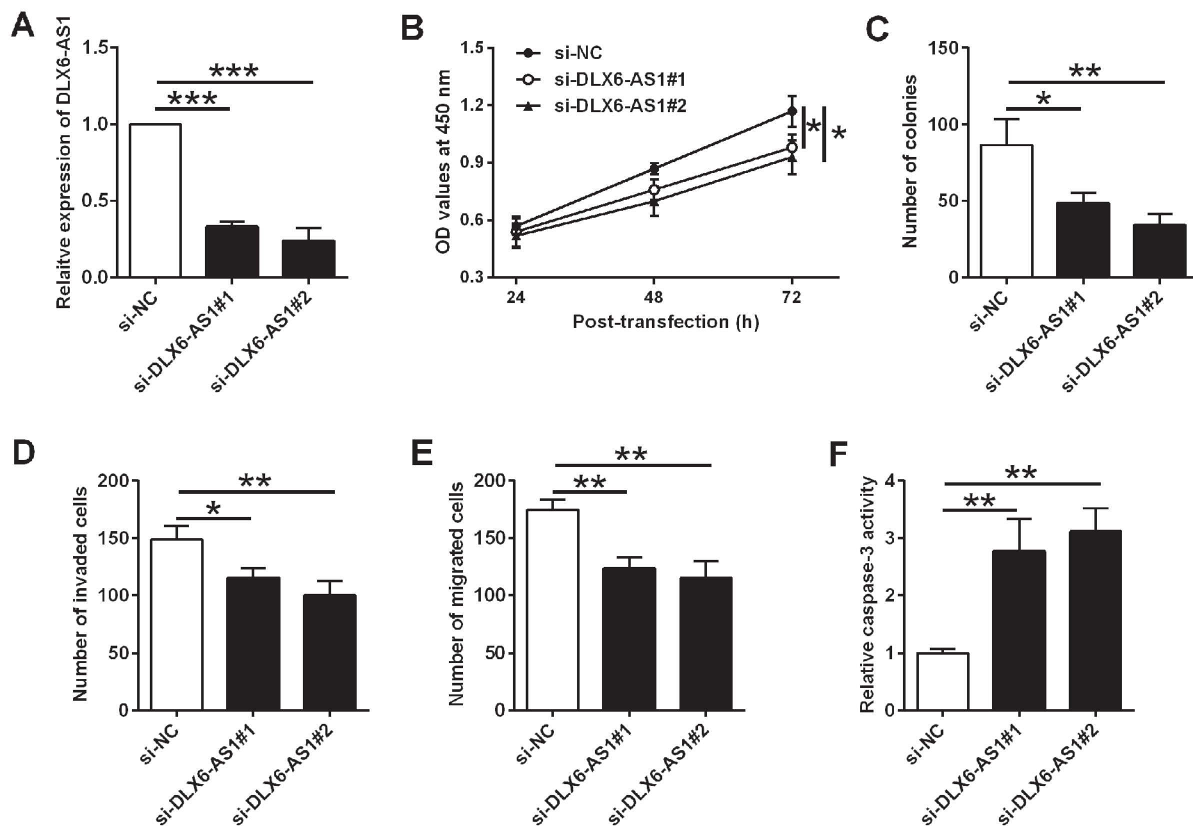

Figure 3. DLX6-AS1 knockdown inhibited SW480 cell proliferation, invasion, and migration, but induced SW480 cell apoptosis. **A**, QRT-PCR detection of DLX6-AS1 in SW480 cells following transfection with si-NC or si-DLX6-AS1#1, #2. **B**, Cell proliferation, (**C**) cell growth, (**D**) cell invasion, and (E) cell migration in SW480 cells following transfection with different siRNAs were determined by CCK-8, colony formation, transwell invasion, and migration assays, respectively. **F**, Caspase-3 activity in SW480 cells following transfection with different siRNAs was determined by caspase-3 activity assay kit. N = 3. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

DLX6-AS1 siRNAs (#1 and #2) showed a reduced expression level of DLX6-AS1 when compared to cells transfected with scrambled siRNA (si-NC; Figure 3A). The functional assays showed that cell proliferation and cell growth of SW480 cells following DLX6-AS1 siRNAs transfection were significantly attenuated when compared to cells with si-NC transfection (Figure 3B and 3C). The invasive and migratory potentials of SW480 cells as determined by transwell invasion and migration assays were effectively suppressed upon the knockdown of DLX6-AS1 (Figure 3D and 3E). In a consistent manner, DLX6-AS1 knockdown also effectively increased the caspase-3 activity of SW480 cells (Figure 3F).

DLX6-AS1 Knockdown Inhibited In Vivo Tumor Growth of SW480 Cells

The DLX6-AS1 knockdown effects were further assessed in a nude mice xenograft model. The nude mice were inoculated with SW480 expressing sh_NC or sh_DLX6-AS1, and the tumor growth from the sh_DLX6-AS1 group was slower than that from sh_NC group (Figure 4A). Consistently, the tumor weight was reduced in sh_DLX6-AS1 group when compared to sh_NC group (Figure 4B). The qRT-PCR results showed that DLX6-AS1 was down-regulated in the tumor tissues from sh_DLX6-AS1 group when compared to that from sh_NC group (Figure 4C). The determination of caspase-3 activity showed that relative caspase-3 activity was significantly higher in sh_DLX6-AS1 group than that in sh_NC group (Figure 4D).

DLX6-AS1 Regulated the Activity of PI3K/AKT/mTOR Signalling in CRC Cells

The Western blot assay was performed to determine the effects of DLX6-AS1 on the PI3K/AKT/mTOR signalling activities in CRC cells.

As shown in Figure 5A, DLX6-AS1 overexpression significantly increased the protein expression levels of p-PI3K, p-AKT and p-mTOR, but had no significant effects on the t-PI3K, t-AKT, and t-mTOR protein expression levels in HCT116 cells (Figure 5A). On the other hand, DLX6-AS1 knockdown caused a significant decrease in the protein expression levels of p-PI3K, p-AKT, and p-mTOR without affecting the t-PI3K, t-AKT, and t-mTOR protein expression levels in SW480 cells (Figure 5B).

Inhibition of mTOR Attenuated the Effects of DLX6-AS1 Overexpression on HCT116 Cell Proliferation, Invasion, Migration, and Apoptosis

To further gain insight into the role of PI3K/AKT/mTOR signalling in DLX6-AS1-mediated effects, we pre-treated the HCT116 cells with the mTOR inhibitor, CCI-799. The pre-treatment of CCI-799 significantly attenuated the enhanced effects of DLX6-AS1 overexpression on HCT116 cell proliferation, invasion, and migration (Fig-

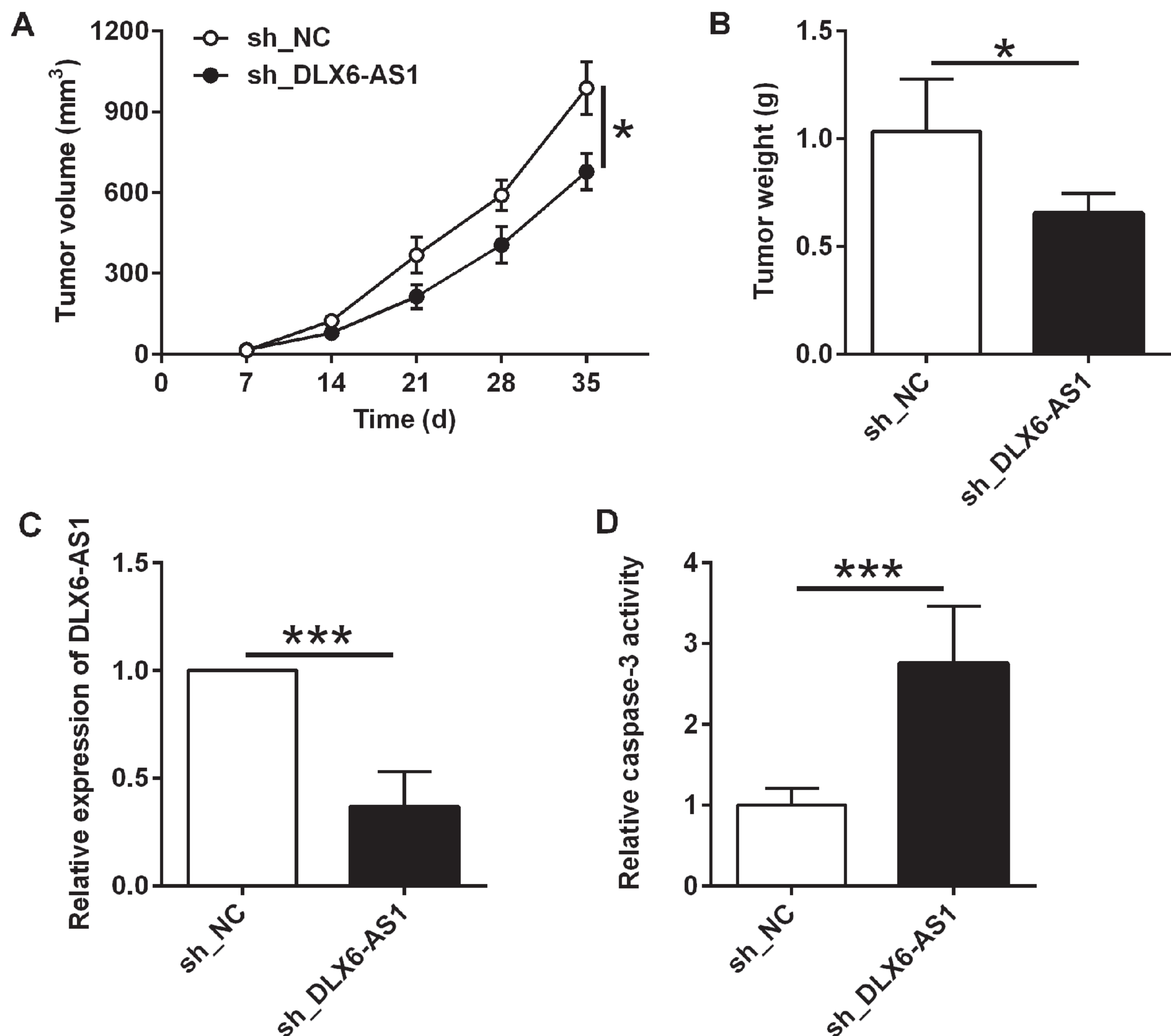

Figure 4. DLX6-AS1 knockdown inhibited *in vivo* tumor growth of SW480 cells. **A**, Tumor volume was reduced in nude mice inoculated with SW480 cells expressing sh_DLX6-AS1. **B**, Tumor weight was lower in nude mice inoculated with SW480 cells expression sh_DLX6-AS1. **C**, QRT-PCR detection of DLX6-AS1 in tumor tissues. **D**, Caspase-3 activity of the tumor tissues were evaluated with caspase-3 activity assay kit. N = 5. *$p < 0.05$ and ***$p < 0.001$.

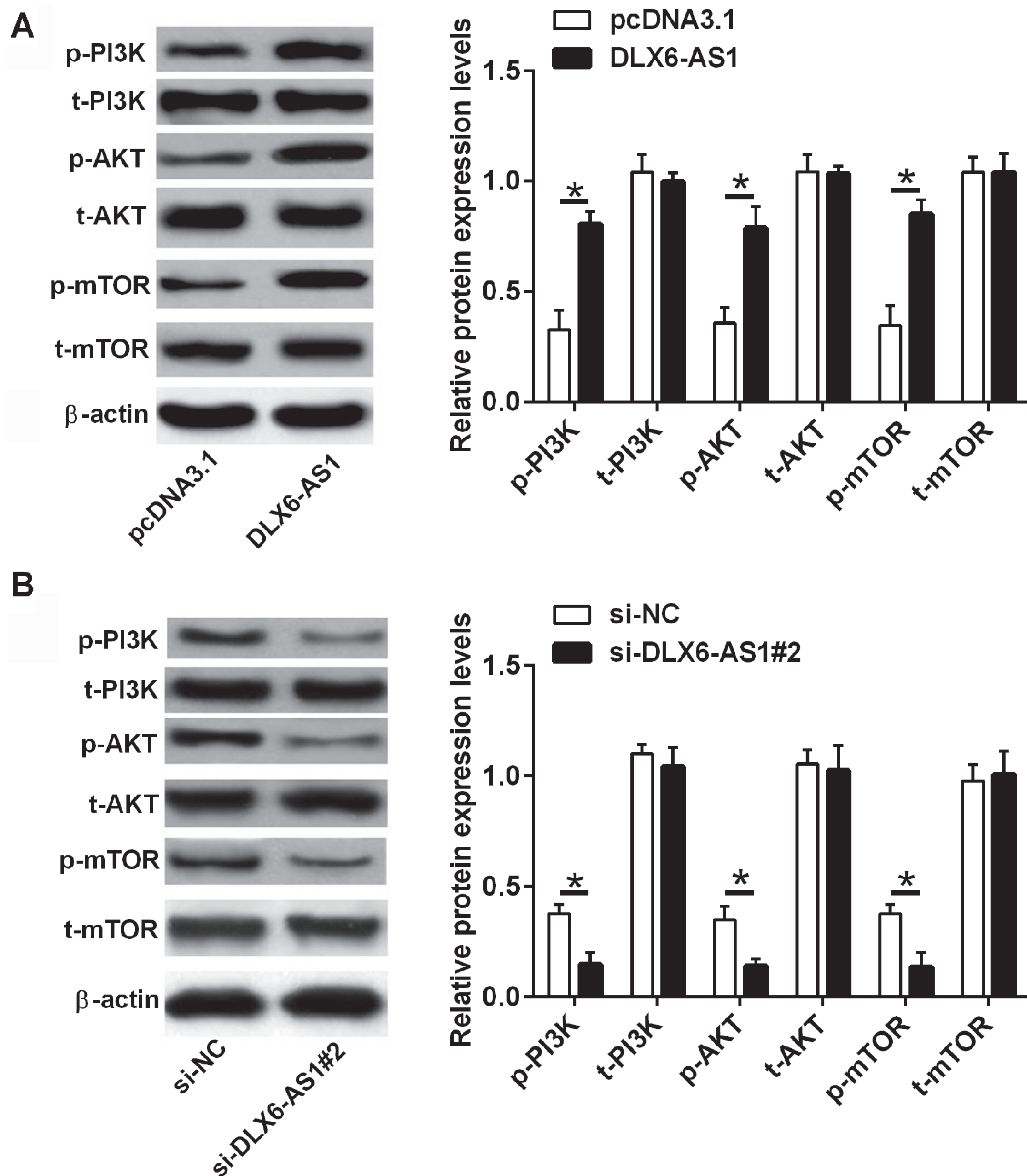

Figure 5. DLX6-AS1 regulated the activity of PI3K/AKT/mTOR signalling in CRC cells. **A**, Western blot detection of p-PI3K, t-PI3K, p-AKT, t-AKT, p-mTOR and t-mTOR protein expression levels in HCT116 cells following transfection with pcDNA3.1 or pcDNA3.1-DLX6-AS1. **B**, Western blot detection of p-PI3K, t-PI3K, p-AKT, t-AKT, p-mTOR, and t-mTOR protein expression levels in SW480 cells following transfection with si-NC or si-DLX6-AS1#2. N = 3. *$p < 0.05$.

ures 6A-6D). In addition, the decreased caspase-3 activity in HCT116 cells with DLX6-AS1 overexpression was partially restored by the CCI-799 pre-treatment (Figure 6E).

Discussion

Tumor recurrence and metastasis largely contributed to the poor survival of CRC patients, although various mechanisms underlying genetic and epigenetic modulations in CRC have been deciphered[17,18]. As such, the identification of novel molecular targets would aid us with a better strategy for the treatment of CRC. Here, we first reported the up-regulation of DLX6-AS1 in CRC tissues and cell lines, and high expression of DLX6-AS1 was significantly associated with advanced T stage and distant metastasis. In the

in vitro studies, we showed that DLX6-AS1 overexpression promoted CRC cell proliferation, invasion, and migration, but suppressed caspase-3 activity; on the other hand, DLX6-AS1 knockdown exerted tumor-suppressive effects on CRC cells. Animal studies showed that DLX6-AS1 knockdown suppressed *in vivo* tumor growth in a nude mice xenograft model. More interestingly, DLX6-AS1 regulated CRC cell proliferation, invasion, and migration, as well as apoptosis via targeting PI3K/AKT/mTOR signalling pathways in CRC cells. Collectively, these data suggested the oncogenic role of DLX6-AS1 in CRC.

DLX6-AS1 is located on chromosome 7q21.3 and is an antisense RNA of DLX6. DLX6 belongs to the DLX gene family, and Morini et al[19] found that DLX6 was associated with the metastatic potential of human breast cancer cells (MDA-MB-231). In this study, DLX6-AS1 was identified to be up-regulated CRC tissues and cell lines. Our findings were consistent with previous studies in other types of malignant tumors, where the up-regulation of DLX6-AS1 was found in lung cancer[13], renal cell carcinoma[14], glioma[20], ovarian cancer[21], and osteosarcoma[22]. In these studies, different regulatory mechanisms of DLX6-AS1 on these cancer cells were also proposed. In lung cancer, DLX6-AS1 knockdown suppressed lung cancer cell proliferation, invasion, and migration while induced apoptosis via suppressing pinoresinol reductase 1 expression by acting as a sponge for miR-144[23]. LncRNA DLX6-AS1 could promote cell proliferation, invasion, and migration via miR-197-5p/E2F transcription factor 1 axis in glioma[20]. In addition, DLX6-AS1 could target Notch signalling pathway to promote ovarian cancer cell proliferation and metastasis[21]. Our data from gain- and loss-of-function studies revealed that DLX6-AS1 promoted CRC cell proliferation and metastasis while inhibited cell apoptosis, and the suppressive effects of DLX6-AS1 knockdown on tumor growth were also observed in *in*

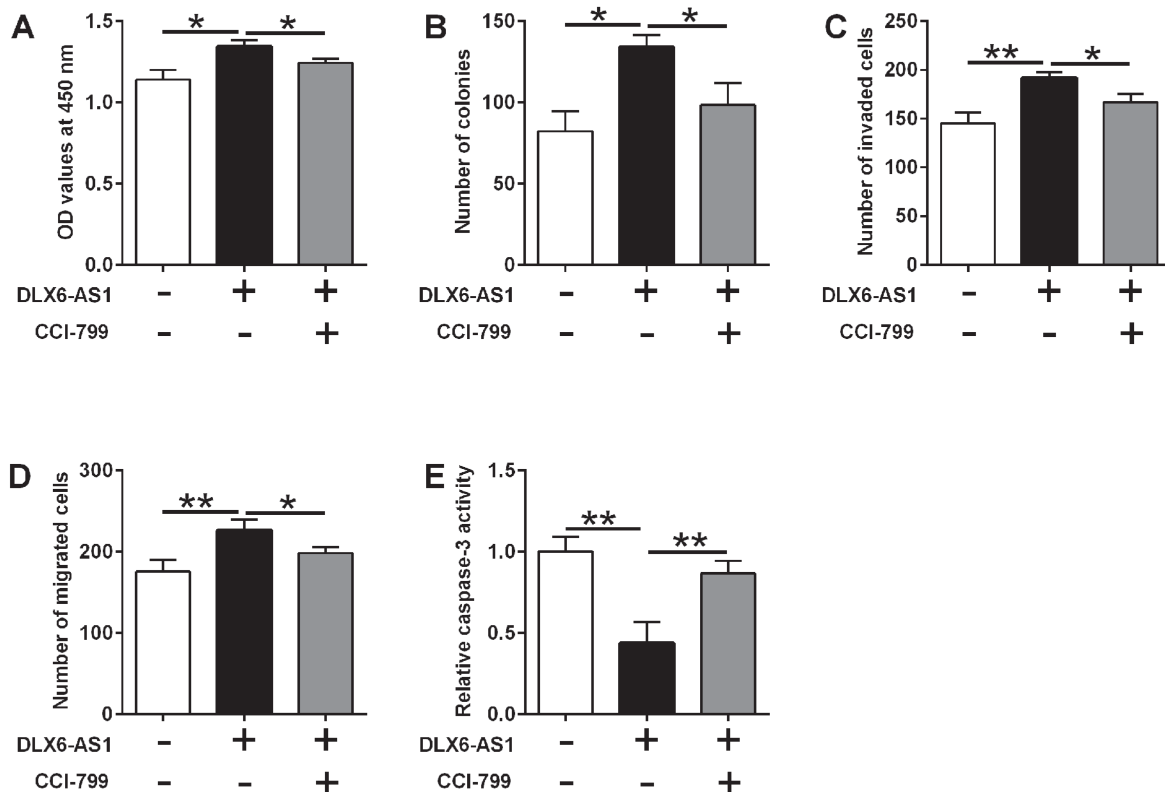

Figure 6. Inhibition of mTOR attenuated the effects of DLX6-AS1 overexpression on HCT116 cell proliferation, invasion, migration, and apoptosis. **A**, Cell proliferation, (**B**) cell growth, (**C**) cell invasion, (**D**) cell migration, and (**E**) caspase-3 activity in CCI-799 (20 μM)-treated HCT116 cells following transfection with pcDNA3.1 or pcDNA3.1-DLX6-AS1 were determined by CCK-8, colony formation, transwell invasion, and migration assays and caspase-3 activity kit assay, respectively. N = 3. *$p < 0.05$ and **$p < 0.01$.

vivo studies. Collectively, these data implied that DLX6-AS1 may act an oncogene to enhance CRC progression.

A large body of evidence has shown that PI3K/AKT/mTOR signalling pathway plays an important role in regulating cancer cell proliferation, metastasis, as well as apoptosis, which contributes to the progression of CRC[24]. Indeed, several lncRNAs have been identified to regulate PI3K/AKT/mTOR signalling pathways in CRC. Li et al[25] showed that lncRNA small nucleolar RNA host gene 7 promoted CRC progression via activating PI3K/AKT/mTOR signalling. Yu et al[26] also showed that lncRNA u50535 regulated PI3K/AKT pathway to promote CRC growth and metastasis. In addition, the lncRNA colorectal neoplasia differentially expressed was regulated by insulin/insulin growth factors and was found to control the CRC cell metabolism via targeting the PI3K/AKT/mTOR pathway[27]. In our study, we showed that DLX6-AS1 overexpression caused an increase in the p-PI3K, p-AKT and p-mTOR protein expression levels, and DLX6-AS1 had the opposite effects, suggesting DLX6-AS1 could activate the PI3K/AKT/mTOR signalling via phosphorylation of these mediators. In addition, blockade of the signalling pathway by using mTOR inhibitor partially abolished the enhanced effects on DLX6-AS1 overexpression on CRC cell proliferation and metastasis. All in all, our data indicated that DLX6-AS1 promoted CRC cell proliferation and metastasis by activating the PI3K/AKT/mTOR signalling pathway.

Conclusions

In summary, our study for the first time demonstrated that DLX6-AS1 was up-regulated in CRC tissues and cell lines, and high expression of DLX6-AS1 correlated with poor clinical characteristics. Further mechanistic researches showed that DLX6-AS1 promoted CRC cell proliferation, invasion, and migration while inhibited cell apoptosis via targeting the PI3K/AKT/mTOR signalling pathway, suggesting the key role of DLX6-AS1 in CRC progression. The present study may provide some novel insights into mechanisms underlying DLX6-AS1-mediated CRC progression.

Acknowledgments

This study was supported by Special Funds of China Influenza Surveillance Network (No. [2009]43 and [2009]91).

Author's Contributions

JZ and YZ designed the study. JZ, WX, and BC performed the experiments. YW, PY, WS, and SL collated the data. JZ, YZ, CM, and RW carried out data analyses and produced the initial draft of the manuscript. KH, NY, and LW contributed to drafting and polishing the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Conflicts of interest

The authors declare no conflicts of interest.

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