Original Article

AIBP promotes cell proliferation and migration through the ERK1/2-MAPK signaling pathway in hepatocellular carcinoma

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Background: As a highly aggressive cancer, hepatocellular carcinoma (HCC) is often found at an advanced stage and has a poor prognosis. Therefore, in addition to the surgical treatment of HCC, the drug therapy for HCC is still under continuous exploration. The primary apolipoprotein of high-density lipoproteins (HDLs) is apolipoprotein A-I binding protein (AIBP), which has a significant impact on cholesterol metabolism, angiogenesis, and a wide range of inflammatory disorders, including cancer. The AIBP function in HCC is, however, yet unknown. This study aims to reveal the underlying mechanisms of AIBP influencing HCC proliferation and migration through mitogen-activated protein kinase (MAPK) pathways.

Methods: AIBP expression and its clinical prognostic association were investigated using The Cancer Genome Atlas (TCGA) data. The AIBP expression was studied in human HCC tissues using immunohistochemistry (IHC) and western blotting. Colony formation assays (CFAs) and cell counting kit-8 (CCK-8) were used to determine *in vitro* cell proliferation. Cell migration and invasion were evaluated using wound-healing and transwell assays. A xenograft tumor model was employed to investigate HCC cell proliferation in nude mice.

Results: Tissues from HCC patients had much increased AIBP expression compared to nearby normal tissues. The prognosis for patients was bleak when AIBP expression was high. When AIBP was overexpressed in SMMC-7721 cells, the cells may become more proliferative, migrative, and invasive. In contrast, the HCC-LM3 cells' ability to proliferate, migrate, and invade was drastically decreased once AIBP was knocked down *in vitro*. Furthermore, *in vivo* research showed that AIBP overexpression may enhance cell proliferation in HCC. Finally, we discovered that AIBP could control the MAPK signaling pathway-involved genes expression, including P-MEK, MEK, c-Myc, P-ERK1/2, and ERK1/2, and that GDC-0994, a specific ERK1/2 inhibitor, could suppress the AIBP overexpression induced cell migration and proliferation abilities.

Conclusions: These findings demonstrated that the ERK/MAPK signaling pathway might be stimulated by AIBP in HCC tissues, leading to increased cell invasion, migration, and proliferation. It was hypothesized that AIBP might act as a useful prognostic and diagnostic marker for HCC.

Keywords: Migration; proliferation; apolipoprotein A-I binding protein (AIBP); hepatocellular carcinoma (HCC); ERK/MAPK

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Introduction

More than 85% of all primary liver tumors are hepatocellular carcinoma (HCC), making it the most prevalent form of primary liver cancer (1,2). It is still one of the most prevalent malignancies, ranking sixth in terms of incidence and third in terms of mortality from cancer (3). However, despite decades of steady improvement in diagnosing and treating HCC and several treatment techniques such as chemotherapy, radiation, surgery, and radiofrequency ablation for early HCC, the HCC prognosis remains poor owing to its fast development and high recurrence rate. Only 18% of liver cancer patients survived for 5 years following surgery, and over 70% of those patients had a recurrence or distant metastases within 2 years (2-6). Therefore, new diagnostic and therapeutic methods must be established, and the molecular pathogenesis of HCC must be elucidated.

The adrenal gland, thyroid, liver, heart, kidney, and testes are only a few of the many tissues that produce the secreted apolipoprotein A-I binding protein (AIBP) (7,8). Previous research has indicated that AIBP may increase cholesterol efflux from endothelial cells and macrophages, consequently decreasing atherosclerosis incidence (9). In addition, AIBP suppresses neuroinflammation by decreasing lipid rafts and diminishing the inflammatory signal from lipopolysaccharide-induced TLR4 dimers and the production of cytokines by microglia (10,11). Through the NOTCH signaling pathway, AIBP may also suppress angiogenesis (12). When AIBP and APOA-I are expressed in intestinal tumors, they have a striking anticancer impact on colorectal cancer (13). Even though AIBP metabolism occurs in the liver and kidney, its precise mechanism on the HCC incidence and progression and its therapeutic implications in liver cancer remain unknown, and it is the first time that AIBP is used to evaluate the occurrence, development and prognosis of HCC.

The mitogen-activated protein kinase (MAPK) pathway has been shown to have substantial involvement in the initiation and progression of HCC in previous research (2). Four different types of MAPKs—ERK, P38, JNK, and ERK5—make up the pathway (14). Phosphorylation of extracellular regulated kinase (ERK) causes its translocation from the cytosol to the nucleus, affecting transcription factors downstream to impact the c-Jun, c-fos, ELK-1, c-Myc, and other genes expression involved in cancer invasion, migration, and proliferation (15). It has been shown that the ERK1/2 inhibitor GDC-0994 is very effective in treating pancreatic cancer (16).

Increasing AIBP expression levels are seen in HCC, which may have implications for patient prognosis. Studies have demonstrated that AIBP may profoundly affect HCC cell invasion, migration, and proliferation. On this premise, we further studied the regulatory function of its potential molecular pathways to further elaborate and prove the important role of AIBP in the occurrence and development of HCC. Our results further show that AIBP stimulates the HCC malignant phenotype by ERK/MAPK signaling pathway activation, and ERK inhibitors efficiently halt this development. These findings offer novel perspectives on the causes of HCC. As a result of its propensity to stimulate the expansion and metastasis of HCC cells, AIBP is widely regarded as a prime therapeutic target. We present this article in accordance with the MDAR and ARRIVE reporting checklists (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2101/rc).

Highlight box

Key findings

- Apolipoprotein A-I binding protein (AIBP) is associated with the progression of hepatocellular carcinoma (HCC) and AIBP might act as a useful prognostic and diagnostic marker for HCC.

What is known and what is new?

- High expression of AIBP is related to the proliferation, migration and invasion of HCC.
- AIBP may promote the invasion, migration and proliferation of HCC by activating ERK/MAPK signaling pathway.

What is the implication, and what should change now?

- The role of AIBP in the ERK/MAPK signaling pathway needs further study.

Methods

Patients and tissue samples

From June 2012 to February 2017, 91 pairs of HCC patients who had radical surgery for HCC at the Affiliated Hospital of Nantong University were selected. Patients who declined to participate, received palliative treatment or a liver transplant, and those with distant metastases or other malignant tumors were excluded. The differential expression between the tumor and nearby tissues was studied using immunohistochemistry (IHC) labeling in 91 samples, including HCC, and matched neighboring tissues. Twenty pairs were used for western blotting.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of the Affiliated Hospital of Nantong University (No. 2020-L093) and informed consent was taken from all the patients.

IHC staining

To study the AIBP localization and expression levels, tissue microarrays (TMAs) were created from 91 HCC patients. Following the paraffin embedding of tissues, they were sliced at 4 μm, dewaxed, hydrated in a gradient ethanol solution, and treated for 5–10 minutes with 3% hydrogen peroxide at room temperature (RT) to quell any remaining endogenous peroxidase activity. The antigen was then removed from TMAs by boiling them in a citric acid solution (pH =6.0) for 40 minutes at 92 ℃. The serum was blocked at RT for 30 minutes with 1% fetal bovine serum (FBS) and discarded without cleaning. anti-AIBP antibodies and TMAs were instantly incubated at 4 ℃ overnight. Following a three-time 1× phosphate buffer saline (PBS) wash, TMAs were incubated at 37 ℃ for 10–30 minutes with a peroxidase-polymerized goat anti-rabbit secondary antibody. The TMAs were incubated with diaminobenzidine for 3–15 minutes after being rinsed three times with 1× PBS. As a final step, the TMAs were thoroughly rinsed in running water, restrained, dehydrated, made transparent, and sealed.

In a blinded fashion, two pathologists compared the proportion of positive cells and the staining intensity under the microscope to determine the level of AIBP expression. Stained cells percentage in cell count: 4 (76–100%), 3 (51–75%), 2 (26–50%), 1 (6–25%), 0 (0–5%); Staining power: 3 (brown yellow), 2 (light yellow), 1 (weak staining), 0 (dyeing strength); The score multiplied by the two was considered: 9–12, strongly positive (+++), 5–8, positive (++), 1–4, weakly positive (+), and 0, negative (–).

Cell culture and transfection

We received HCC-LM-3 and SMMC-7721 human hepatoma cell lines from the Clinical Research Center of the hospital mentioned above. These cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 0.02% tetracycline, and 1% penicillin-streptomycin at 37 ℃ with 5% CO_2.

In order to select the positive clones, 24 hours before transfection, per the manufacturer's guidelines, in a 6-well plate, 1×10^6 cells were plated with AIBP-single-guide RNA (sgRNA) plasmids and its negative control sgRNA (NC-sgRNA). The medium containing the plasmids was removed 48 hours later, and the transfected cells were treated with 3 μg/mL puromycin for 48 hours. Western blot tests verified the AIBP knockdown. Transheep (Shanghai, China) designed and synthesized both the AIBP-sgRNA and NC-sgRNA.

Western blotting analysis

When extracting total proteins from cells and tissues, a cell lysis solution was used, which included protease and phosphatase inhibitor cocktails. Electrophoretic separation of proteins was carried out on a 6–12% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel, which was then blotted to a polyvinylidene fluoride (PVDF) membrane. After nonfat milk (5%) containing Tris-buffered saline Tween (TBST) blocking for 2 hours at RT, membranes were rinsed with 1× PBST three times before being incubated with primary antibodies at 4 ℃ overnight. After three 10-minute incubations at RT with the secondary antibodies, a final three 10- minute incubation with 1× PBS was performed. Finally, a bioimaging system (Bio-Rad, California, USA) was used to observe target bands after the PVDF membrane was smeared with electrochemiluminescence (ECL) luminescent solution; the protein bands quantification was done using ImageJ. At least three separate experiments were conducted for each result.

The Cancer Genome Atlas (TCGA) liver hepatocellular carcinoma (LIHC) database analysis

AIBP mRNA levels were analyzed in normal and malignant tissues by downloading data from TCGA for 374 HCC patients and 50 neighboring samples. Student's *t*-test was used to assess AIBP expression levels and overall survival (OS) rate across groups.

Wound healing and transwell assay

Migration and invasion experiments used transwell chambers (8.0 μm pore size). A total of 2×10^4 cells, as determined by counting, were plated in the upper chamber in the 200 μL serum-free (SF) media. The bottom chamber was placed into the 24 wells with 600 μL of normal media. After 24 hours incubation, cells were stained for 15 minutes

with 0.5% crystal violet, following fixation with methanol for 30 minutes. Three different areas were chosen randomly to determine the total cell number that could pass through the pore. Meanwhile, photographs of each well were obtained using an inverted Nikon research microscope.

Cells at a density of 5×10^5 cells/well in 6-well culture plates were allowed to grow overnight. A 100 µL pipette tip was used to scratch two parallel lines into the cell layer when the cell density reached 95%. The cells were then cultured under normal conditions after being washed with 1× PBS three times. ImageJ software was used to quantitatively analyze microscopy images taken at 0, 24, and 48 hours to determine how quickly the wounds healed.

Proliferation and colony formation assay (CFA)

When doing a CFA, 1,000 cells per well were plated in 6-well plates. After 14 days in culture, the cells were fixed in formaldehyde for 30 minutes, stained with crystal violet for 15 minutes at RT, and then washed twice with PBS. The number of colonies that could be seen was counted manually, and sample images were taken.

A total of 3,000 cells were plated in five replicates onto 96-well plates; 2 hours before each observation, 10 µL CCK-8 and 90 µL SF media were added, and the absorbance was measured at 450 nm using enzyme-linked immunosorbent assay (ELISA) at 24, 48, and 72 hours.

Xenograft mice model

The Ethics Committee of Affiliated Hospital of Nantong University approved the *in vivo* studies (No. S20200315-009), and was conducted according to animal use and feeding standards established by the Committee of the China Animal Protection Association. A protocol was prepared before the study without registration. Male BALB/c nude mice, aged 6 weeks, were bought from Nantong University's Clinical Animal Research Center. First, a xenograft model was established by implanting 100 µL PBS suspended 1×10^6 cells in both mouse groins. The size of the tumor was assessed weekly using the width^2 × length ×0.52. Four weeks after implantation, the mice were euthanized. At the same time, the tumor tissue was weighed.

Statistical analysis

SPSS and GraphPad Prism 8.0.2 were used for the statistical analyses. Statistical significance was determined by comparing results from at least three separate experiments. The variations between the groups were analyzed using a two-tailed Student's *t*-test. The significance level was set at $P>0.05$.

Results

Upregulated of AIBP in human HCC tissues and its role in prognosis prediction

We examined the TCGA database and investigated the AIBP expression in tissues to learn more about the possible function of AIBP in HCC. AIBP mRNA levels were significantly greater in HCC tissues than in nearby normal tissues (*Figure 1A*,*1B*). After that, we used western blotting to discover that in twenty matched fresh HCC tissues, the AIBP expression level was dramatically upregulated (*Figure 1C*,*1D*). Meanwhile, we used IHC staining to confirm the significant expression of AIBP in 91 paired HCC samples (*Figure 1E*,*1F*). These findings demonstrate that AIBP expression is elevated in human HCC tissues.

We next investigated if there is a link between clinicopathological features and AIBP expression in HCC patients. Patients with high AIBP levels had a poorer OS rate than those with low AIBP levels, as shown by the results of a Kaplan-Meier plotter survival study (https://gdc.cancer.gov/support/gdc-webinars/tcga-resources-available-gdc; $P<0.001$, *Figure 1G*). Furthermore, we discovered that AIBP expression was associated with Barcelona Clinic Liver Cancer (BCLC) stage ($P<0.001$), vascular invasion ($P<0.001$), tumor node metastasis (TNM) stage ($P<0.001$), and tumor size ($P=0.02$) based on our data of 91 HCC patients (*Table 1*). Depending on their baseline characteristics, the 91 patients included in the analysis revealed that AIBP was a strong and independent predictive factor of OS in HCC patients. Altogether, our results suggested that AIBP was overexpressed in HCC tumor tissues and may serve as a poor prognostic indicator.

AIBP overexpression promotes in vitro HCC cell invasion, migration, and proliferation

Transfecting SMMC-7721 cells with an AIBP-overexpressing plasmid or a carrier plasmid allowed us to study the AIBP functions in HCC cells, according to the results of the western blotting (*Figure 2A*). AIBP overexpression markedly enhanced HCC cell proliferation, as measured by the CCK-

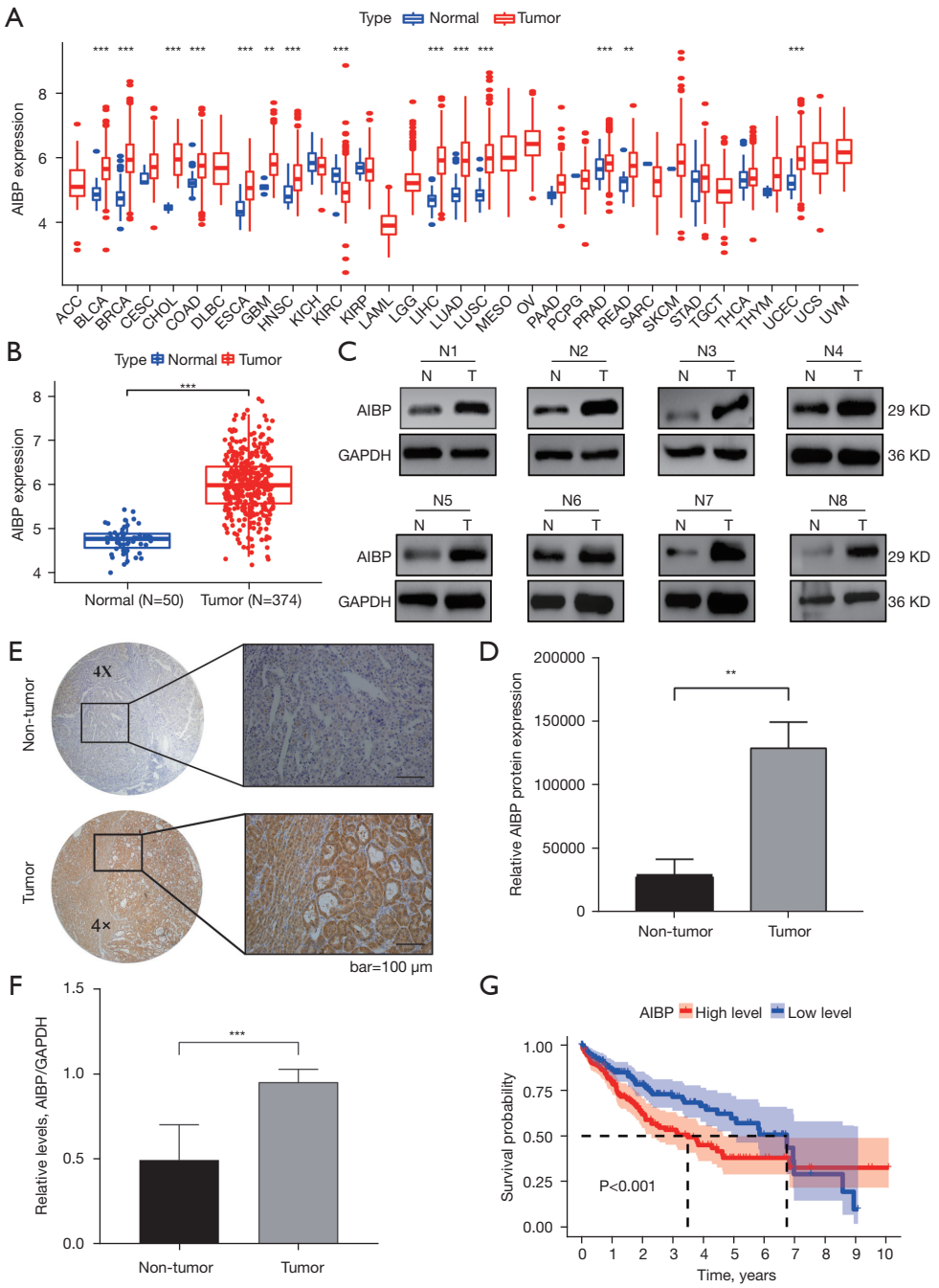

Figure 1 AIBP is overexpressed in HCC and predicts poor prognosis. (A) The AIBP expression level in several cancers. (B) According to the TCGA HCC database, expression of AIBP in the normal liver tissues (n=50) and primary liver tumor tissues (n=374) (P<0.001). (C) The levels of AIBP protein expression, measured by western blotting in tumor and adjacent normal tissues from eight HCC patients. GAPDH served as the internal loading control. ImageJ was used to determine the protein density of AIBP, and the results are shown in the panel. (D) The quantitative protein levels of AIBP in (C). (E) IHC images demonstrating AIBP expression in HCC tumor tissues and matching non-tumor tissues. Analyses were conducted on positive regions' average integrated optical density. (F) The quantitative protein levels of AIBP in (E). (G) Kaplan-Meier analysis of AIBP expression with overall survival in patients with HCC. **, P<0.01; ***, P<0.001. AIBP, apolipoprotein A-I binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; N, normal tissue; T, tumor tissue; HCC, hepatocellular carcinoma; TCGA, The Cancer Genome Atlas; IHC, immunohistochemistry.

Table 1 Associations of AIBP expression with clinicopathological parameters

Variables	Subgroup	AIBP expression			Test	
		Negative (n=58)	Positive (n=33)	Total	χ^2	P
Age	Median	36	19	55	0.178	0.67
	Range	22	14	36		
Sex	Female	11	8	19	0.355	0.55
	Male	47	25	72		
AFP level	<400 mg/L	52	29	81	0.068	0.79
	≥400 mg/L	6	4	10		
HBsAg	Negative	24	13	37	0.034	0.85
	Positive	34	20	54		
Ascites	Yes	44	22	66	0.893	0.34
	No	14	11	25		
Cirrhosis	Yes	33	22	55	0.840	0.35
	No	25	11	36		
Tumor size	>5 cm	16	17	33	5.211	0.02*
	≤5 cm	42	16	58		
Multiple tumors	Yes	17	16	33	3.346	0.06
	No	41	17	58		
Tumor differentiation	Well	50	30	80	0.438	0.50
	Poor	8	3	11		
Vascular invasion	Yes	10	18	28	13.740	<0.001***
	No	48	15	63		
Metastasis	Yes	54	27	81	2.739	0.09
	No	4	6	10		
TNM stage	T1/T2	40	11	51	10.040	<0.001***
	T3/T4	18	22	40		
BCLC stage	A/B	43	13	56	10.727	<0.001***
	C	15	20	35		

*, P<0.05; ***, P<0.001. AIBP, apolipoprotein A-I binding protein; AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen; TNM, tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer.

8 assay (*Figure 2B*). Together with wound-healing assays, Transwell migration and invasion studies revealed that AIBP overexpression increased HCC cell invasion and migration (*Figures 2C,2D*). Additionally, AIBP overexpressed cells exhibited dramatically improved colony-forming abilities (*Figure 2E*). These results demonstrated that AIBP overexpression stimulated the HCC cells *in vitro* migration, invasion, and proliferation.

AIBP knockdown suppresses HCC cell invasion, migration, and proliferation in vitro

In order to confirm the AIBP effects on HCC cells, the CRISPR-Cas9 technology was used to reduce AIBP expression in HCC-LM3 cells (*Figure 3A*). The CCK-8 and CFAs showed that AIBP knockdown dramatically reduced the proliferative abilities of HCC-LM3 cells, in contrast to

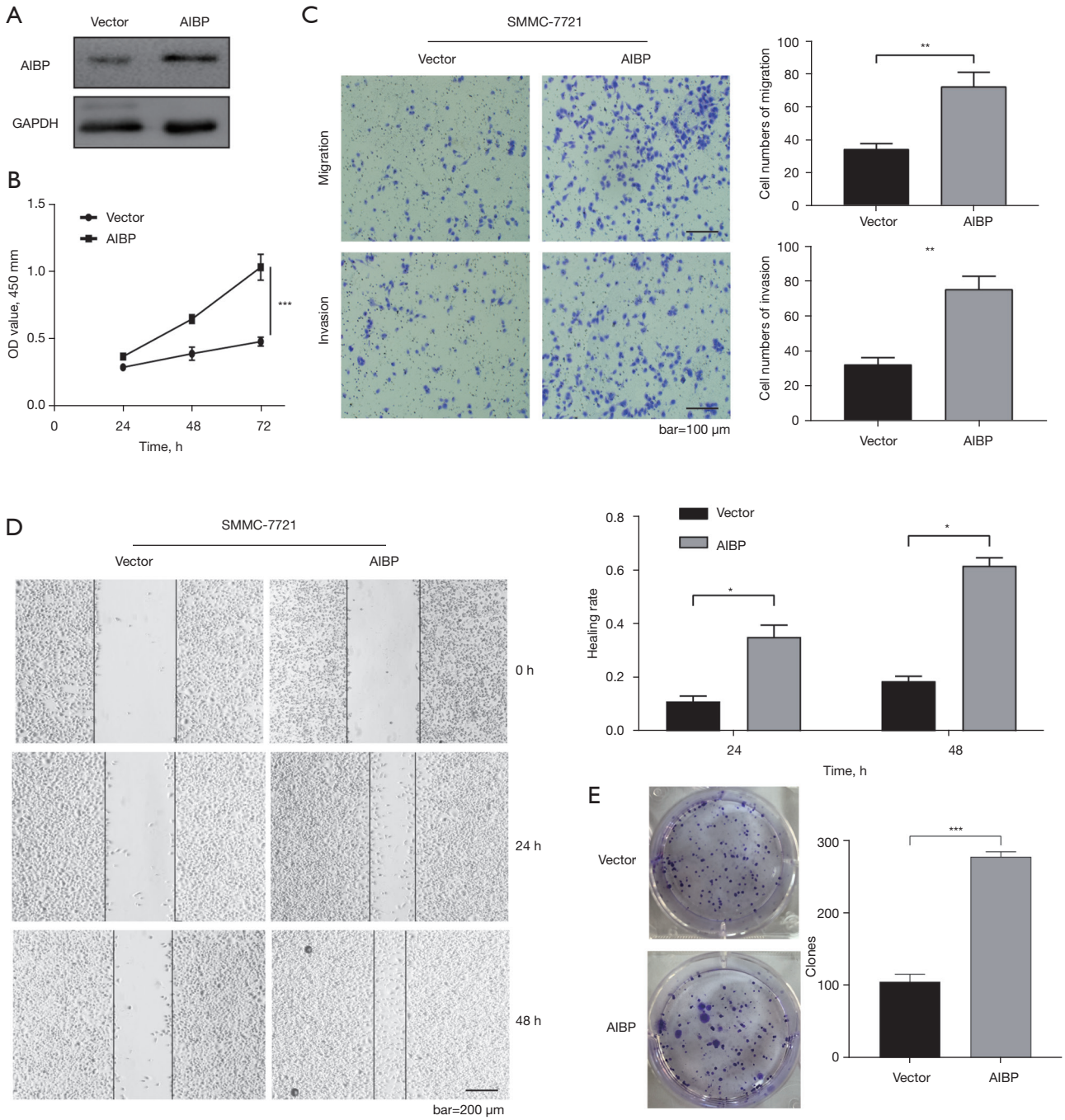

Figure 2 AIBP overexpression promotes HCC cell proliferation, migration, and invasion *in vitro*. (A) Protein expression levels of AIBP were detected by western blotting in SMMC-7721 cells transfected with a blank vector or an AIBP overexpression plasmid. (B) Control and AIBP overexpression HCC cell proliferation was measured using the CCK-8 assay. (C) Transwell assay images showing control cells and AIBP overexpressing SMMC-7721 cells. The migrating cells were stained with crystal violet, under a microscope. Random samples from three fields were counted. (D) Wound healing assay was carried out to examine the migratory capacities of SMMC-7721 cells with or without AIBP overexpression. Images were taken under a microscope and quantified using ImageJ software. (E) Colony formation assays showed colony numbers in HCC cells with or without AIBP overexpression. Colony cells were stained with crystal violet, photographed by camera, and quantified using ImageJ software. *, P<0.05; **, P<0.01; ***, P<0.001. AIBP, apolipoprotein A-I binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; OD, optical density; HCC, hepatocellular carcinoma; CCK-8, cell counting kit-8.

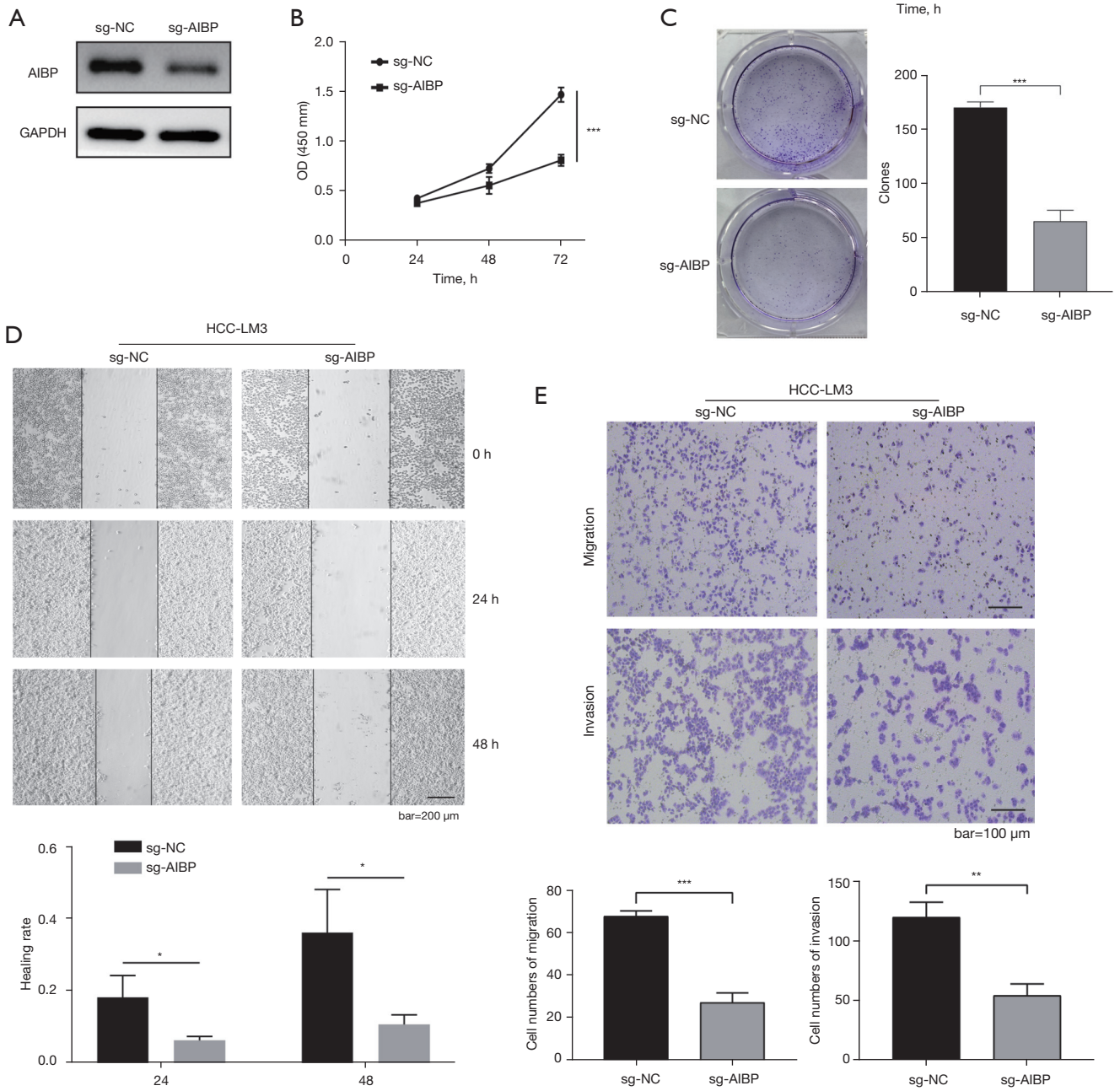

Figure 3 AIBP knockdown inhibited HCC cell proliferation, migration, and invasion *in vitro*. (A) The expression of AIBP was analyzed using western blotting in both AIBP-knockdown and wild-type HCC-LM3 cells. (B) Proliferation of negative control and AIBP knockout HCC-LM3 cells was measured using the CCK-8 assay. (C) The effects of AIBP knockdown on HCC-LM3 cells were investigated using migration and invasion assays. The migrating cells were stained with crystal violet, under a microscope, random samples from three fields were counted. (D) The impact of AIBP knockdown on HCC-LM3 cell migration was examined using wound healing assays. Images were taken under a microscope and quantified using ImageJ software. (E) Numbers of colonies were detected in HCC-LM3 cells with and without AIBP depletion by colony formation assays. Colony cells were stained with crystal violet, photographed by camera, and quantified using ImageJ software. *, P<0.05; **, P<0.01; ***, P<0.001. sg, single-guide RNA; NC, negative control; AIBP, apolipoprotein A-I binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; OD, optical density; HCC, hepatocellular carcinoma; CCK-8, cell counting kit-8.

the prior findings (*Figure 3B*,*3C*). AIBP-sgRNA plasmid-transfected HCC-LM3 cells showed a reduction in invasion and migration ability (*Figure 3D*,*3E*).

AIBP overexpression promotes in vivo HCC cell proliferation

Subcutaneous injections of SMMC-7721 cells, either without or with stably overexpressing AIBP, were then made in nude mice to verify *in vivo* HCC cell proliferation abilities (*Figure 4A*). The AIBP overexpression group had much larger and heavier tumors than the control group (*Figure 4B-4D*). Also, proliferating cell nuclear antigen (PCNA) staining revealed that the tumor cells overexpressing AIBP were more proliferative than the control cells (*Figure 4E*).

AIBP facilitates cell invasion, migration, and proliferation via the ERK1/2-MAPK signaling pathway

The aforementioned findings suggested that AIBP might stimulate cell invasion, migration, and proliferation *in vivo* and *in vitro*; however, the molecular mechanism responsible for these effects is unknown. Consequently, the Kyoto Encyclopedia of Genes and Genomes was utilized to investigate potential AIBP-involved signaling pathways. We discovered that the MAPK signaling pathway was linked to AIBP overexpression (*Figure 5A*). Therefore, we speculate that AIBP controls the biological activity of HCC cells through this pathway. Western blotting findings demonstrated that AIBP could control the gene expression involved in the MAPK signaling pathway (*Figure 5B*). GDC-0994, a selective ERK1/2 inhibitor, was also utilized to show that AIBP is linked to the ERK1/2-MAPK signaling pathway (*Figure 5C*). We discovered that GDC-0994 inhibited ABIP overexpression-induced cell proliferation and migration (*Figure 5D-5G*). These findings showed that AIBP promotes cell invasion, migration, and proliferation in HCC cells through the ERK1/2-MAPK signaling pathway.

Discussion

Regarding cancer-related mortality, HCC ranked third globally (17). Its diagnosis and treatment are still difficult. No substantial decrease in mortality has been seen in patients with HCC despite the availability of several therapeutic options such as surgery, combined radiation therapy, and chemotherapy (2,18-21). As a result, further research into the causes of HCC is required to develop a more comprehensive strategy for treating the disease. This work aimed to explore the potential AIBP functions in HCC cell migration, invasion, and proliferation. According to the findings, AIBP expression was greater in HCC tissues than in surrounding normal tissues. Overexpression of AIBP was also associated with a decreased likelihood of survival for those with HCC. In the current work, we found that overexpression of AIBP promoted ERK phosphorylation, enhancing cell invasion, migration, and proliferation. The results of these studies indicated that AIBP might act as a novel predictive biomarker in HCC and as a novel potential therapeutic target, both of which are critical for improving the HCC patients' prognosis.

Previous research indicated that AIBP promoted macrophages and endothelial cells' cholesterol efflux, therefore controlling lipid metabolism (8,9,12), and was the primary component of high-density lipoprotein (HDL) (22). AIBP's ability to regulate lipid transport by promoting the creation of cell membrane lipid rafts also influences the development of atherogenesis (7,9,10,23). AIBP may influence the inflammatory response because of its ability to control lipid transport (10,11,23,24). In contrast, there is a lack of research comparing AIBP to other tumors. According to Mao *et al.*, AIBP suppresses angiogenesis by controlling Notch signaling (12,22,24,25). The expression of AIBP was drastically reduced in colorectal cancer.

On the other hand, AIBP and APOA-I promote cholesterol efflux, which has a significant impact on the anticancer effect (13). Rather, the present research shows that AIBP is often overexpressed in individuals with liver cancer and that HCC patients have a shorter OS. It is unclear, however, how AIBP specifically supports the malignant phenotypic development of HCC. In the past, the HUGO Gene Nomenclature Committee renamed AIBP to NAXE. AIBP has been credited with transforming R-NADHX into the physiologically active S-NADHX (7,22). Increasing cellular NADH dehydrogenases may result from a splice variant that is mitochondria-resident and functions to reduce cyclic NADHX. The biosynthesis of proteins, nucleic acids, and fatty acids requires adenosine triphosphate (ATP), which is produced by the oxidative respiratory chain and requires NADH dehydrogenases. The findings of this research add to the evidence that AIBP is a driving factor in the progression of liver cancer.

In addition to ERK1/2-MAPK signaling pathway, there are also classical signaling pathways including

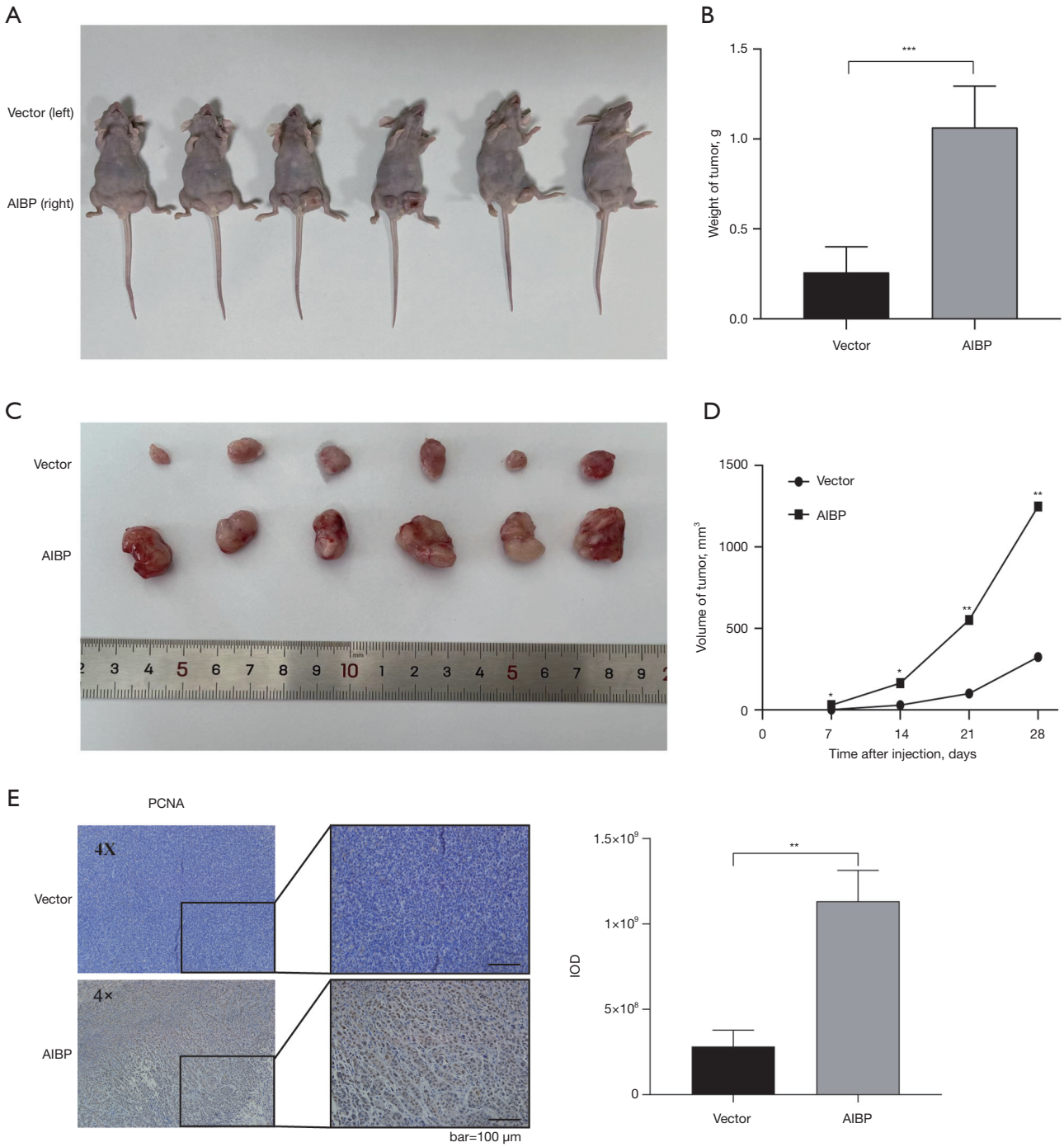

Figure 4 *In vivo* experiment confirmed that overexpression of AIBP enhances tumor growth. (A) Male nude mice were injected subcutaneously with SMMC-7721 cells transfected with AIBP overexpression plasmid or control. Photographs taken 28 days after cell injection showing representative nude mice. (B) Tumor weight, determined and compared between xenografts from each group. (C) Four weeks later, the xenograft tumors' representative images were obtained after euthanizing nude mice. (D) The tumor volumes were measured every week after the injection. (E) Images of xenograft tumors stained with hematoxylin and eosin to show their representative appearance. *, P<0.05; **, P<0.01; ***, P<0.001. AIBP, apolipoprotein A-I binding protein; PCNA, proliferating cell nuclear antigen; IOD, integrated optical density.

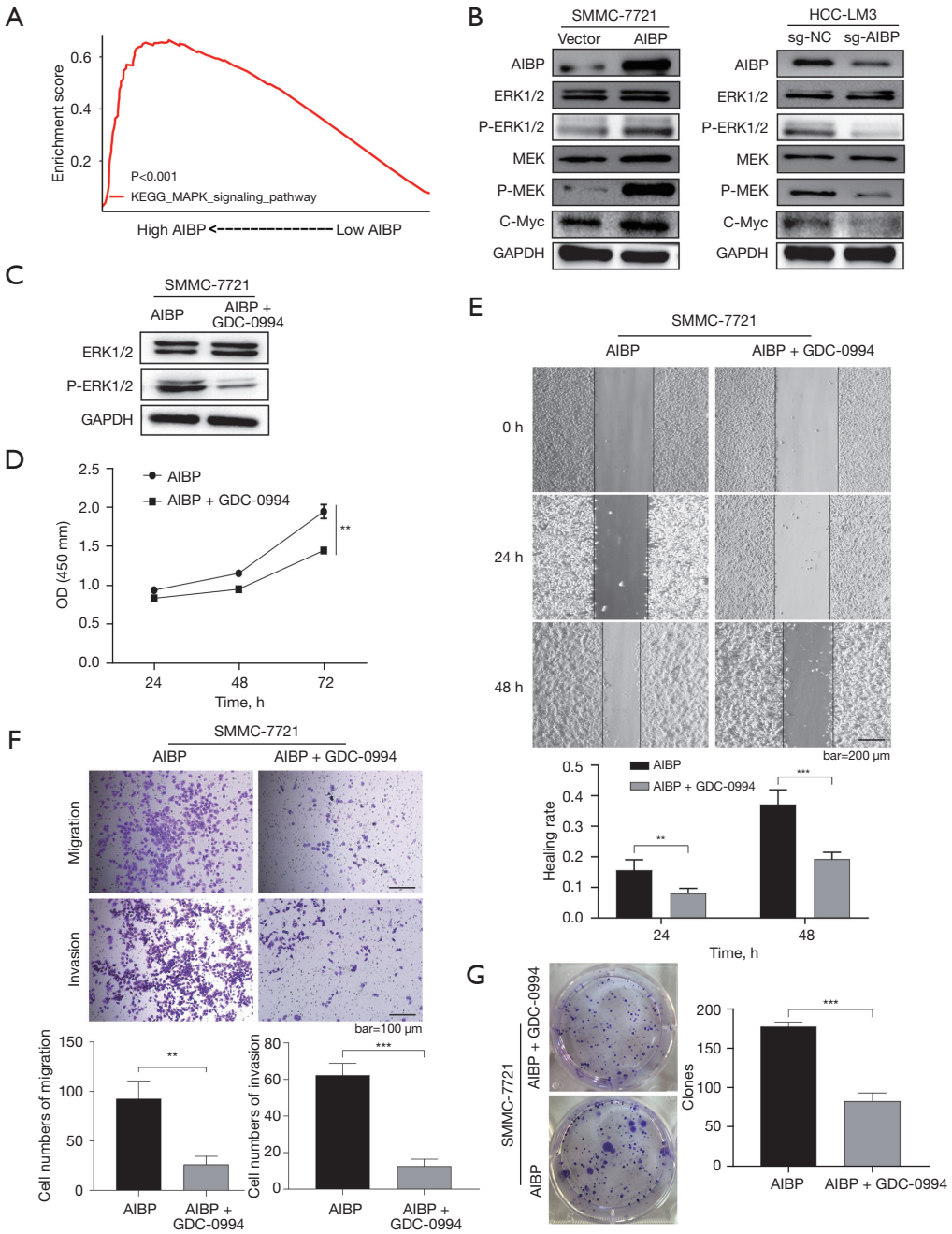

Figure 5 Therapeutic effects of ERK1/2 inhibitor GDC-0994 on the malignant behavior of HCC cells. (A) High AIBP expression was associated with the MAPK signaling pathway, satisfying the threshold of P<0.001. (B) Western blotting analysis of MAPK-related genes in HCC-LM3 cells with or without AIBP knockdown and SMMC-7721 cells with or without AIBP overexpression. (C) The ERK and P-ERK expression levels in the indicated SMMC-7721 cells, treated with GDC-0994 (the ERK1/2 inhibitor, 2 μM, 48 hours). (D) CCK8 assays showed that AIBP-mediated cell growth could be rescued in SMMC-7721 cells treated with GDC-0994. (E,F) The migration and invasion capacity was rescued in cells treated with GDC-0994. The migrating cells were stained with crystal violet. Images of two experiments were taken under a microscope and quantified using ImageJ software. (G) The growth ability of SMMC-7721 cells could be rescued by GDC-0994. Colony cells were stained with crystal violet, photographed by camera, and quantified using ImageJ software. **, P<0.01; ***, P<0.001. KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinase; AIBP, apolipoprotein A-I binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; OD, optical density; HCC, hepatocellular carcinoma.

mTOR, TGF-β, Wnt/β-catenin and so on. Firstly, phosphatidylinositol 3 kinase (PI3K)-protein kinase B (PKB/AKT)-mechanistic target of rapamycin complex 1 (mTORC1) is a classical signaling pathway that regulates cell metabolism and growth. The PI3K-AKT-mTOR signaling pathway affects many cellular functions and plays an important role in a variety of cellular processes such as proliferation, angiogenesis, apoptosis, and metastasis. In the present clinical trials, AKT inhibitors Palomid 529 and perifosine can significantly enhance the sensitivity of tumor cells to radiotherapy. Secondly, the NOTCH pathway can bind to the NOTCH receptor on the membrane during signal transduction, and then undergo a continuous proteolytic cleavage cascade to release the NOTCH intracellular domain (NICD). NICD will be transferred to the nucleus and bind to transcription factors to induce the expression of NOTCH target genes. It regulates a variety of cell biological processes including proliferation, stem cell maintenance, differentiation and death. Thirdly, Wnt is a secreted protein that undergoes translation and modification—lipid modification and glycosylation to activate Wnt/β-catenin pathway. Wnt/β-catenin pathway also plays an important role in angiogenesis, metastasis, chemoresistance and tumor metabolism. Previous studies have shown that β-catenin can cooperate with other proto-oncogenes such as c-Met, K-RasV12, activated AKT, LKB1 and Nrf2 to lead to HCC development.

Multiple mechanisms contribute to the unique expression of the ERK1/2-MAPK signaling pathway in HCC, making it a prime therapeutic target in recent years (26). MAPK pathways are often seen as cascades of MAPK, the middle kinase (MAPKK), and the upstream kinase (MAPKKK). MAPK, in response to P38, JNK, and mitogens, is categorized as an ERK pathway (14,27) based on various input signals. Patients with HCC who have an active MAPK pathway tend to have a bad prognosis. ERK/MAPK inhibitors are employed extensively in phase 1 and 2 clinical cancer treatments (15,16). Examples of such inhibitors are GDC-0994 and PD329501. Our results showed that AIBP overexpression led to an aggressive phenotype in HCC cells via promoting ERK phosphorylation. However, ERK inhibitor therapy restored the HCC cells' malignant phenotype. These findings indicated that downregulating AIBP expression or intervening in the AIBP-MAPK axis may reduce tumor invasion, migration, and proliferation.

In the current treatment of patients with HCC: radical surgery, TACE as well as ablation has long been the cornerstone of therapy for patients with early-stage HCC (stage I–II) to reduce the recurrence rate and improve the OS prognosis of patients, Whether the early-stage HCC patients with high AIBP expression are suitable to targeted inhibition of AIBP warrants further study. However, AIBP is expected to be used in early detection and diagnosis of HCC patients. For the treatment of advanced HCC (stage III–IV), since most patients lose the opportunity for surgery due to distant metastasis or diffuse tumor infiltration, the more favorable options are chemotherapy combined with tumor targeted therapy, and palliative surgery combined with radiotherapy and chemotherapy if conditions permit. Recently, in phase III clinical trial, single-agent tislelizumab, camrelizumab and durvalumab was shown to offer improved OS. So, we look forward to combining with targeted inhibition of AIBP can be reserved for patients with advanced liver cancer and high AIBP expression.

The research presented here is not completely free of limitations. Firstly, it is still unclear how AIBP controls P-ERK1/2 expression and which genes are influenced by the ERK1/2-MAPK signaling pathway. Secondly, the function of GDC-0994 in xenograft tumor models has not been established. However, this investigation showed that AIBP plays a significant part in the growth and prognosis of HCC.

Conclusions

In conclusion, we revealed that AIBP was elevated in HCC tissues and controlled the invasion migration and proliferation of HCC cells via the ERK1/2-MAPK signaling pathway. Therefore, it is anticipated that AIBP would be a useful clinical prognostic predictor of HCC and that targeting AIBP would expand the therapeutic options for HCC.

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Footnote

Reporting Checklist: The authors have completed the MDAR and ARRIVE reporting checklists. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2101/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2101/coif). The authors have no conflicts of interest to declare.

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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of the Affiliated Hospital of Nantong University (No. 2020-L093) and informed consent was taken from all the patients. The Ethics Committee of Affiliated Hospital of Nantong University approved the *in vivo* studies (No. S20200315-009), which were conducted according to animal use and feeding standards established by the Committee of the China Animal Protection Association.

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