Heliyon 10 (2024) e35906

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

LncRNA HOXB-AS3 promotes proliferation, migration, and invasion of gallbladder cancer cells by activating the MEK/ERK pathway

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ARTICLE INFO

Keywords:
Gallbladder carcinoma
Long non coding RNA
HOXB-AS3
MEK/ERK

ABSTRACT

Background: LncRNA HOXB-AS3 are associated with tumor progression in several types of carcinomas, yet, its possibly biological role in gallbladder carcinoma(GBC) remains unclear. Therefore, this study aimed to investigate the biological function of HOXB-AS3 in GBC.

Methods: To know the potential function of HOXB-AS3 in gallbladder carcinoma, real-time polymerase chain reaction was used to detected the expression of HOXB-AS3 in gallbladder carcinoma cells. The colony formation assay and cell counting kit-8 assay was performed to measured cell viability. Flow cytometry was to analyse cell apoptosis and cell cycle. Cell invasion and migration were determined by the transwell invasion assay and wound-healing assay. A nude mice xenograft tumor model was performed to investigate the biological function of HOXB-AS3 in vivo.

Results: The results indicated that HOXB-AS3 was significantly elevated in gallbladder carcinoma tissues and cell lines. We used siHOXB-AS3 to knockdown the expression levels of HOXB-AS3. And knockdown HOXB-AS3 expression depressed gallbladder cancer cell viability and induced cell apoptosis. In addition, the gallbladder carcinoma cell cycle was obviously arrested at the G1 phase. Cell invasion and migration were markedly suppressed following knockdown HOXB-AS3 expression. Furthermore, the features of siHOXB-AS3 in gallbladder cancer cells could be reversed by the ERK1/2 phosphorylation agonist Ro 67–7476. Finally, we confirmed that HOXB-AS3 promoted the growth of transplanted tumors in vivo.

Conclusion: HOXB-AS3 promoted gallbladder carcinoma cell proliferation, invasion and migration by activating the MEK/ERK signaling pathway. HOXB-AS3 contributed to gallbladder cancer

Abbreviations: LncRNA, long non coding RNA; GBC, gallbladder carcinoma; HOXB-AS3, HOXB cluster antisense RNA 3.

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https://doi.org/10.1016/j.heliyon.2024.e35906
Received 22 September 2023; Received in revised form 5 August 2024; Accepted 6 August 2024
Available online 8 August 2024

tumorigenesis and metastasis, making it a viable therapeutic target for gallbladder cancer treatment.

1. Introduction

Gallbladder carcinoma (GBC), the most common biliary-tract malignancy, ranks as the sixth most common gastrointestinal carcinoma globally, with an incidence rate of 2/100 000 [1,2]. And GBC exhibits a distinctive geographical distribution, with a heightened incidence observed in China, Korea, India, Japan, Pakistan in comparison to other nations globally [3,4]. The absence of specific symptoms, biomarkers, or noticeable physical signs in the early stages of GBC frequently results in a diagnosis being made at a relatively advanced stage [5]. To date, the only curative approach currently considered for GBC patients is complete surgical resection [6], with palliative treatments such as radiotherapy, chemotherapy and molecular targeted therapy. However, despite advancements over the past few decades, the prognosis of gallbladder carcinoma had been not obviously improved [7]. Therefore, it is of great importance to understand the fundamental pathogenesis of gallbladder carcinoma and thus to enhance our comprehension of treatment strategies for GBC.

Long non coding RNA(lncRNA) constitute a specialized class of RNA with weakly or no protein-coding ability, composed of more than 200 nucleotides [8,9]. In previous studies, lncRNAs were confirmed to play essential roles in cell apoptosis, proliferation, invasion and metastasis in many malignant cancers, and to regulate gene expression at different levels, such as translational, transcriptional, and post-transcriptional processes [10–13]. The HOXB cluster antisense RNA 3 (HOXB-AS3) has been annotated as a long non-coding RNA (lncRNA) within the NONCODE databases. Initially identified by Huang J, HOXB-AS3 has been shown to exert an inhibitory effect on colon carcinoma tumorigenesis through metabolic reprogramming [14]. Then, Zhuang et al. have proved that HOXB-AS3 promotes the development of ovarian cancer through the activation of the Wnt/β-catenin signaling pathway [15]; Jiang et al. have reported that lncRNA HOXB-AS3 is able to stimulate the proliferation of lung cancer [16]; Ying X et al. reported that HOXB-AS3 promotes cell proliferation and inhibits apoptosis by regulating ADAM9 expression through targeting miR-498-5p in endometrial carcinoma [17]. However, it has been not previously reported whether the HOXB-AS3 played a role in GBC progression. Consequently, we aimed to study the function of lncRNA HOXB-AS3 in gallbladder carcinoma progression.

Genes belonging to the MEK-ERK signaling pathway play a crucial role in numerous types of human cancers. For example, MAPK (mitogen-activated protein kinase) members superfamily exhibit close associations with cellular processes essential for apoptosis, proliferation, and survival [18]. The MEK-ERK signaling pathway plays a prominent role in the regulation of cellular proliferation, migration, invasion and survival in GBC [19]. However, whether long non coding RNA HOXB-AS3 can modulate this signaling pathway remains unclear.

In the present study, we found that long non codding RNA HOXB-AS3 was obviously elevated in GBC tissues as well as GBC cell lines. And knockdown the expression of HOXB-AS3 depressed gallbladder cancer cell viability and induced cell apoptosis with the GBC cell cycle blocked in the G1 phase. Further, gallbladder cancer cell invasion and migration were markedly suppressed following knockdown of HOXB-AS3 expression. Meanwhile, knockdown of HOXB-AS3 significantly reduced the phosphorylation levels of MEK and ERK, suggesting that HOXB-AS3 may contribute to the progression of gallbladder carcinoma through the MEK-ERK signaling pathway. To confirm this possibility, we used the ERK1/2 phosphorylation agonist Ro 67–7476 to our further study. It was found that the ERK1/2 phosphorylation agonist could offset the effect of HOXB-AS3 decreasing. Collectively, our findings indicate that HOXB-AS3 acts as a promoter of gallbladder carcinogenesis through the activation of the MEK-ERK signaling pathway. Notably, this is the first study to confirmed that lncRNA HOXB-AS3 plays a role in the tumorigenesis of gallbladder carcinoma through the activation of the MEK-ERK signaling pathway.

2. Materials and methods

2.1. Expression analysis of HOXB-AS3

The lncRNA datase GSE74048 was downloaded from the Gene Expression Omnibus database(GEO, www.ncbi.nlm.nih.gov/geo). The GSE74048 dataset comprises the comprehensive expression profiling of long non-coding RNAs in human gallbladder cancer, as ascertained through microarray technology. This analysis encompassed 3 individual pairs of human gallbladder carcinoma tissues(N = 3), along with their corresponding matched peri-carcinomatous tissues(N = 3) [20]. To determine whether the expression of HOXB-AS3 in GBC tissues was different from that in matched peri-carcinomatous tissues, we downloaded this datasets for further study. After downloading the dataset, Perl programming language software (version 5.34.0, https://www.perl.org/) and the R programming language software (version 4.0.5, https://www.r-project.org/) were used to obtain the HOXB-AS3 expression data (fold changes) .

2.2. Cell culture

The human intrahepatic-biliary epithelial cell (HIBEpic), along with human gallbladder carcinoma cell lines(NOZ and GBC-SD), were obtained from Guangzhou Jennio Biotech Co., Ltd(China). All cells were cultured at 37 °C with 5 % CO_2 in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific Co., USA) supplemented with 10 % fetal bovine serum (FBS, Thermo Fisher

Scientific Co., USA) and 1 % penicillin-streptomycin. When confluence reached 70 %, the cells were regularly digested and passaged, and fresh culture medium was routinely replaced every 48–72 h. After 5–6 times passaged, cells were used for further experiments.

2.3. Cell infection

The GBC-SD and NOZ cells were randomly seeded into 6-well plates at a density of 3×10^5 cells/well the day before transfection. When the cells reached 70 % confluence, 20 nM small interfering RNA (siRNA) and the Erk1/2 phosphorylation agonist Ro 67–7476 (1 μM) [21] were transfected into GBC-SD and NOZ cells using lipofectamine 3000 (Thermo Fisher Scientific Co., USA) in DMEM medium at room temperature. The transfection reagents were then replaced with DMEM medium containing 10 % FBS after 12-h incubation in 5 % CO_2 at 37 °C. After 48–72 h, HOXB-AS3 expression level was detected by quantitative reverse transcription polymerase chain reaction and subsequent experiments were performed.

2.4. RT real-time PCR(qPCR)

To purify total RNA from NOZ, GBC-SD and HIBEpic cells, the Trizol reagent (Thermo Fisher Scientific Co., USA) was utilized according to the manufacturer's instructions. Subsequently, the concentration of the extracted RNA was precisely quantified using a biological spectrometer (Thermo Fisher Scientific Co., USA). With 1 μg of this purified total RNA as the input, reverse transcription was conducted to synthesize cDNA using a HiFiScript cDNA kit (CWBio Co.,Guangzhou, China) at a temperature of 42 °C for 20 min. Thereafter, reverse-transcribed real-time PCR (qPCR) was conducted utilizing the Roche Real-Time PCR System, with SYBR Premix *Taq*II (CWBio Co., Guangzhou, China). The optimized PCR cycling conditions comprised an initial denaturation step at 95 °C for 5 min, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 1 min, and a subsequent temperature ramp to 65 °C for 1 min to ensure complete extension of the PCR products. Lastly, a final extension step was executed at 65 °C for 5 min to finalize the synthesis of all PCR products.

2.5. Western blot

After 72 h of transfection, total protein was extracted using the protein extraction kits (CWBio Co., Guangzhou, China). The concentration of the extracted protein was precisely determined using an enhanced BCA assay kit(Beyotime, China). Proteins (20 μg) were separated by 7.5%–10 % SDS-PAGE gels and then the proteins were transferred to the PVDF membranes. Following a 35-min blocking step at room temperature using a rapid blocking buffer provided by Epizyme Co, the membranes were subsequently incubated with the primary antibodies at 4 °C for an overnight period. The following primary antibodies were used: Anti-ERK (1:1000, Cell signaling technology), MEK (1:1000, Cell signaling technology), pERK (1:1 000, Cell signaling technology), pMEK (1:1000, Cell signaling technology), GAPDH (1:3000, Cell signaling technology). After the primary antibody incubation, the membranes were further incubated with secondary antibody (1:5000, CWBio Co., Guangzhou, China) for 1.5 h at room temperature. Following this, the proteins on the membrane were visualized using a chemiluminescence detection kit (Biosharp, China). For semi-quantitative analysis of the blot data, Image J software, version 1.8.1, was utilized.

2.6. Colony formation assay

GBC-SD and NOZ cells were randomly seeded into 6-well plates at a density of 1000 cells/well and then cultured in DMEM supplemented with 10 % FBS overnight at 37 °C. Then, GBC-SD and NOZ cells were treated with siHOXB-AS3##1, siHOXB-AS3##2 or siNC(negative control), and the cells were maintained in culture with regular medium changes for up to 14 days. To prepare the colonies for counting, they were first fixed with 20 % methanol at room temperature for 20 min, followed by staining with 1 % crystal violet solution(Beyotime, China) for an additional 30 min at room temperature. Finally, the colonies were counted using a light microscope(Olympus, Japan).

2.7. Cell counting kit 8 assay (CCK-8)

Gallbladder carcinoma cells GBC-SD and NOZ were randomly seeded into the 96-well plates at a density of 5000 cells per well overnight. Then, the NOZ and GBC-SD cells were transfected with siHOXB-AS3##1, siHOXB-AS3##2 or siNC(negative control) for 0, 24, 48, or 72 h. 10 μL CCK-8 reagent (LiankeBio, China) was added to each well, and incubated for 3 h at 37 °C. Optical density (OD) was then measured by a microplate reader (Thermo Fisher Scientific, USA) at 450 nm.

2.8. Wound healing assay

NOZ and GBC-SD cells were randomly seeded into 6-well plates and then cultured in DMEM supplemented with 10 % FBS until the NOZ and GBC-SD cells were 90 % confluent. After a scratch wound was created in the cell monolayer with a 200 μl pipette tip, DMEM was then added to the 6-well plates. At 0 h and 72 h, migration of NOZ and GBC-SD cells was photographed. To quantify the extent of migration, the wound distance at both 0 and 72 h was measured using ImageJ software. The percentage of gap closure, which served as an indicator of cell migration, was calculated as follows: (0 h average distance-72 h average distance)/(0 h average distance) *100 %.

2.9. Transwell invasion assay

After 24 h transfection, gallbladder carcinoma cells GBC-SD and NOZ were enzymatically detached using trypsin at 37 °C and resuspended in DMEM media devoid of fetal bovine serum (FBS). To facilitate cell invasion, the upper chamber membrane was precoated with a 1:8 dilution of Matrigel (Solarbio, China) solution and incubated at 37 °C for 1.5 h. Subsequently, 10 0000 NOZ and GBC-SD cells, suspended in DMEM without FBS, were seeded into the upper chamber, while the lower chamber was filled with DMEM supplemented with 10 % FBS to serve as a chemoattractant. After a 36-h incubation period at 37 °C, invading cells were fixed with 4 % paraformaldehyde for 25 min at room temperature, followed by staining with 0.1 % crystal violet for another 25 min under the same conditions. Invading NOZ and GBC-SD cells were visually inspected and enumerated using a microscope (Olympus, Japan) by assessing five randomly chosen fields of view.

2.10. Flow cytometry of cell apoptosis

To assess apoptosis rate of GBC-SD and NOZ cells, flow cytometry was determined. Initially, the gallbladder carcinoma cells were treated with si-NC or si-HOXB-A3 and then were cultured for 72 h in 6-well plates. Following this incubation period, The cells were enzymatically detached using trypsin and a total of 100 000 cells of suspended cells were gathered. Then, these cells were stained using an FITC-annexin -V Apoptosis kits (LiankeBio Co., China). The stained cells were instantly examined by flow cytometry (Beckman, USA). The proportion of apoptotic cells was subsequently determined using Flowjo software (BD Bioscience, 10.6.2).

2.11. Flow cytometry of the cell cycle

After being transfected with either si-HOXB-AS3 or si-NC, NOZ and GBC-SD cells were cultured in 12-well plates for 48 h. Then, the NOZ and GBC-SD cells were enzymatically detached using trypsin at 37 °C and total 20 000 cells of suspended cells were collected. Propidium iodide (LiankeBio, China) was used to stain the harvested cells. The cell cycle phases of NOZ and GBC-SD cells, categorized as G1/G0, S, and G2/M stages, were analyzed, and the proportions of each phase were determined using Modfit software (Verity Software House, version 3.1).

2.12. Tumor xenograft model

In the study, except for in-vitro studies described above, in-vivo experiment were also performed. Female BALB/c-nude mice with aged 4–6 weeks were purchased from Gempharmatech-GD Laboratory. ($N = 10$, Guangzhou, China) and were divided into 2 groups (si-HOXB-AS3 and si-NC) randomly. The animals were kept in specific-pathogen-free (SPF) animal laboratory. Equal numbers of si-HOXB-AS3 and si-NC GBC-SD cells (4×10^6 cells) were subcutaneously injected into the nude mice. Tumor volumes were measured by a vernier caliper and were calculated using the following formula: $V=(S^2 \times L)/2$ (S, the shortest tumor length; L, the longest tumor length). All nude mice were killed after 40days and the xenograft tumors were collected for immunohistochemistry.

2.13. Statistical analysis

All the experiments in this study were performed at least three times. Data from at least three independent experiments were presented as the mean ± standard deviation. All statistical analyses in this study were performed using GraphPad Prism(GraphPad software, version 8.0). Statistical analyses were conducted using One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, and a P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Long non coding RNA HOXB-AS3 was elevated in gallbladder carcinoma

The expression levels of HOXB-AS3 in gallbladder cancer and para-cancer tissues were obtained from the GEO dataset GSE74048. HOXB-AS3 was markedly up-regulated in GBC tissues compared with matched peri-carcinomatous tissues ($n = 3$, $P < 0.05$, Fig. 1A). Furthermore, the expression levels of HOXB-AS3 in human intrahepatic biliary epithelial cell (HIBEpic) and the GBC cell lines GBC-SD and NOZ were detected by qPCR. HOXB-AS3 was significantly increased in NOZ and GBC-SD cells compared with HIBEpic cells ($P < 0.05$, Fig. 1B). These results demonstrated that HOXB-AS3 was highly expressed in GBC tissues and cell lines, suggesting a possible link between HOXB-AS3 and gallbladder cancer.

HOXB-AS3 expression in gallbladder carcinoma tissues and corresponding para-cancer tissues were obtained from the GEO dataset GSE74048. Notably, when compared to matched peri-carcinomatous tissues, HOXB-AS3 was significantly up-regulated in gallbladder carcinoma tissues ($n = 3$, $P < 0.05$, Fig. 1a). Furthermore, qPCR analysis was conducted to assess the expression levels of HOXB-AS3 in human intrahepatic-biliary epithelial cells (HIBEpic) and two gallbladder carcinoma cell lines, NOZ and GBC-SD. The results indicated that, compared to HIBEpic cells, HOXB-AS3 expression was markedly increased in NOZ and GBC-SD cells ($P < 0.05$, Fig. 1b). These findings collectively demonstrated that HOXB-AS3 is highly expressed in gallbladder carcinoma tissues and cell lines, suggesting a potential link between HOXB-AS3 and gallbladder carcinoma.

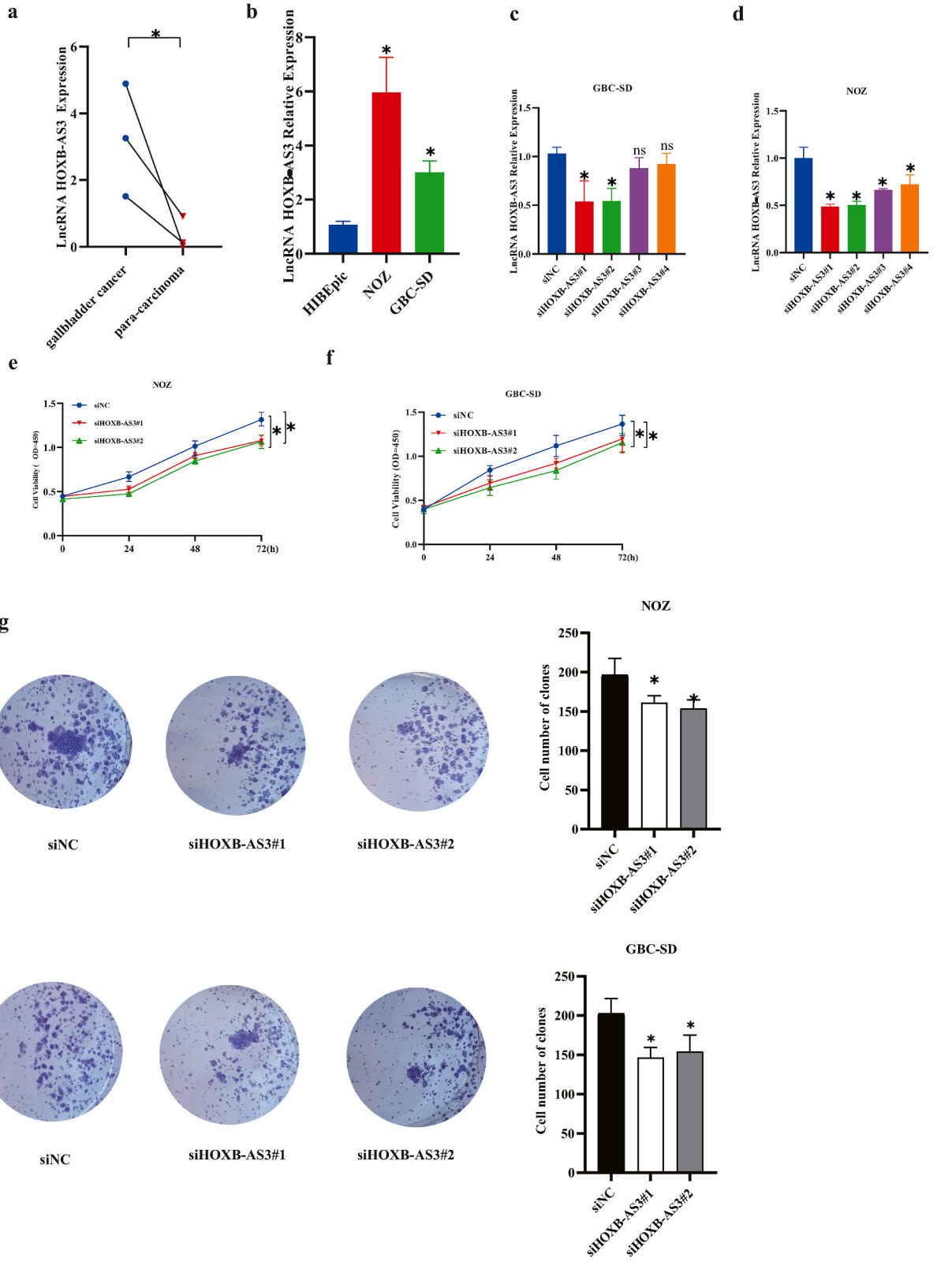

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Fig. 1. ***HOXB-AS3 promoted GBC-SD and NOZ cells proliferation***. (a) Expression levels of HOXB-AS3 in gallbladder carcinoma tissues and peri-carcinomatous tissues(n = 3). (b)qPCR to detect the expression levels of HOXB-AS3 in gallbladder carcinoma cells and HIBEpic cells. (c-d) lncRNAHOXB-AS3 was down-regulated after treating with siRNA in gallbladder carcinoma cells. (e–h) CCK-8 assay and colony formation assay to detected the cell proliferation of gallbladder carcinoma cells after inhibiting HOXB-AS3, respectively. Data at least three independent experiments were presented as the mean ± standard deviation. *p < 0.05, ns: no significance.

3.2. HOXB-AS3 promoted GBC-SD and NOZ cells proliferation

To investigate the role of HOXB-AS3 in gallbladder carcinoma, 4 small interfering RNAs (siRNAs) specifically targeting HOXB-AS3, along with one non-specific siRNA (si-NC), were utilized. The transfection of these siRNAs resulted in a significant down-regulation of HOXB-AS3 expression in both NOZ and GBC-SD cells (Fig. 1c and d). Furthermore, knockdown of HOXB-AS3 led to a marked suppression of proliferation in these cells, which was evidenced by both the CCK-8 assay (Fig. 1e and f) and the cell colony formation assay (Fig. 1g and h). These findings collectively indicated that HOXB-AS3 promotes the proliferation of gallbladder carcinoma cells.

3.3. HOXB-AS3 promoted GBC cell cycle progression and suppressed cell apoptosis

Inhibition of HOXB-AS3 resulted in the blockage of the NOZ and GBC-SD cell cycle at the G1 phase (Fig. 2a and b). Furthermore, HOXB-AS3 siRNA induced apoptosis in NOZ and GBC-SD cells (Fig. 2c and d). Collectively, these results validated that HOXB-AS3 facilitates cell cycle progression and suppresses cell apoptosis in gallbladder carcinoma.

3.4. HOXB-AS3 induced gallbladder carcinoma cell migration and invasion

Transwell and wound-healing assays were used to detect the effects of HOXB-AS3 on migration and invasion of NOZ and GBC-SD cells. Knockdown of HOXB-AS3 significantly impaired wound closure in gallbladder carcinoma cells (Fig. 3a and b). Additionally, transwell assays revealed that HOXB-AS3 knockdown markedly reduced the invasion capacity of NOZ and GBC-SD cells (Fig. 3c and d). These findings confirmed that HOXB-AS3 knockdown suppressed the migration and invasion abilities of gallbladder cancer cells.

3.5. HOXB-AS induced the phosphorylation levels of MEK and ERK in GBC cells

HOXB-AS3 knockdown notably decreased the phosphorylation levels of ERK and MEK in NOZ and GBC-SD cells, while the expression of ERK and MEK remained unchanged (Fig. 4a and b). These findings suggest that HOXB-AS3 promotes MEK and ERK phosphorylation in gallbladder cancer cells.

3.6. HOXB-AS3 induced proliferation, migration and invasion of GBC cells through activation of the MEK-ERK signaling pathway

HOXB-AS3 plays pivotal roles in promoting GBC cell proliferation, invasion, and migration, and its knockdown notably alters MEK and ERK phosphorylation levels in GBC cells. To further validate that HOXB-AS3 regulates GBC cell proliferation, invasion, and migration through the MEK/ERK pathway, GBC-SD cells were co-transfected with siHOXB-AS3 and the ERK1/2 phosphorylation agonist Ro 67–7476.As shown in Fig. 5a, siHOXB-AS3+DMSO significantly reduced the phosphorylation levels of ERK compared with the siNC + DMSO group. When GBC cells were transfected with siHOXB-AS3 and Ro 67–7476 simultaneously, the phosphorylation levels of ERK increased compared with the siHOXB-AS3+DMSO group. Also, when GBC cells were transfected with siNC and Ro 67–7476 simultaneously, the levels of ERK phosphorylation obviously increased compared with the siHOXB-AS3+DMSO group. These results indicated that the low levels of ERK phosphorylation induced by siHOXB-AS3 can be reversed by the ERK1/2 phosphorylation agonist Ro 67–7476.

Compared with the siNC + DMSO group, siHOXB-AS3+DMSO suppressed GBC cell proliferation, but was it could be reversed by Ro 67–7476 at the CCK-8 assay (Fig. 5b). The apoptotic rate of GBC-SD cells increased when HOXB-AS3 was knocked down but decreased in the presence of Ro 67–7476 (Fig. 5c). Compared with the siNC + DMSO group, HOXB-AS3 knockdown resulted in the cell cycle being arrested at the G1 phase, and the rate of G1 phase cells was increased by Ro 67–7476 (Fig. 5d). Compared with the siNC + DMSO group, siHOXB-AS3+DMSO suppressed GBC cell migration and invasion, but was reversed by Ro 67–7476, which was confirmed by the wound healing and transwell invasion assays, respectively (Fig. 5e and f).

Overall, these results indicate that HOXB-AS3 promotes proliferation, invasion, and migration of GBC cells through activation of the MEK-ERK signaling pathway.

3.7. HOXB-AS promoted xenograft tumor growth in vivo

To ascertain whether HOXB-AS3 influences the tumorigenesis of gallbladder carcinoma in vivo, we established a xenograft model. On the 40th day, the mice were euthanized, and the tumors were subsequently weighed and photographed.(Fig. 6a–d). The tumor volume and weight increased significantly in the siHOXB-AS group compared with control group($P < 0.05$). Immunohistochemistry was performed to verify the effect of HOXB-AS knockdown upon tumor proliferation. And the expression levels of Ki67, pERK and pMEK was lower in the tumors of the siHOXB-ASgroup mice (Fig. 6e). These results indicate that knockdown of HOXB-AS expression

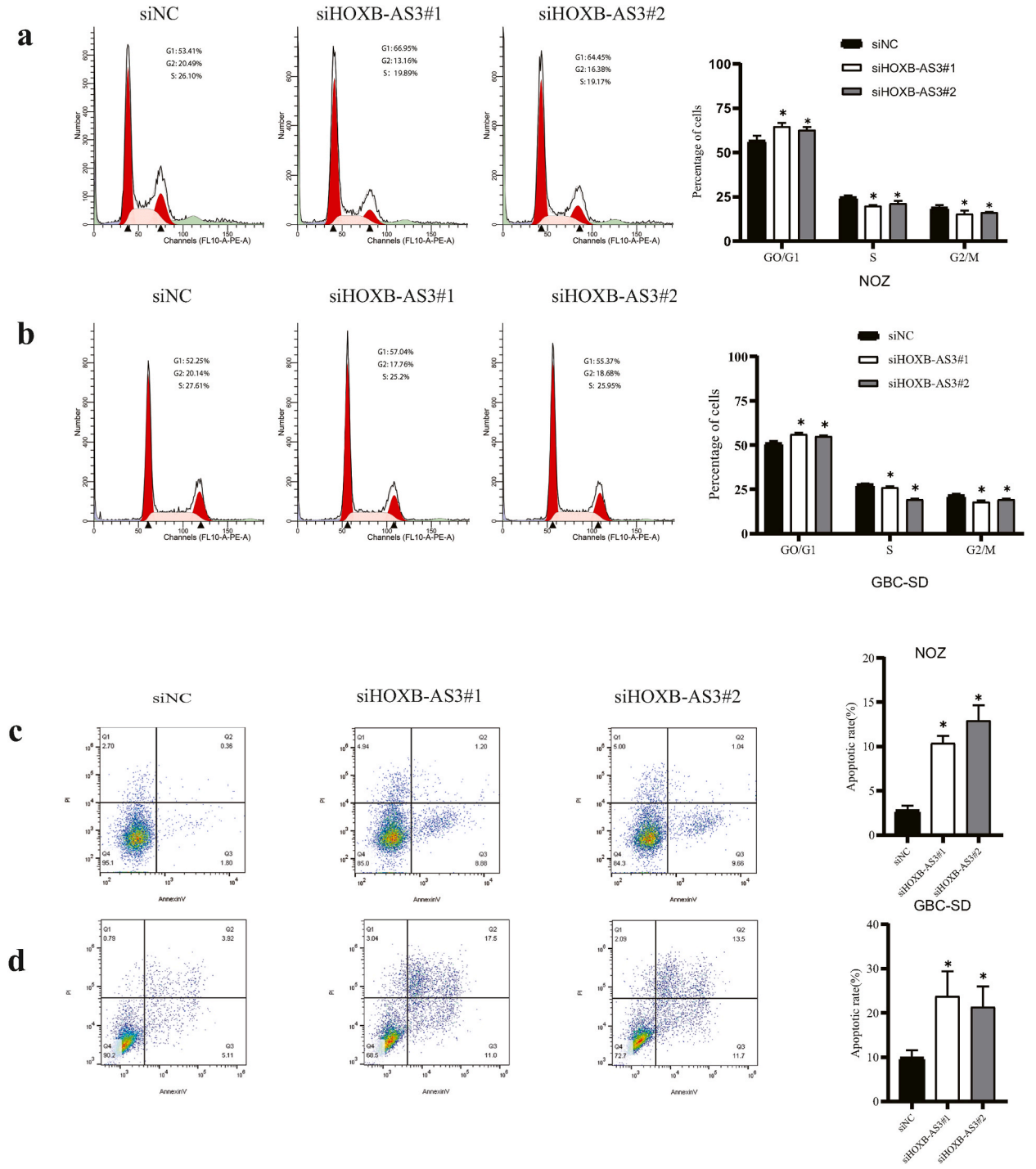

Fig. 2. ***HOXB-AS3 induced gallbladder carcinoma cell cycle progression and inhibited cell apoptosis***. (a–b) Flow cytometry of gallbladder carcinoma cell cycle in NOZ and GBC-SD cell that treated with siNC or siHOXB-AS3. (c–d) Flow cytometry of cell apoptosis in gallbladder carcinoma cells that treated with siHOXB-AS3 and siNC. Data at least three independent experiments were presented as the mean ± standard deviation. *p < 0.05.

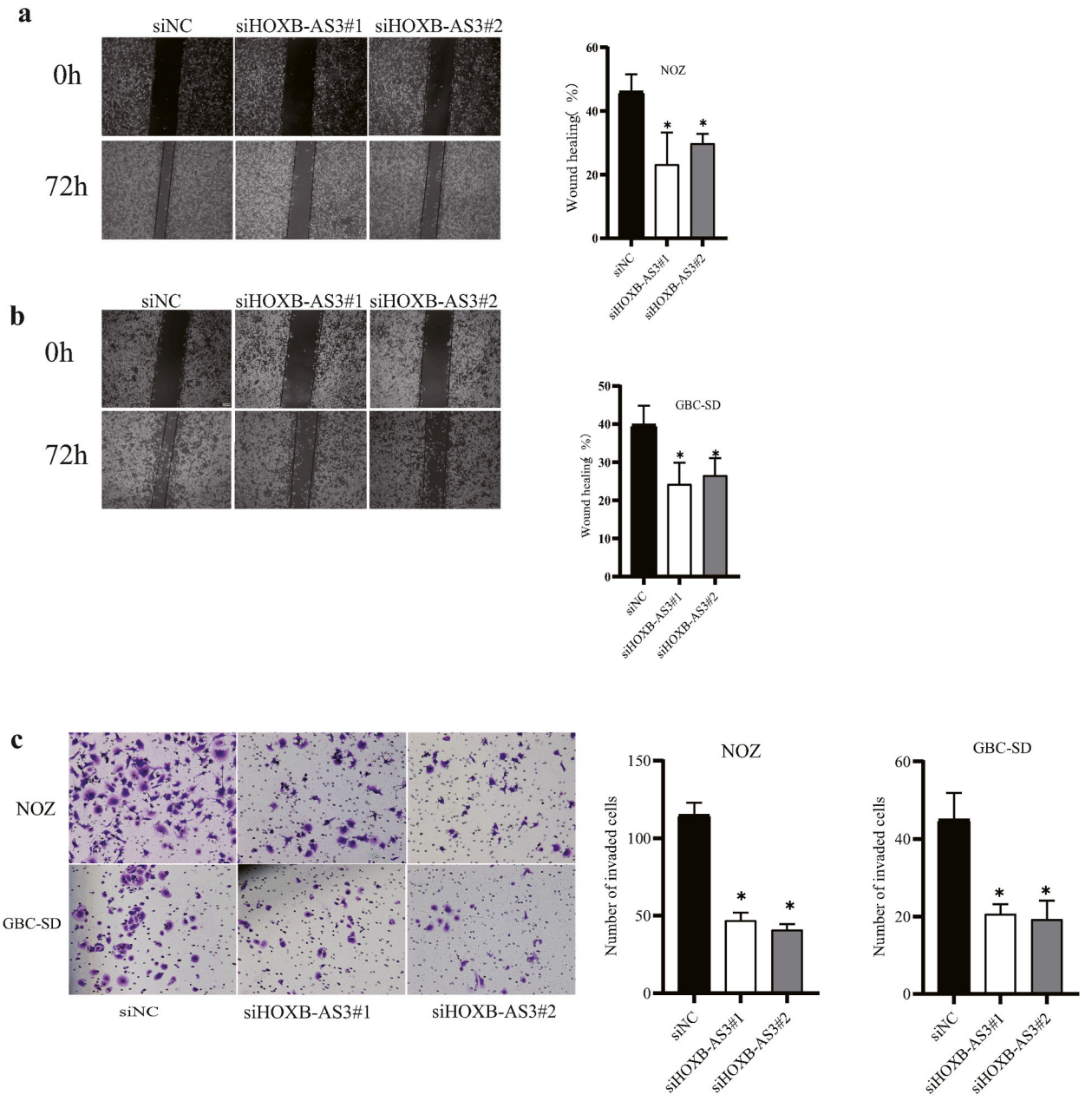

Fig. 3. ***HOXB-AS3 promoted the migration and invasion of gallbladder carcinoma cell.*** (a–b) The migration ability of gallbladder carcinoma cells that treated with siNC or siHOXB-AS3 was detected by wound-healing assay. (c-d)The invasion ability of gallbladder carcinoma cells that treated with siNC or siHOXB-AS3 was detected by transwell assay. Data at least three independent experiments were presented as the mean ± standard deviation. *p < 0.05.

suppresses xenograft tumor growth in vivo.

4. Discussion

Gallbladder carcinoma (GBC) leads to be a highly lethal illness and is a major threat to human health [22,23]. Increasing evidences suggest that several long non coding RNAs play a role in gallbladder carcinoma progression. For example, the OIP5-AS1 promotes gallbladder carcinoma cell migration and invasion [24]. FOXD2-AS1 is able to inhibits GBC progression via MLH1 methylation [25]. By interacting with LET, the lncRNA EPIC1 can promotes proliferation and inhibits apoptosis of gallbladder carcinoma cells [26]. LINC01410 is reported to promote gallbladder carcinoma progression by targeting STAT5 through the ERB pathway [27]. In recent years, the novel molecule HOXB-AS3 has attracted more and more attention. It plays an important role in many cancers such as colon

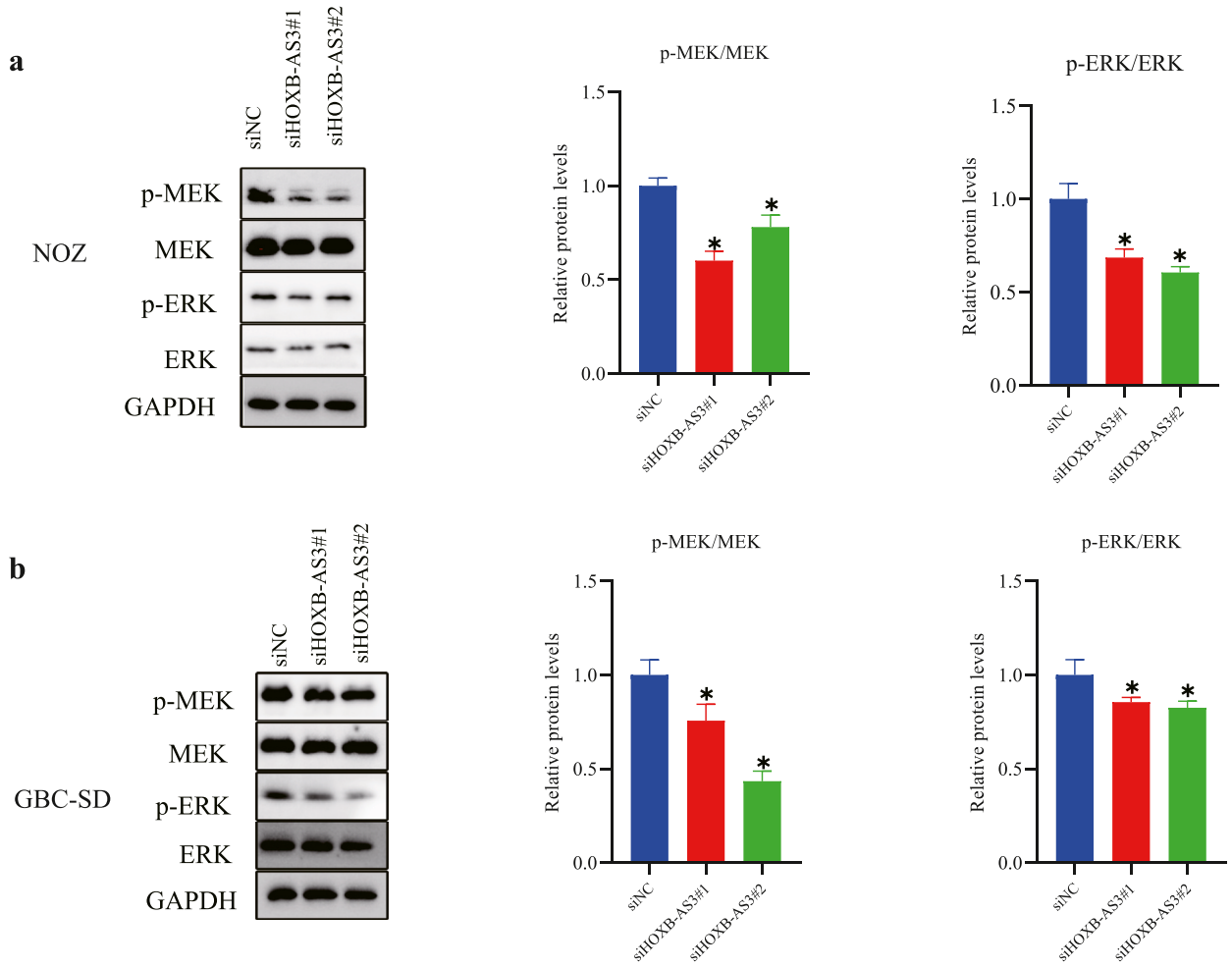

Fig. 4. ***HOXB-AS induced the phosphorylation levels of ERK and MEK in gallbladder carcinoma cells.*** (a–b) West blot to detected the protein expression levels of total MEK, pMEK, ERK and pERK in NOZ and GBC-SD cell that treated with siHOXB-AS3 and siNC. Data at least three independent experiments were presented as the mean ± standard deviation. *p < 0.05.

carcinoma, ovarian cancer, endometrial carcinoma and lung cancer [14–17]. However, whether the long non coding RNA HOXB-AS3 played a important role in the progression of gallbladder carcinoma has not been previously reported. Therefore, we investigated the function of lncRNA HOXB-AS3 in the progression of GBC, following the identification of its high expression in gallbladder carcinoma cell lines and tissue.

In the present study, we observed a significant up-regulation of HOXB-AS3 expression in GBC tissue and cell lines. Furthermore, HOXB-AS3 knockdown significantly reduced GBC cells proliferation, invasion, migration, and promoted cell apoptosis. The MEK-ERK signaling pathway plays a crucial role in cell proliferation, differentiation, survival, and migration [28]. MEK/ERK signaling has been previously linked to the oncogenesis of numerous human tumors, such as pancreatic cancer, retinoblastoma and gastric cancer [29–31]. In our study, we discovered that the knockdown of HOXB-AS3 led to the inhibition of MEK and ERK phosphorylation in gallbladder carcinoma cells.

Consequently, to confirm the role of activation of the MEK-ERK signaling pathway in the progression of GBC, the ERK1/2 phosphorylation agonist Ro 67–7476 was used in a subsequent study. Experiments showed that the features of siHOXB-AS3 in GBC cells could be reversed by the ERK1/2 phosphorylation agonist Ro 67–7476; this suggested that lncRNA HOXB-AS3 promoted GBC cell proliferation, invasion, migration and suppressed apoptosis via the MEK/ERK pathway. Also, we confirmed that HOXB-AS promoted xenograft tumor growth in vivo. Our study is the first to reveal that HOXB-AS3 contributes to the tumorigenesis of GBC through activation of the MEK-ERK signaling pathway.

However, this study has some limitations. Firstly, the sample size of GBC tissue was small(3 vs 3), which might lead to selection bias. Consequently we are trying to obtain enough gallbladder carcinoma tissues from gallbladder cancer patients in our hospital for further research. Secondly, we discovered that long non coding RNA HOXB-AS3 promoted GBC cell proliferation, invasion, migration and suppressed apoptosis through activation of the MEK-ERK pathway. Nevertheless, no evidence has emerged to suggest that HOXB-AS3 possesses the ability to directly phosphorylate ERK or MEK. As a result, future research endeavors should concentrate on

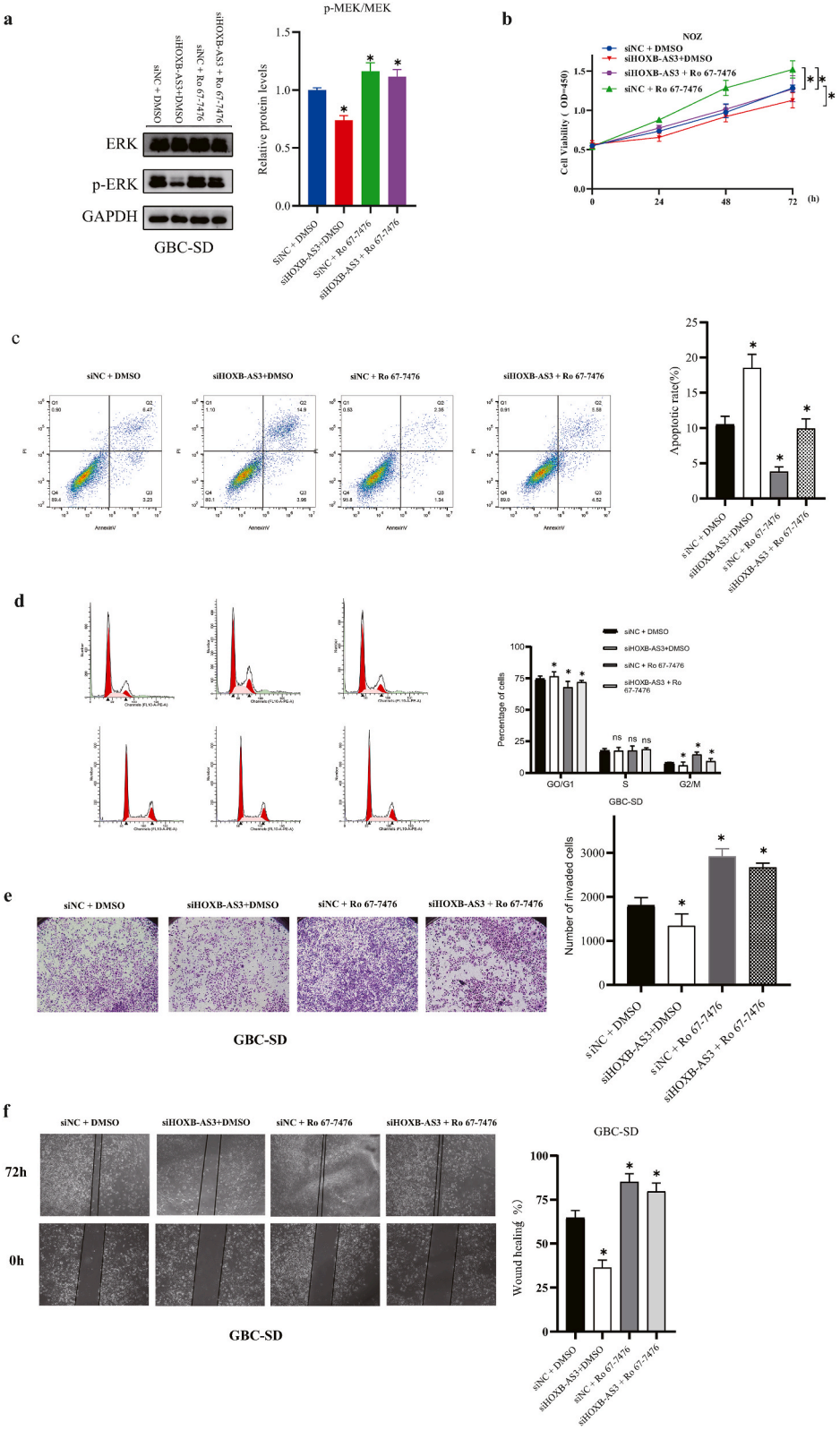

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Fig. 5. ***HOXB-AS3 induced proliferation, migration and invasion of gallbladder carcinoma cells via activating MEK-ERK signaling pathway.*** (a) West blot were performed to detected the protein expression levels of total ERK and pERK in GBC-SD cell that treated with siHOXB-AS3 or ERK1/2 phosphorylation agonist Ro 67–7476. (b) CCK-8 assays to detected cell viability of GBC-SD cell (c–d) Flow cytometry to detect the cell cycle and apoptosis of gallbladder carcinoma cell. (e-f)Wound-healing assay & Transwell assays to detect the invasion ability of migration ability in GBC-SD cell that treated with Ro 67–7476 or siHOXB-AS3 , respectively. Data at least three independent experiments were presented as the mean ± standard deviation. *p < 0.05.

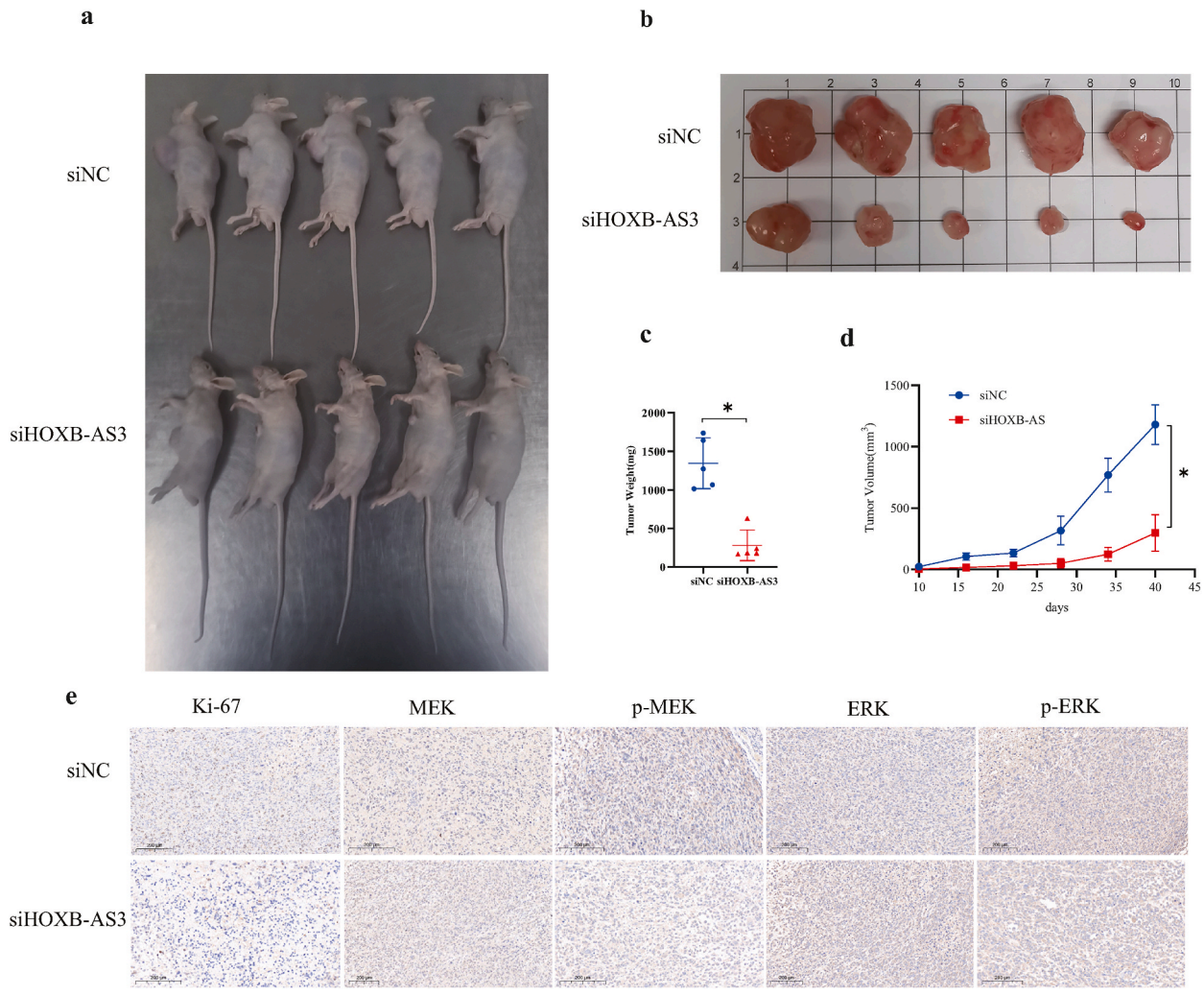

Fig. 6. *HOXB-AS promoted xenograft tumor growth in nude mice* (a–b) Photographs of the nude mice and xenograft tumors treated with siHOXB-AS3 or siNC. (c) Growth curve of xenograft tumor volumes.(d)Weights of the tumors in the xenograft mouse model. (e) Immunohistochemistry were detected the expression levels of pMEK, MEK, pERK, ERK and Ki-67 of xenograft tumor.

deciphering precise regulatory mechanism of long non coding HOXB-AS3 in gallbladder carcinoma.

5. Conclusion

HOXB-AS3 promoted gallbladder cancer cell proliferation, invasion and migration, and suppressed apoptosis through the MEK-ERK pathway. HOXB-AS3 contributes to gallbladder carcinoma progression, making it a viable therapeutic target for gallbladder carcinoma treatment.

Ethical statement

The Institutional Review Board of The Sixth Affiliated Hospital of Sun Yat sen University approved this study.(Approval number:

IACUC-2022081501).

Funding

This study was supported by the National Natural Science Foundation of China (granted number: 30671987 & 8180102403) and National Key Clinical Discipline.

Data availability

Data associated with this study are available in:https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74048. All data of the study are available for reasonable request.

CRediT authorship contribution statement

Jiayan Wu: Writing – original draft. **Jiandong Yu:** Data curation. **Hongquan Zhu:** Formal analysis. **Zhiping Chen:** Methodology. **Yongling Liang:** Software. **Qin Chen:** Investigation. **Guolin Li:** Project administration. **Yunle Wan:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35906.

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