



LncRNA prostate androgen-regulated transcript 1 (PART 1) functions as an oncogene in osteosarcoma via sponging miR-20b-5p to upregulate BAMBI

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Background: Osteosarcoma (OS) is an aggressive bone cancer that most commonly affects adolescents and children. Emerging studies have shown that long noncoding RNA (lncRNA) performs essential roles in the occurrence and development of many tumors. Prostate androgen-regulated transcript 1 (PART 1) has been reported as a tumor oncogene; despite this, the mechanisms underlying its involvement in OS are unclear.

Methods: OS and paired normal tissue samples were obtained, and gene expressions were detected by real time-quantitative polymerase chain reaction (RT-qPCR). The functions of PART 1 in OS cell proliferation, invasion, and migration were determined by Cell Counting Kit-8 (CCK-8) and Transwell assays. Furthermore, the binding sites of PART 1 and miR-20b-5p as well as those between miR-20b-5p and bone morphogenic protein and activin membrane-bound inhibitor homolog (BAMBI) were verified by bioinformatics analysis and dual-luciferase reporter assay.

Results: Our study found obvious overexpression of PART 1 in OS tissues and cells. Furthermore, PART 1 overexpression facilitated OS cell proliferation, invasion, and migration. Further mechanistic investigations revealed that PART 1 could sponge to miR-20b-5p, which was expressed at a low level in OS tissues and cells. Importantly, miR-20b-5p overexpression inhibited OS cell proliferation, invasion, and migration. Additionally, BAMBI was confirmed as a downstream gene of miR-20b-5p, and its expression was reversely modulated by miR-20b-5p and positively modulated by PART 1. Rescue experiments suggested that BAMBI was involved in PART 1-mediated promotion of OS progression.

Conclusions: PART 1 serves as a competing endogenous RNA to promote OS tumorigenesis via its regulation of the miR-20b-5p/BAMBI axis, which may provide a promising therapeutic biomarkers for OS patients.

Keywords: Osteosarcoma (OS), prostate androgen-regulated transcript 1 (PART 1), miR-20b-5p, BAMBI

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1 Introduction

2 Osteosarcoma (OS) is a bone malignancy originating from
3 osteoid bone tissue which has a high mortality rate (1,2).
4 Despite the effectiveness of therapeutic modalities for
5 OS, including surgical resection, neoadjuvant, or adjuvant
6 radiotherapy or chemotherapy, the rates of mortality
7 and metastasis among patients with the disease are still
8 extremely high. Moreover, for patients with metastatic
9 or recurrent disease, the survival outlook is dismal (3,4).
10 Multiple pathophysiological and pathological processes,
11 such as epithelial-mesenchymal transition (EMT), drug
12 resistance, autophagy, and the invasion, migration,
13 apoptosis, and proliferation of OS cells are closely related
14 to OS development (5-7). Unfortunately, the mechanism
15 underlying OS progression has not been fully uncovered.
16 Therefore, determining the key molecules implicated in OS
17 may prove helpful to efforts to develop effective prevention
18 and treatment measures.

19 Recently, a new series of noncoding RNAs (ncRNAs)
20 have been found, including circular RNAs (circRNAs), long
21 ncRNAs (lncRNAs, RNA transcripts >200 bp in length) and
22 microRNAs (miRNAs, RNA transcripts ~22 bp in length),
23 all of which have important effects on tumor development
24 and the modulation of basic protein effectors of cellular
25 functions (8,9). As the 2 main members of the ncRNA
26 family, lncRNAs and miRNAs play pivotal roles in OS
27 tumorigenesis.

28 An increasing bank of evidence has confirmed that
29 numerous lncRNAs play key roles in multiple pathological
30 and physiological cellular processes, including cell
31 invasion, differentiation, apoptosis, and proliferation (10).
32 Dysregulation of lncRNA expression has been found to
33 have oncogenic effects (e.g., PROX1-AS1 in prostate
34 cancer and NCK1-AS1 in urinary bladder cancer) (11,12)
35 or tumor suppressive effects (e.g., TSLNC8 in breast
36 cancer and RP11-422N16.3 in hepatocellular carcinoma)
37 (13,14) during carcinogenesis. So far, a number of lncRNAs
38 have been reported to possess promising prognostic or
39 diagnostic value for OS (15,16); however, the role of
40 prostate androgen-regulated transcript 1 (PART 1) in this
41 malignancy is largely unknown. Recently, studies by showed
42 that PART1 regulated the apoptosis of chondrocytes in
43 osteoarthritis (17). A recent study by investigated the
44 functions of PART1 in hepatocellular carcinoma and
45 found that PART1 served as oncogenic lncRNA through
46 sponging miR-590-3p to upregulate HMGB2 expression in
47 hepatocellular carcinoma (18). Accordingly, we hypothesize
48

that PART1 may play a key role in OS development. 49

In recent decades, miRNAs have also been found to 50
serve as epigenetic regulators in disease development. 51
MiRNAs repress gene expression and participate in gene 52
silencing via direct interaction with the 3'-untranslated 53
region (UTR) of target messenger RNAs (mRNAs), 54
leading to the repression of mRNA translation or 55
degradation (19). miRNAs participate in a variety 56
of pathological and biological processes, including 57
carcinogenesis, metabolism, and embryonic development 58
(20). Several crucial activities of miRNAs in OS have 59
been reported (21). Specifically, lncRNAs have been 60
demonstrated to be endogenously competing RNAs 61
which target miRNAs to inhibit miRNA-associated gene 62
degradation. 63

In the present study, we investigated the expression and 64
roles of PART 1 in OS, as well as the potential underlying 65
regulatory mechanism. We elucidated that PART 1 serves 66
as a competing endogenous RNA in OS by sponging miR- 67
20b-5p. We present the following article in accordance with 68
the MDAR reporting checklist (available at <http://dx.doi.org/10.21037/atm-21-658>). 69
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71 Methods

72 Tissue samples

73 Forty-six pairs of OS tissue samples and matched non- 74
cancerous tissues were harvested from patients who 75
underwent excision surgery for OS in our hospital. All 76
of the patients were radiation and chemotherapy naive. 77
Liquid nitrogen was used to freeze the tissue samples before 78
the extraction of total RNA. All patients signed a written 79
informed consent form. All procedures in our study were 80
carried out in accordance with the Helsinki Declaration 81
(as revised in 2013). The study was approved by the Ethics 82
Committee Board of our Hospital. 83
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89 Cell lines and cell culture

90 OS cell lines [HOS (TCHu167) and MG-63 (TCHu124)] 91
and human fetal osteoblastic cell line (hFOB) 1.19 92
were acquired from the Type Culture Collection of the 93
Chinese Academy of Sciences (Shanghai, China). Cells 94
were maintained in Dulbecco's Modified Eagle Medium 95
(DMEM; Invitrogen, Carlsbad, CA, USA) with 10% fetal 96
bovine serum (FBS; Invitrogen) in a humidified chamber
containing 5% CO₂ at 37 °C.

97 *Cell transfection*

98 The miR-20b-5p inhibitor and mimic were designed by
99 Gene Pharma (Shanghai, China). The whole sequences
100 of PART 1 and bone morphogenic protein and activin
101 membrane-bound inhibitor homolog (BAMBI) were
102 cloned into pcDNA3.1 vector to overexpress PART 1 and
103 BAMBI, respectively. For knockdown of PART 1, its small
104 interfering RNAs (siRNAs) were synthesized as si-PART 1
105 by Gene Pharma (Shanghai, China). Lipofectamine 2000
106 (Invitrogen) was employed to transfect the above plasmids
107 into HOS and MG-63 cells.
108
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110 *Real time-quantitative polymerase chain reaction (RT- 111 qPCR) assay*

112 TRIzol reagent (Invitrogen) was utilized for the extraction
113 of total RNA from OS tissue samples and cultured cell lines,
114 afterwards, a reverse transcription reaction was performed
115 using a reverse transcription kit (Takara Bio Company,
116 Shiga, Japan). qRT-PCR was completed with SYBR[®] Green
117 PCR Master mix (Thermo Fisher Scientific, Inc., MA,
118 USA) on an ABI Prism 7500 Sequence Detection system
119 (Applied Biosystems; Thermo Fisher Scientific, Inc.) in
120 adherence to the manufacturers' protocols and with U6 or
121 GAPDH serving as an internal control. The $2^{-\Delta\Delta Ct}$ method
122 was used for measurement of relative gene expressions. The
123 primers used were as follows: PART1 Forward, 5'-AAG
124 GCC GTG TCA GAA CTC AA-3' and Reverse, 5'-GTT
125 TTC CAT CTCA GCC TGG A-3'; miR-20b-5p forward,
126 5'-ACA CTC CAG CTG GGC AAA GTG CTC ATA
127 GT-3' and reverse, 5'-TGG TGT CGT GGA GTC G-3';
128 BAMBI forward, 5'-CTC AAA TTC CCC ACT CAC
129 CCA-3' and reverse, 5'-GCT GAT ACC TGT TTC CTT
130 GTC CTG-3'; U6 forward, 5'-CTC GCT TCG GCA
131 GCA CA-3' and reverse, 5'-AAC GCT TCA CGA ATT
132 TGC GT-3'; GAPDH forward, 5'-AAT CCC ATC ACC
133 ATC TTC CA-3' and reverse, 5'-TGG ACT CCA CGA
134 CGT ACT CA-3'.
135
136

137 *Cell Counting Kit-8 (CCK-8) assay*

138 To determine cell viability, a CCK-8 assay was carried out
139 as instructed by the manufacturer. OS cells were inserted
140 into a 96-well plate and harvested at 24, 48, 72 or 96 hours
141 post transfection. Then, after the indicated amount of time,
142 CCK-8 was added to each well and the cells were incubated
143 for a further 1 hour. The absorbance was detected at
144

450 nm using a microplate reader (Bio-Rad Laboratories, 145
Hercules, CA, USA). 146

147 *Transwell assay*

148 The migration and invasion abilities of cells were detected 149
by Transwell assay. Cell migration ability was determined 150
using 6.5-mm Transwell chambers (8.0 μ m pore size; BD 151
Biosciences, Franklin Lakes, NJ, USA), and cell invasion 152
ability was assessed using Transwell chambers precoated 153
with Matrigel (BD Biosciences). Briefly, OS cells were 154
resuspended in serum-free medium and then seeded into 155
the apical chambers, with the bottom chambers filled with 156
DMEM containing 10% FBS. After 24 hours of incubation, 157
non-invasive or non-migratory cells were removed with 158
cotton swabs. Cells located in the lower chamber were fixed 159
and stained. Finally, cells in 5 randomly selected visual fields 160
were quantified under a light microscope (Olympus Corp., 161
Tokyo, Japan). 162
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165 *Western blot*

166 Total protein extraction was accomplished using RIPA 167
buffer (Beyotime, Shanghai, China). After measurement 168
of the protein concentration using a bicinchoninic acid 169
protein assay kit (Beyotime), the protein samples were 170
subjected to sodium dodecyl sulfate-polyacrylamide gel 171
electrophoresis (SDS-PAGE) separation and transferred 172
onto a polyvinylidene difluoride (PVDF) membrane. The 173
membrane was blocked with 5% skim milk and incubated 174
with specific primary antibodies against BAMBI (ab203070; 175
1:1,000, Abcam, Cambridge, MA, USA) and GAPDH 176
(ab9485; 1:2000, Abcam, Cambridge, MA, USA) at 4 °C. 177
After incubation overnight, the membrane was incubated 178
with horseradish peroxidase (HRP)-conjugated goat anti- 179
rabbit (1:2,000, Abcam, Cambridge, MA, USA) secondary 180
antibody for 2 hours at room temperature. Finally, the 181
signals were detected using an electrochemiluminescence 182
(ECL) kit (Thermo Fisher Scientific, Inc.). GAPDH was 183
used as the internal control. 184
185

186 *Luciferase reporter assay*

187 Dual-luciferase reporter assay (Promega, Madison, WI, 188
USA) was performed to verify the relationships between 189
PART1 or BAMBI and miR-20b-5p. After that, the wild- 190
type PART1 and mutant PART1 sequences harboring 191
predicted miR-20b-5p binding sites were synthesized and 192

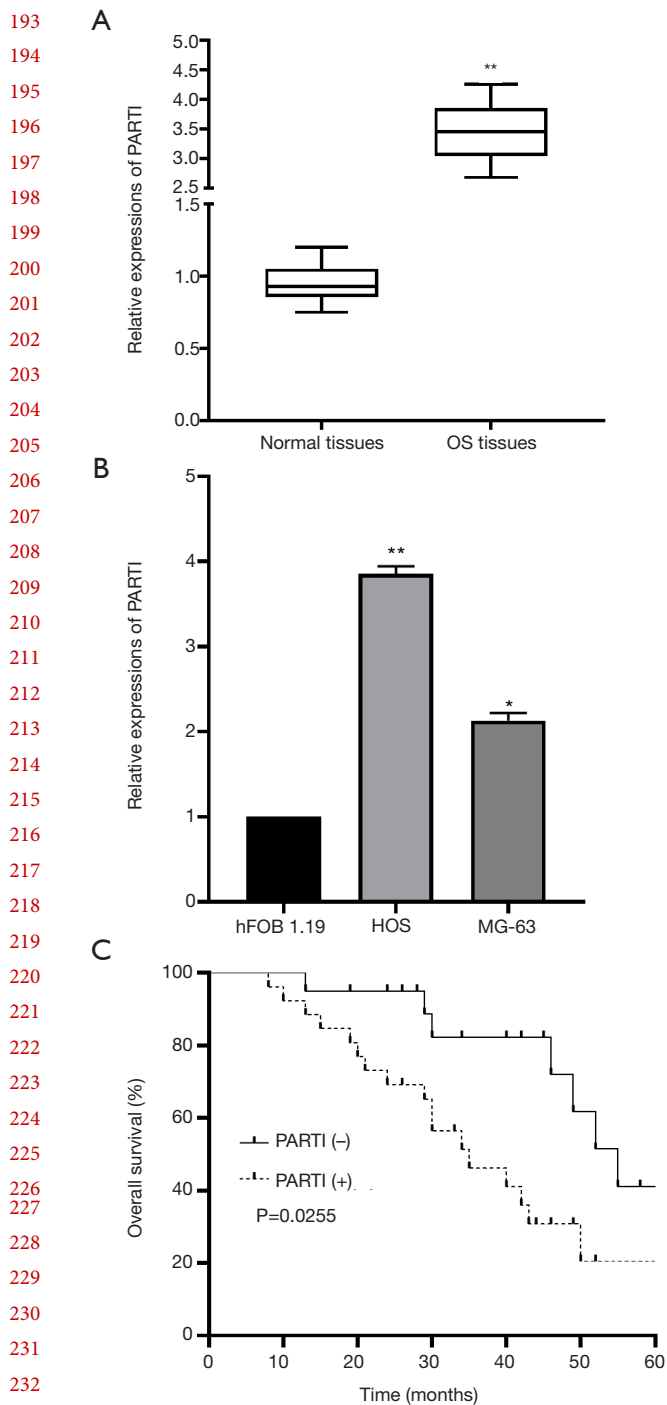


Figure 1 Overexpression of PART 1 in OS indicated shorter overall survival. (A,B) real time-quantitative polymerase chain reaction (RT-qPCR) analysis demonstrated that PART 1 expression was upregulated in OS tissues and cells. (C) High PART 1 expression was associated with shorter overall survival in OS patients. OS, osteosarcoma; PART 1, prostate androgen-regulated transcript 1. *, $P < 0.05$; **, $P < 0.01$.

inserted into the pGL3-control vector (Promega, Madison, WI, USA) to construct the luciferase reporter vector of PART1-WT and PART1-Mut. Similarly, the wild-type BAMBI 3'-untranslated regions (UTR) and mutant BAMBI 3'-UTR sequences containing embracing predicted miR-20b-5p binding sites were synthesized and inserted into the pGL3-control vector for the construction of the luciferase reporter vectors of BAMBI-WT and BAMBI-Mut. Following this, the luciferase reporter vectors were cotransfected into OS cells with NC-mimics or miR-20b-5p mimics using Lipofectamine 2000 (Invitrogen) for the execution of the dual-luciferase reporter assay, respectively. Finally, cells were harvested in 48 hours post transfection and the luciferase activities of luciferase reporter vectors were evaluated via the dual-luciferase reporter assay kit (Promega).

Statistical analysis

All of the above experiments were performed in triplicate. SPSS 17.0 version (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses. Data were tested using Student's t-test or one-way analysis of variance with Tukey's post-hoc test. The relationships between the expressions of miR-20b-5p and PART 1, PART 1 and BAMBI were assessed by Spearman's or Pearson's correlation analysis. The overall survival of the OS patients was determined with Kaplan-Meier curve together with log-rank test. $P < 0.05$ was considered to indicate significant difference.

Results

High PART 1 expression in OS tissue indicated a poor prognosis

To determine the clinical significance of PART 1 in OS, we firstly detected the expression level of PART 1 in OS tissues and matched non-tumor tissues. The RT-qPCR results demonstrated that PART 1 expression was significantly increased in OS tissues compared to non-tumor tissues (Figure 1A). Similarly, upregulation of PART 1 was also observed in OS cells (Figure 1B). The survival analysis indicated that OS patients with PART 1 upregulation had strikingly shorter overall survival compared to the patients with lower PART 1 expression (Figure 1C). Overall, these results showed that PART 1 was upregulated in patients with OS and indicated a poor prognosis.

240 **PART 1 accelerated OS cell proliferation, invasion, and** 241 **migration**

242 Having detected the aberrant up-regulation of PART 1 in
243 OS tissues, we next performed functional assays, including
244 a CCK-8 assay and Transwell assays, to determine the
245 functions of PART 1 in the progression of OS. The cell
246 lines MG-63 and HOS were transfected with pcDNA3.1-
247 PART 1 or si-PART 1. The results of RT-qPCR verified
248 that PART 1 was successfully overexpressed in MG-
249 63 cells and was knocked down in HOS cells following
250 transfection with pcDNA3.1-PART 1 or si-PART 1
251 (*Figure 2A*). The CCK-8 assay showed that pcDNA3.1-
252 PART 1 significantly elevated the viability of MG-63
253 cells, whereas the proliferative ability of HOS cells was obviously
254 reduced by si-PART 1 transfection (*Figure 2A*). Also,
255 the Transwell assays revealed that PART 1 upregulation
256 promoted the migration and invasion abilities of MG-63
257 cells (*Figure 2B*). In contrast, PART 1 knockdown notably
258 reduced HOS cell migration and invasion (*Figure 2B*).
259 Taken together, these observations suggested that PART
260 1 upregulation contributed to the malignant progression
261 of OS.
262

263 **PART 1 acted as a sponge of miR-20b-5p in OS cells**

264 To determine the molecular mechanisms participating
265 in PART 1-mediated OS progression, miRNAs could
266 potentially serve as targets for PART 1 were predicted with
267 Starbase. Results showed that PART 1 contained conserved
268 binding sites for miR-20b-5p (*Figure 3A*). Subsequently,
269 a luciferase reporter assay was performed to verify the
270 correlation of PART 1 with miR-20b-5p. The miR-20b-5p
271 mimics noticeably decreased the luciferase activities of the
272 PART 1-wt plasmid; however, we failed to observe a notable
273 difference in the luciferase activities of the PART 1-mut
274 plasmid (*Figure 3B*). Next, the expression levels of miR-
275 20b-5p in cells transfected with pcDNA3.1-PART 1 or si-
276 PART 1 were measured by RT-qPCR. When PART 1 was
277 overexpressed, miR-20b-5p expression was decreased, while
278 PART 1 knockdown dramatically increased miR-20b-5p
279 expression (*Figure 3C*). Similarly, the regulatory functions of
280 miR-20b-5p in PART 1 expression were also investigated.
281 As shown in *Figure 3D*, miR-20b-5p inhibition resulted in
282 significant upregulation of PART 1, whereas the opposite
283 effect was observed with miR-20b-5p overexpression.
284 Additionally, in OS tissues, a significant decrease in miR-
285 20b-5p expression was detected (*Figure 3E*), and a negative
286
287

correlation between the expressions of PART 1 and miR-
20b-5p was also confirmed (*Figure 3F*).

miR-20b-5p inhibited proliferation, invasion and **migration in OS cells**

The expression levels of miR-20b-5p in OS cells were
further analyzed. A remarkable decrease in miR-20b-5p in
OS cells was verified (*Figure 4A*). HOS and MG-63 cells
were transfected with miR-20b-5p mimics or inhibitor
to induce miR-20b-5p overexpression or inhibition,
respectively. The transfection was confirmed to have been
successfully completed by RT-qPCR (*Figure 4B*). The
regulatory effects of miR-20b-5p on OS cell proliferation,
invasion, and migration were subsequently investigated.
The results showed that miR-20b-5p overexpression
inhibited OS cell proliferation, invasion, and migration,
while miR-20b-5p silencing exerted the opposite functions
(*Figure 4C,D*).

BAMBI served as a target of miR-20b-5p in LAC cells

We further explored the mechanism underlying the
promotion of OS progression by the PART 1/miR-20b-
5p axis. TargetScan showed that BAMBI contained binding
sites of miR-20b-5p (*Figure 5A*). The direct binding of
miR-20b-5p to the 3'-UTR of BAMBI at putative sites
was confirmed by the results of a luciferase reporter
assay (*Figure 5B*). BAMBI was significantly inhibited by
miR-20b-5p overexpression and promoted by miR-20b-
5p inhibition (*Figure 5C,D*). Furthermore, BAMBI was
markedly upregulated in OS tissue samples compared to the
para-carcinoma tissues (*Figure 5E*). In addition, a positive
correlation of the expressions of BAMBI and PART 1 was
found to exist in OS tissues (*Figure 5F*).

PART 1 promoted OS tumorigenesis by sponging miR- 20b-5p to upregulate BAMBI

To determine whether the miR-20b-5p/BAMBI axis was
implicated in the oncogenic functions of PART 1 in OS
cells, a rescue assay was carried out. MiR-20b-5p inhibitor
and pcDNA-BAMBI were transfected into OS cells
together with si-PART 1. As shown in *Figure 6A*, BAMBI
expression was significantly downregulated by si-PART 1,
and this reduction was PART 1 ally reversed by silencing
of miR-20b-5p or BAMBI overexpression. Additionally, we
found that the suppressive effects of PART 1 knockdown

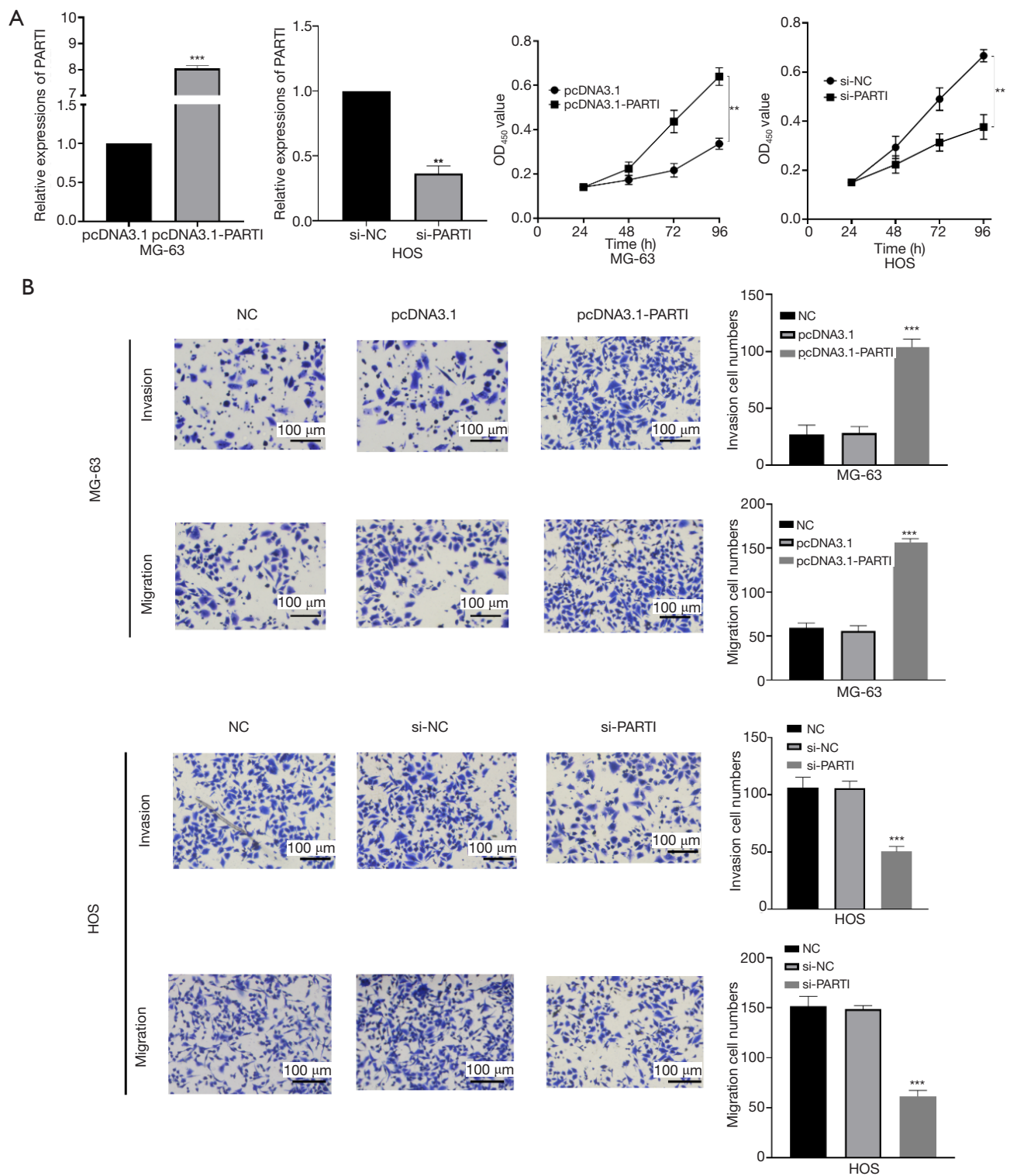


Figure 2 PART 1 accelerated OS cell proliferation, invasion, and migration. (A) Successful upregulation or downregulation of PART 1 in OS cells was confirmed by RT-qPCR and upregulation promoted OS cell viability, as demonstrated by Cell Counting Kit-8 assay. (B) Transwell assays indicated that PART 1 upregulation contributed to OS cell invasion and migration ($\times 100$ magnification). The cells were stained with crystal violet. OS, osteosarcoma; PART 1, prostate androgen-regulated transcript 1. **, $P < 0.01$; ***, $P < 0.001$.

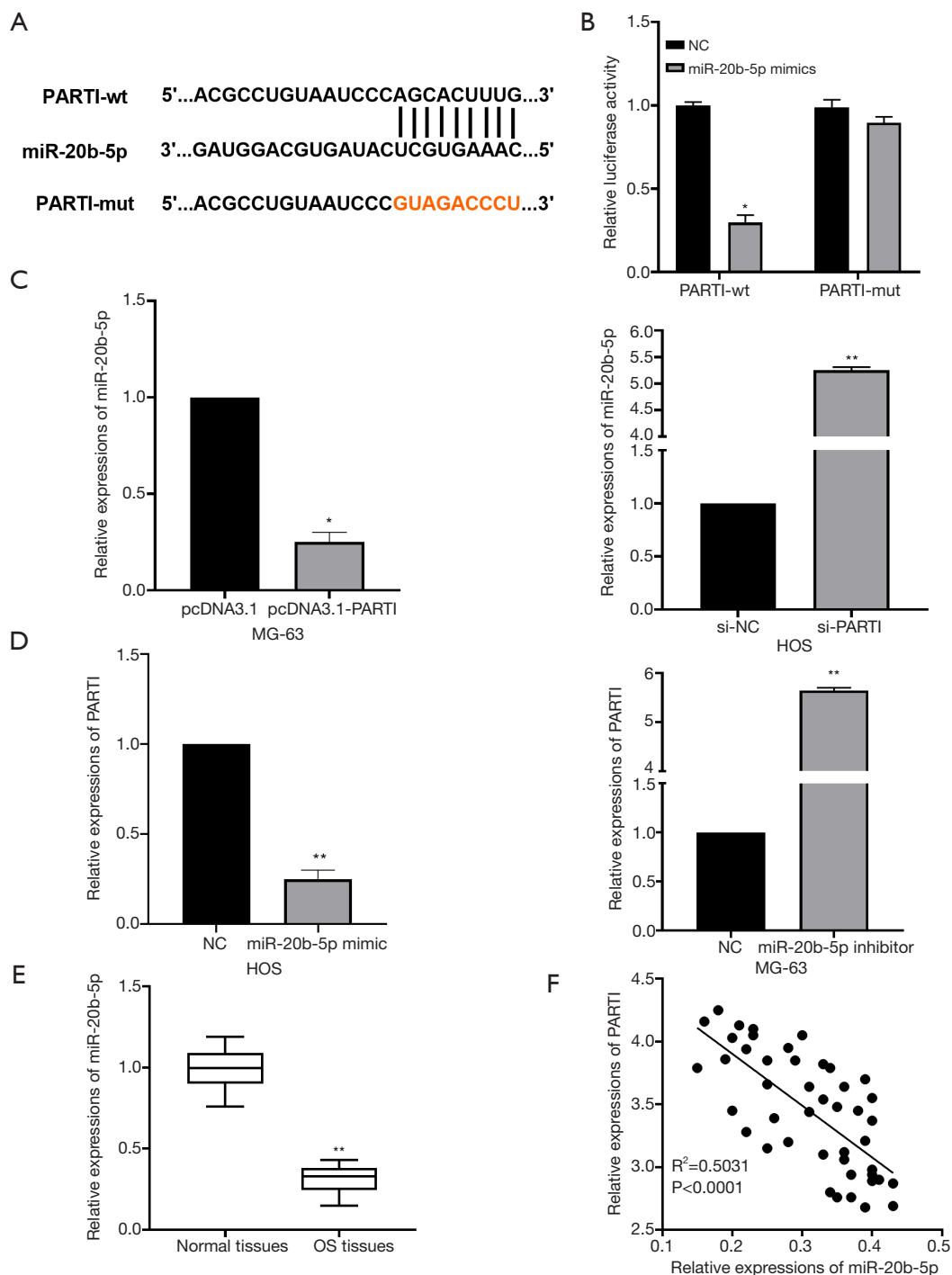


Figure 3 PART 1 acted as a sponge of miR-20b-5p in OS cells. (A) Putative binding sites of miR-20b-5p in the 3'-untranslated region of PART 1 were obtained from Starbase. (B) Relative luciferase activity of OS cells transfected with PART 1-wt/mut reporter plasmid and miR-20b-5p mimic. (C,D) The regulatory relationship of PART 1 and miR-20b-5p was confirmed by RT-qPCR analysis. (E) Downregulated miR-20b-5p expression was identified in OS tissues. (F) A negative correlation between the expressions of PART 1 and miR-20b-5p in OS tissues was confirmed. OS, osteosarcoma; PART 1, prostate androgen-regulated transcript 1. *, $P<0.05$; **, $P<0.01$.

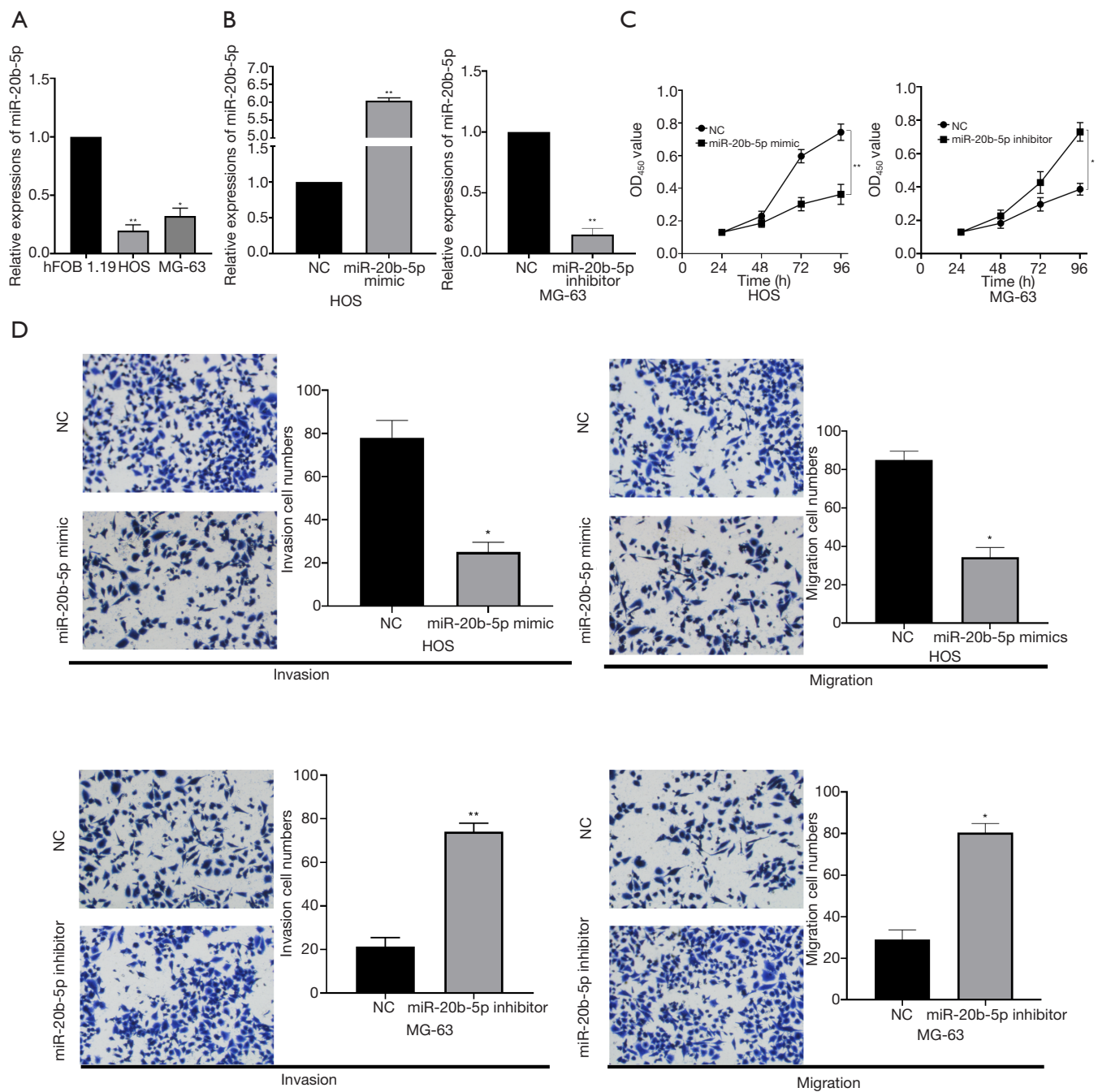


Figure 4 miR-20b-5p inhibited the proliferation, invasion, and migration in OS cells. (A) miR-20b-5p was downregulated in OS cells. (B) miR-20b-5p was efficiently overexpressed and silenced by miR-20b-5p mimics and inhibitor, respectively. (C, D) miR-20b-5p inhibited the proliferation, invasion, and migration in OS cells. The cells were stained with crystal violet in transwell assay ($\times 100$ magnification). OS, osteosarcoma. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

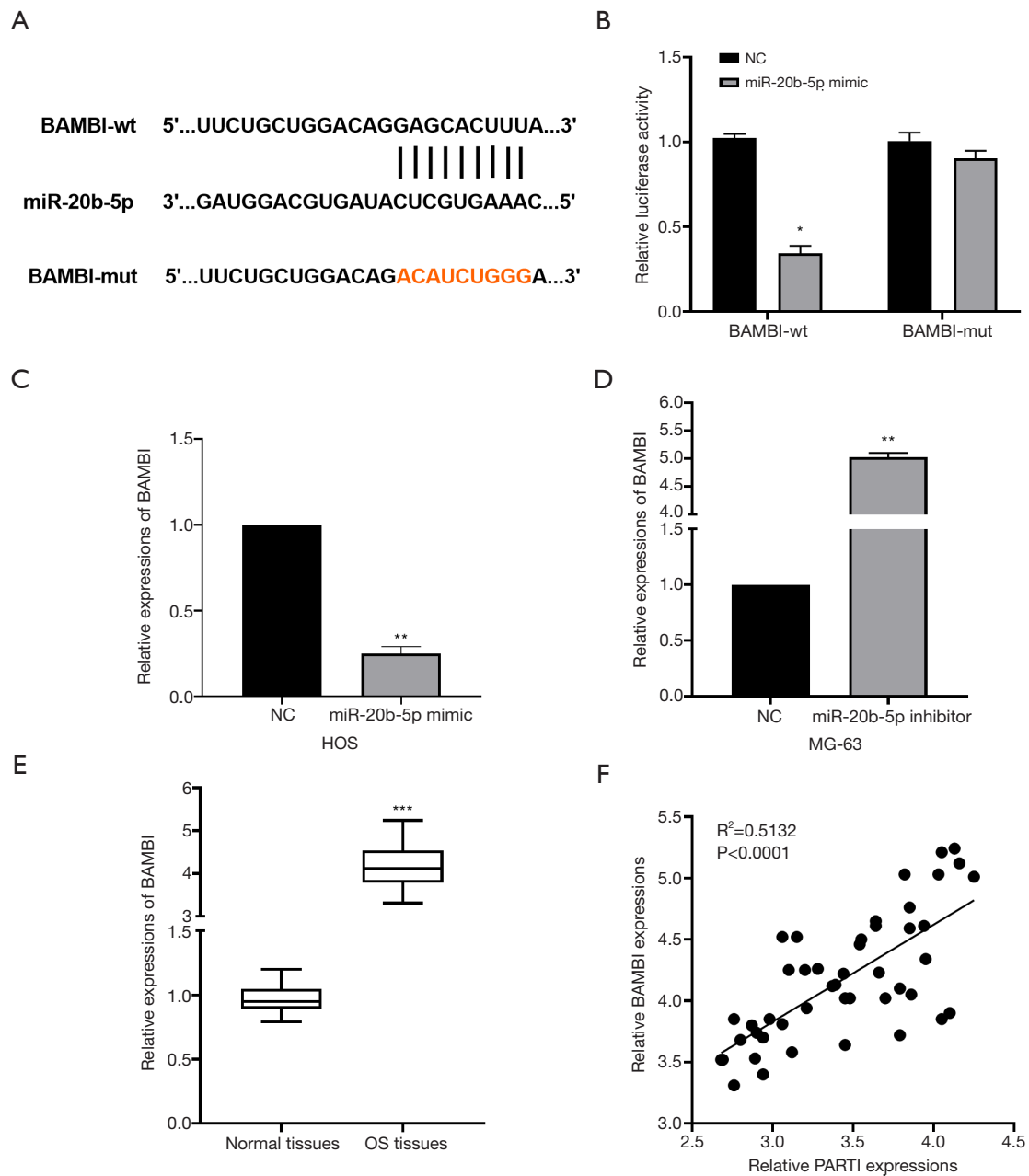


Figure 5 BAMBI served as a target of miR-20b-5p in OS cells. (A) Putative binding sites of miR-20b-5p in the 3'-UTR of BAMBI were predicted by TargetScan. (B) miR-20b-5p mimic significantly decreased the relative luciferase activity of BAMBI-wt reporter plasmid. (C,D) BAMBI expression was regulated by miR-20b-5p in OS cells. (E,F) High BAMBI expression was identified in OS tissues, and was positively correlated with PART 1 expression. OS, osteosarcoma. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

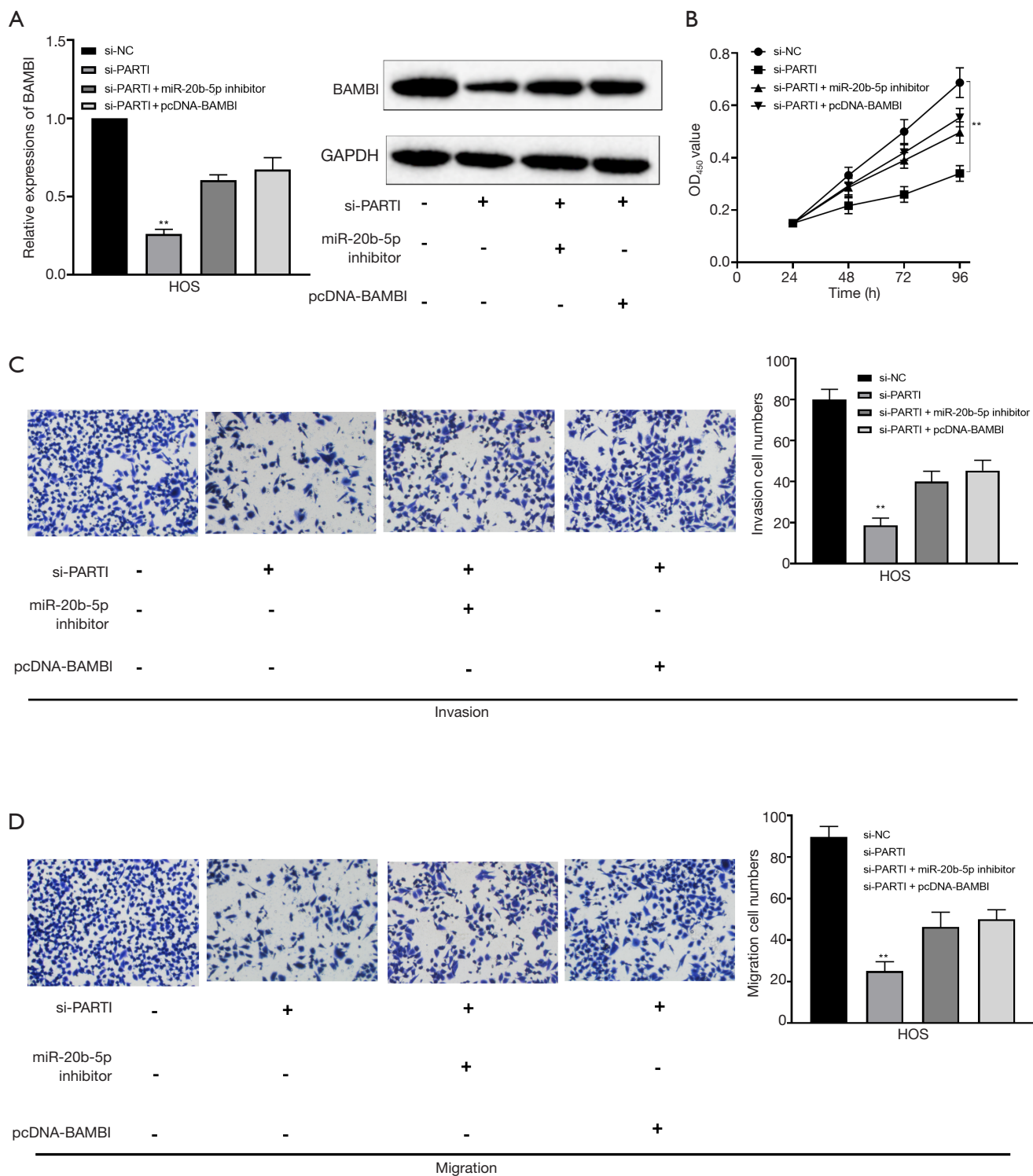


Figure 6 PART 1 promoted OS tumorigenesis by sponging miR-20b-5p to upregulate BAMBI. (A) BAMBI expression was silenced by si-PART 1, and this reduction could be PART 1 ally reversed by miR-20b-5p inhibitor or pcDNA-BAMBI. (B,C,D) miR-20b-5p inhibitor or pc DNA-BAMBI could reverse the inhibitory effects of si-PART 1 in OS cell proliferation, invasion, and migration. The cells were stained with crystal violet in transwell assay (×100 magnification). OS, osteosarcoma; PART 1, prostate androgen-regulated transcript 1. **, P<0.01.

on OS cell proliferation, migration, and invasion could be dramatically attenuated by miR-20b-5p silencing or BAMBI overexpression (Figure 6B,C,D). All of these data revealed PART 1 to be a modulator of OS cell malignancy through its sponging of miR-20b-5p to promote BAMBI expression.

Discussion

OS is a common bone malignancy in adolescents and teens. Tumor recurrence and metastasis are 2 primary factors for the high mortality rate of OS (22). There is mounting evidence showing that lncRNA is important in a variety of biological cellular processes (23,24). Previous studies have indicated that dysregulation of lncRNAs is correlated with tumor progression and patient outcomes (25). Recently, lncRNAs have been found to play crucial roles in OS. For instance, Ding *et al.* found that CRNDE (colorectal neoplasia differentially expressed) facilitated OS cell proliferation, invasion, and EMT via the Wnt/beta-catenin signaling pathway, following activation by SP1 (26). Furthermore, Cui *et al.* found that TMPO antisense RNA 1 promoted OS tumorigenesis by regulating the miR-199a-5p/WNT7B axis (27). Also, a study by Zhu *et al.* showed that PCAT6 promoted OS progression by sponging miR-185-5p and activating the transforming growth factor beta signaling pathway (28). Yet, the impact of PART 1 on the biological behavior of OS cells has remained unclear. Therefore, the aim of the present study was to elucidate the roles and mechanisms of PART 1 in OS.

lncRNA PART 1 is known as an androgen-regulated and prostate-specific gene (29). PART 1 overexpression in the prostate gland has been confirmed as being related to prostate tumor initiation (30). In recent years, aberrant PART 1 expression has also been confirmed in other tumors. For instance, Xuan *et al.*'s study indicated PART 1 was an independent predictor of prognosis in glioma patients (31), while Zhou *et al.* found that PART 1 regulated colorectal cancer via activation of the Wnt/beta-catenin pathway and regulation of miR-150-5p/miR-520h/CTNNB1 (32). Moreover, Zhu *et al.* found that PART 1 contributed to non-small cell lung cancer progression via the JAK-STAT signaling pathway (33). In our study, we found that PART 1 was upregulated in OS, with its overexpression promoting the viability, invasion, and migration of OS cells.

Accumulating studies have demonstrated that lncRNAs may serve as ceRNAs in carcinogenesis. ceRNAs can sponge miRNAs, reducing their binding to the target genes and thereby modulating their expression (34). In our study,

miR-20b-5p, which was predicted as a target of PART 1, was found at low levels in OS tissues and cells. Further investigation of the potential mechanisms indicated that BAMBI served as a direct target of miR-20b-5p in OS cells. PART 1 was found to act as a promoter of OS tumorigenesis by sponging miR-20b-5p to upregulate BAMBI.

In conclusion, the data of the present study revealed that high levels of PART 1 and low levels of miR-20b-5p are expressed in OS. PART 1 upregulation notably contributed to OS cell proliferation and cell mobility. Further study showed that the anti-OS functions of PART 1 were exerted via its sponging of miR-20b-5p to up-regulate BAMBI. Our findings may provide novel diagnostic markers for OS and enrich our knowledge of OS progression.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All patients signed a written informed consent form. All procedures in our study were carried out in accordance with the Helsinki Declaration (as revised in 2013). The study was approved by the Ethics Committee Board of our Hospital.

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