Expression and Functional Identification of INPP4B in Gallbladder Cancer Patients and Gallbladder Cancer Cells

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Abstract

Purpose: Inositol polyphosphate 4-phosphatase type II (INPP4B) is a negative regulator of PI3K-Akt signaling pathway and plays a contradictory role in different types of cancers. However, its biological role in human gallbladder cancer (GBC) remain unclear. Here we aimed to investigate the expression, clinical significance and biological function of INPP4B in GBC clinical dates and GBC cell lines.

Methods: The INPP4B protein expression levels in gallbladder cancer tissues and normal gallbladder tissues were detected by immunohistochemistry, and the clinical significance of INPP4B was analyzed. Knockdown and overexpression of INPP4B on GBC-SD and SGC-996 cells were used to identify INPP4B function in vitro, using cell proliferation assay, clonogenic assay, apoptosis detection, cratch wound-healing assay and transwell assay.

Results: INPP4B was up-regulated in human GBC tissues compared with normal gallbladder tissues, and was related to histopathological differentiation. Here, we observed that INPP4B was highly expressed in high-moderate differentiated compared to low-undifferentiated. Additionally, we found that INPP4B expression was not associated with overall survival in GBC patients and was not an independent prognostic factor. Furthermore, when we stratified the relationship between INPP4B expression and prognosis of GBC from histopathological differentiation, we found that INPP4B played a contradictory role in GBC progression at different degrees of differentiation. In addition, INPP4B knockdown inhibited tumorigenicity in vitro, and INPP4B overexpression induced tumorigenicity in vitro, which may play a role as an oncoprotein.

Conclusions: These findings implicated that INPP4B may play a dual role in the prognosis of GBC with different degrees of differentiation, and might act as an oncogene in gallbladder cancer cells.

Introduction

Gallbladder cancer (GBC) is the most common malignant tumor of the biliary system, which has the characteristics of gender, geographical location and race difference [1, 2]. In recent years, although the diagnosis and treatment of GBC has been greatly improved, the prognosis of GBC patients has not been significantly improved, with a five-year survival rate of less than 5% and an average survival of 6 months [3]. Therefore, elucidating the molecular mechanisms of GBC progression is critical to facilitate the development of new diagnostic and therapeutic strategies.

PI3K-Akt signaling pathway plays an important role in tumor cell proliferation, differentiation, angiogenesis, invasion and metastasis [4, 5]. Hyper-activation of PI3K-Akt signaling has been shown to be the driving factor of tumor initiation and progression [6, 7]. PI3K-Akt signaling pathway has been considered as a therapeutic target for a variety of malignant tumors, and many clinical trials have been conducted to investigate the therapeutic effects of PI3K-Akt pathway inhibitors on human cancer[8]. Recently, Inositol polyphosphate-4-phosphatase type II (INPP4B), as a phosphoinositide phosphatase, has been proved to be a negative regulator of the PI3K-Akt signaling and plays a role of tumor suppressor

gene (TSG) [9–12]. Low expression of INPP4B in several type of cancers is associated with poor clinical outcomes [13–15]. Knockdown of INPP4B could enhance breast cancer, melanoma and prostate cancer cell lines proliferation and migration, suggesting its TSG role in these cancer cells [11, 15, 16]. However, recently, some studies have reported that INPP4B is highly expressed in colon cancer, acute myeloid leukemia and melanoma, which plays a role of oncogene and has a positive correlation with poor clinical outcomes [17–19]. Even within the same tumor, different researchers have come to different conclusions about the role of INPP4B in oncogene and TSG[16, 18–20]. Above the previous studious indicate that the expression and functional role of INPP4B remains controversial in cancers, which seems to be related to tumor types. However, to date, the expression level, clinical prognostic value and biological functional of INPP4B in GBC have not been studied. To help comprehensive understand the potential value of INPP4B in GBC, it will be very useful to detect its expression level, evaluate its clinical prognostic significance and study its cell function in vitro.

In the present study, we detected the expression of INPP4B in GBC tissues and non-tumorous tissues by immunohistochemistry. We found that the expression of INPP4B in GBC tissues was significantly higher than that in non-tumorous tissues. The INPP4B protein expression was decreased in low-undifferentiated of GBC tissues but increased in high-moderate differentiated tissue. In addition, high INPP4B expression was associated with a favourable prognosis in patients high-moderate differentiated, while poor prognosis in patients with low-undifferentiated. We further explored the biological function of INPP4B in GBC cells in vitro. We found that INPP4B knockdown inhibited tumorigenicity, while INPP4B overexpression induced tumorigenicity in vitro. These results suggested that INPP4B plays a critical role in GBC and may provide a potential target for the treatment of GBC.

Materials And Methods

Patients and tissue specimens

In the present study, a total of 127 GBC patient tissues and 47 non-tumorous tissues were collected from the Armed Police Corps Hospital of Anhui (Hefei, China) using for analysising INPP4B expression by immunohistochemistry staining. Detailed clinicopathological parameters were described in Table 1. Patients did not receive any anticancer treatment prior to surgery.

Table 1
Relationship between INPP4B expression and clinicopathological variables (n = 127).

Clinicopathological variables	Total	INPP4B expression		*p* value
		positive (58)	negative (69)	
Gender				0.862
Male	36	16	20	
Female	91	42	49	
Age (y)				0.984
< 68	59	27	32	
≥ 68	68	31	37	
Tumor size (cm)				0.116
< 2	67	35	32	
≥ 2	60	23	37	
Differentiation				**0.026**
High/moderate	81	43	38	
Low/undifferentiated	46	15	31	
Depth of invasion				0.754
T1/T2	39	17	22	
T3/T4	88	41	47	
Lymph node metastasis				0.405
Yes	51	21	30	
No	76	37	39	
TNM				0.735
I/II	83	37	46	
III/IV	44	21	23	
Gallstones				0.321
Yes	84	41	43	
No	43	17	26	

Note: TNM, tumor-node-metastasis; AFP, alpha fetoprotein; CEA, carcino-embryonic antigen; CA199, carbohydrate antigen 199; $p < 0.05$ was defined statistically significant.

Clinicopathological variables	Total	INPP4B expression		*p* value
		positive (58)	negative (69)	
AFP (ug/L)				0.999
< 20	122	56	66	
≥ 20	5	2	3	
CEA (ng/ML)				0.771
< 5	100	45	55	
≥ 5	27	13	14	
CA199 (U/ML)				0.735
< 37	83	37	46	
≥ 37	44	21	23	

Note: TNM, tumor-node-metastasis; AFP, alpha fetoprotein; CEA, carcino-embryonic antigen; CA199, carbohydrate antigen 199; $p < 0.05$ was defined statistically significant.

RNA preparation, reverse transcription and real-time qPCR

Total RNA was extracted from GBC cells using TRIzol Reagent (Invitrogen). Reverse transcription (RT) was performed using ReverTra Ace qPCR RT Master Mix (Toyobo) to obtain first-strand cDNA following the manufacturer's instructions. qPCR was then performed to amplify the cDNAs with the SYBR-Green mix (Toyobo, Japan) on an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, USA). The PCR primers were used for amplification as followings: INPP4B, 5'-ACGCAGGAAAGTCAGGCTAA-3' (forward), 5'-TGCCAGGTAACACC ATTTCTT-3' (reverse); GAPDH was used as endogenous control, 5'-ATCAAGAAGGTGGTGAAGCAGG-3' (forward), 5'-CGTCAAAGGTGGAGGAGTGG- 3' (reverse). INPP4B relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. Each sample was done in triplicate.

Immunohistochemistry (IHC)

Immunohistochemistry (IHC) was used to detect the expression of INPP4B in 4-μm formalin-fixed paraffin-embedded human GBC tissues and non-tumorous tissues. The IHC process and staining evaluation were performed as previously reported [21, 22]. The tissue sections were incubated with INPP4B primary antibody (Abcam, ab81269, 1:50) and negative controls with normal rabbit IgG (Beyotime Institute of Biotechnology, A7016). The immunohistochemical staining results were evaluated by two independent pathologists who did not know the patient's clinical information. The immunoreactivity score (IRS) were determined by "staining intensity × staining percentage". The specimen INPP4B expression levels were defined as negative ($INPP4B^-$, IRS < 4) and positive ($INPP4B^+$, IRS ≥ 4).

Cell culture and lentivirus infection

GBC-SD and SGC996 GBC cell lines were obtained from Genechem (Shanghai, China) and cultured in RPMI1640 medium supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml) in a 5% CO2 humidified incubator at 37 °C. In order to establish GBC-SD cell and SGC996 cell with stable overexpression of INPP4B, the two cell lines were infected with INPP4B overexpression lentiviral (GV492-INPP4B, OEINPP4B) and control lentiviral (GV492, OECtrl, purchased from Genechem, Shanghai, China) respectively. In addition, for establishing stable INPP4B knockdown GBC-SD cell and SGC996 cell, the two cell lines were infected with shRNA INPP4B lentiviral (GV248-shINPP4B) and control lentiviral (GV248-shCtrl, purchased from Genechem, Shanghai, China). The target sequence of shINPP4B was as 5'-CCATCTGAGTATCCCATCTAT-3'. Overexpression and knockdown efficiency of target genes were detected by q-PCR.

Cell proliferation assay and clonogenic assay

Cell proliferation assay and clonogenic assay were employed to measure the role of INPP4B on GBC cell viability and proliferation capacity. The detailed cell proliferation assay processes was performed as described previously [23, 24]. Briefly, different lentivirus (OEINPP4B, OECtrl; shINPP4B, shCtrl) infection GBC cell lines (GBC-SD and SGC-996) were seeded into 96-well plates (about 2000 cells/well) in sextuple, and cell viability was determined by MTT (Genview, JT343) at each 24 h interval following the manufacturer's instructions. The number of viable cells was determined by measuring absorbance at OD490 nm using a Universal Microplate Reader (BioTek Instruments, Inc.). Each experiment was performed in three times. The detailed colony formation assay processes was performed as described previously [25, 26]. In short, different lentivirus infection GBC cell lines were seeded into 6-well plates (about 800 cells/well) and the medium was replaced every three days. After two weeks, the colonies were fixed by 4% polyoxymethylene, stained with Giemsa, and counted by inverted microscope. Each assay was repeated in three times.

Apoptosis assay

The detailed process of apoptosis analysis has been described in previous studies [27]. In brief, according to the manufacturer's instructions, the apoptosis of different lentivirus infection GBC cell lines was analyzed using the Annexin V-APC apoptosis detection kit (eBioscience, 88-8007). The apoptosis rate was analyzed by flow cytometry (FACSCalibur; BD Biosciences).

Scratch wound-healing assay and transwell assay

Cell migration and invasion ability were assessed by scratch wound-healing assay and transwell assay and performed as previously described [28, 29]. In short, for scratch wound-healing assay, different lentivirus infection GBC cell lines were seeded in 96-well plates (about 5 × 105 cell/well). The next day, after the cells attached to the bottom, linear wounds were created by a scratch tester at the center of cell monolayer. In different time points, the wounds images were taken under Celigo instrument, and the migration area of different lentivirus infection GBC cell lines were analyzed by this software. Briefly, For transwell assay, different lentivirus infection GBC cell lines in 0.2 mL serum-free medium (about 1×10^5 cells/well) were added to the upper chamber of 24-well plates with 8-μm pores (Corning, 3422), and

0.6 mL DMEM (Corning, 10-013-CVR) containing 30% FBS (Ausbian, A11-102) was added to the lower chamber. The plate was incubated for 24 h, non-invading cells were removed with cotton swabs, then the remaining cells were fixed with 4% paraformaldehyde for 30 minutes, stained with 0.5% crystal violet, and counted under a microscope at 200 × magnification.

Statistical analysis

SPSS 16.0 software (SPSS, Inc.) and GraphPad Prism 5 were used for statistical analysis. Pearson $\chi 2$ test was used to assess the relations between INPP4B expression and clinicopathologic characteristics. Data are expressed as mean ± SEM. Student's t test was used to evaluate the statistical significance of two groups. Survival analysis was performed by Kaplan-Meier method and Cox proportional hazards regression model. $p < 0.05$ was considered statistically significant. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and #$p < 0.0001$, ns, not significant).

Results

INPP4B is increased in GBC tissues, and serves as a prognostic factor

INPP4B protein expression in tumors are still controversial and has not been studied in GBC tissues. We analyzed the protein expression of INPP4B in 127 GBC tissue samples and 47 non-tumorous tissues by immunohistochemistry. As a result, INPP4B staining was mainly located in the cell cytoplasm, and the positive rate was significantly higher in GBC tissues than in non-tumorous tissues ($p = 0.008$, data not shown). Representative images are shown in Fig. 1A. The results revealed that INPP4B was highly expressed in GBC and was mainly located in the cytoplasm.

In addition, when we further elucidated the correlation between INPP4B expression and GBC clinicopathological parameters, we found that INPP4B expression was closely associated with histopathological differentiation, and the immunoreactivity score (IRS) of high-moderate differentiated tissues was markedly higher than that of low-undifferentiated tissues (Table 1 and Fig. 1B). These data suggested that INPP4B may play an important role in the progression of GBC.

Based on the above findings, we speculated that INPP4B might be related to GBC prognosis. However, Kaplan-Meier analysis and log-rank test revealed that INPP4B expression was not associated with overall survival (OS) in GBC patients. Multivariate analysis showed that INPP4B was not an independent predictor factor (Fig. 1C and Table 2). However, when we stratified the relationship between INPP4B expression and prognosis of GBC patients in terms of histopathological differentiation, we got some interesting findings. In high-moderate differentiation group, we found that GBC patients with $INPP4B^{+}$ showed better prognosis than that of patients with $INPP4B^{-}$ (Fig. 1D); while in low-undifferentiated group, we found that GBC patients with $INPP4B^{+}$ showed worse prognosis than that of patients with $INPP4B^{-}$

(Fig. 1E). These results indicate that INPP4B plays a contradictory predictor role in GBC progression with different histopathological differentiation.

Table 2
Univariate and multivariate analysis of the correlation between clinicopathological parameters and prognostic significance of GBC patients (n = 127).

Variables	Univariate analysis	*p* value	Multivariate analysis	*p* value
	HR(95%CI)		HR(95%CI)	
Gender (male vs. female)	1.316(0.860–2.013)	0.205		NA
Age (y) (< 68 vs. ≥68)	1.265(0.871–1.837)	0.217		NA
Tumor diameter (cm) (< 2 vs. ≥2)	1.424(0.983–2.063)	0.061		NA
Differentiation (low/undifferentiated vs. high/moderate)	0.566(0.387–0.827)	**0.003**	0.667(0.428–1.041)	0.075
Depth of invision (T1/TI vs. T3/T4)	1.683(1.101–2.572)	**0.016**	1.359(0.850–2.172)	0.200
Lymph node metastasis (no vs. yes)	1.829(1.249–2.676)	**0.002**	2.537(1.123–5.730)	**0.025**
TNM stages (I/II vs. III/IV)	1.653(1.123–2.433)	**0.011**	0.616(0.263–1.441)	0.264
Gallstones	1.026(0.698–1.508)	0.896		NA
AFP (< 20 vs. ≥20)	2.107(0.853–5.207)	0.106		NA
CEA (< 5 vs. ≥5)	1.236(0.790–1.932)	0.353		NA
CA199 (< 37 vs. ≥37)	1.198(0.816–1.758)	0.356		NA
INPP4B expression (positive vs. negative)	0.681(0.467–0.993)	0.086		NA

Note: Variables with *p* values more than 0.05 in the univariate models were not adapted (NA) in the multivariate analysis. $p < 0.05$ was defined statistically significant and was given in bold. CI: confidence interval. HR: Hazard ratio.

INPP4B regulates GBC cells proliferation in vitro

Given the high expression of INPP4B in GBC tissue and its correlation with the clinical prognosis of GBC patients, we inferred that INPP4B might regulate GBC cells growth. To confirm our hypothesis, we chose

GBC-SD and SGC996 cells for in vitro assay. Stable overexpression, knockdown and control of INPP4B in GBC-SD and SGC996 cells were established by different lentiviruses infection, respectively. Subsequently, we examined the effects of INPP4B on the growth and proliferation of GBC-SD and SGC996 cells using MTT and colonigenic assays. As shown in Fig. 2A, 2B and 2C, blocking the expression of endogenous INPP4B markedly inhibited the proliferation and colony formation of GBC-SD and SGC996 cells, whereas overexpression of INPP4B weakly promoted the proliferation and colony formation of these cells. In summary, our findings suggest that blocking the expression of endogenous INPP4B has a greater effect on the proliferation of GBC cells than overexpression.

INPP4B regulates GBC cells apoptosis in vitro

Previous studies suggested that INPP4B is involved in the apoptosis of tumor cells [15, 30]. The apoptosis levels of different lentivirus infection GBC-SD and SGC-996 cells were analysed by flow cytometry. Our results showed that both INPP4B overexpression and knockdown significantly increased the apoptosis rate of GBC-SD cell (Fig. 3A and 3B). However, in SGC-996 cell, INPP4B overexpression markedly reduced the apoptosis rate, and INPP4B knockdown significantly increased the apoptosis rate (Fig. 3C and 3D). Our results suggest that INPP4B regulates apoptosis in GBC cells, but the regulatory effects are different in different cell lines.

INPP4B promotes GBC cells migration and invasion in vitro

Scratch wound-healing assay and transwell assay were used to further investigate the effect of INPP4B on the migration and invasion ability of GBC cells. Scratch wound-healing assay confirmed that INPP4B overexpression markedly increased GBC-SD and SGC996 cells migration ability, while INPP4B knockdown had an opposite effect on the migration ability of these cells (Fig. 4A and 4B). Consistent with this results, transwell assays demonstrated that INPP4B overexpression notably increased GBC-SD and SGC996 cells invasion ability, while INPP4B knockdown had an opposite effect on the invasion ability of these cells (Fig. 5A and 5B). Taken together, These dates suggest that INPP4B promotes GBC cells metastasis and invasion ability in vitro.

Discussion

INPP4B, as a phosphoinositide phosphatase, has been reported to be low in expression, and plays a tumor suppressive role in human prostate cancer, breast cancer and ovarian cancers by negatively regulating PI3K-Akt signaling [10, 11, 13]. More recently, some unexpected findings indicate that INPP4B is significantly upregulated, which plays an oncogene role in AML, melanoma and colon cancer by activating SGK3, and is associated with the patients prognosis [17–19,31,]. To date, previous studies on the role of INPP4B in tumor suppressor gene and oncogene has been controversial, even in the same tumor. Guo et al. reported that INPP4B is frequently upregulated in human colon cancer tissues and cell lines and promotes tumorigenesis [18]. Sung et al. and Ma et al. reported that INPP4B is down-regulated

and have a tumor suppressor role in colorectal tumors [20]. However, the expression and clinical significance of INPP4B in human GBC and its biological function on GBC cells have not been studied.

In our present study, we first revealed that INPP4B is highly expressed in GBC tissues compared with non-tumor tissues and is associated with GBC patient prognosis in different histopathological differentiation group. When we investigated the correlation between INPP4B expression and clinicopathological parameters and clinical prognosis, we got some interesting findings. Table 1 and Fig. 1B revealed that INPP4B protein expression was associated with histopathological differentiation, and INPP4B expression in high-moderate differentiation tissues was higher than that in low-undifferentiated tissues. When we did not stratify the relationship between INPP4B expression and GBC prognosis, survival analysis and Cox regression analysis showed that INPP4B was not associated with overall survival in GBC patients and was not an independent prognostic factor (shown in Table 2 and Fig. 1C). When we stratified the relationship between INPP4B expression and GBC prognosis according to differentiation grade, and we further found that GBC patients with high INPP4B expression had a better prognosis in high-moderate differentiation group, but a worse prognosis in low-undifferentiated group, which played a contradictory role. These results exhibited the dual role of INPP4B in different histopathological differentiation of GBC prognosis. This findings seem to be consistent with the report of Yang et al. Yang et al. study revealed that INPP4B plays a tumor suppressor role in non-metastatic colorectal cancer stem-like cells (CR-CSLCs) and plays an oncogene role in metastatic CR-CSLCs according to different mechanisms, although INPP4B is weakly expressed in non-metastatic CR-CSLCs and highly expressed in metastatic CR-CSLCs [32]. These results suggest that even for the same tumor, INPP4B tends to promote tumor progression in more malignant tissues and cells, while inhibits tumor progression in relatively less malignant tissues and cells, regardless of its expression. Indeed, this phenomenon was also observed in another type of tumor we studied (date not shown). This may be one of the reasons that different researchers have come to different conclusions about tumor progression in INPP4B. Next, we further studied the function of INPP4B in GBC cells.

Previous studies on the role of INPP4B in tumor cells have shown that INPP4B has the function of tumor suppressor gene or oncogene in different tumor cells. INPP4B acts as a tumor suppressor gene in breast cancer cells and prostate cancer cells. Its knockdown can promote the proliferation and motility of breast cancer cells [10], and its overexpression can inhibit the migration, invasion and angiogenesis of prostate cancer cells [33]. While, INPP4B acts as an oncogene in AML cells and colon cancer cells. INPP4B promotes the growth of AML cells [17], and INPP4B silencing inhibits colon cancer cell proliferation and retards colon cancer xenograft growth [18]. In our study, we explored the function of INPP4B in two GBC cell lines (GBC-SD, SGC996) by proliferation, colony formation, apoptosis, migration and invasion. Our result shown that knockdown of INPP4B in GBC-SD cell and SGC996 cell significantly suppressed proliferation, colony formation, migration and invasion ability; by contrast, overexpression of INPP4B in these two GBC cells notably increased migration and invasion ability, but weakly promoted cell proliferation and colony formation at different level. These findings suggest that the tumor suppressor gene INPP4B plays a potential carcinogenic role in GBC. When we analyzed the effect of INPP4B on the apoptosis of these two GBC cell lines, we got some interesting phenomena. Overexpression of INPP4B in

SGC996 cell significantly reduced its apoptosis rate, while knockdown of INPP4B notably increased the apoptosis rate. But for GBC-SD cell, it was confusing that both overexpression and knockdown of INPP4B all increased the apoptosis rate, which may be due to the multiple complex carcinogenic signaling pathways of INPP4B on different cells, which needs further study. In addition, this also reflects the heterogeneity of the two cell lines used in our study, so more biological function tests should be performed to validate the results using more cell lines and provide evidence for targeted therapy in different patients. In conclusion, our study suggest that INPP4B could be a potential marker for diagnosis and drug targeted therapy of GBC.

In summary, we first identified that INPP4B is upregulated in china GBC tissues by immunohistochemistry, and it plays a contradictory prognostic role in the progression of GBC patients with different histopathological differentiation. We found that GBC patients with high expression of INPP4B have a better prognosis in high-moderate differentiation patients but a worse prognosis in low-undifferentiated patients. In vitro cell experiment further confirmed that INPP4B may play an oncogene role in GBC cells. We found that INPP4B knockdown could inhibit proliferation and colony formation, decrease cell migration and invasion capability, and increase in the apoptosis rate of GBC-SD and SGC996 cells; while INPP4B overexpression has an opposite effect on the biological behaviors of GBC-SD and SGC996 cells, except that it also increases the apoptosis rate of GBC-SD cells. These findings suggest that INPP4B may play an important role in the pathogenesis and development of GBC. However, this study has some limitations that cannot be ignored. First, The number of enrolled patients in this study was relatively small, and more cases could more accurately assess INPP4B expression and its relationship with prognosis in GBC. Second, the reason why both INPP4B overexpression and INPP4B knockdown all can increase the apoptosis rate of GBC-SD cells. Finally, this study only proves that INPP4B plays an important role in the development of GBC from clinical significance to cell function studies. We need to further explore the more accurate mechanism regulation of INPP4B in the progression of GBC, which can help us provide new methods for the clinical treatment of GBC. This is what we plan to study in future work.

Conclusions

In conclusion, our study is the first to assess the clinical significant and function of INPP4B in GBC. Our results demonstrat that INPP4B is highly expressed in GBC tissues and is significantly associated with poor overall survival in low-undifferentiated GBC patients, and with better overall survival in high-moderate differentiated GBC patients. In addition, we find that INPP4B can promote wound healing, migration, invasion and proliferation in vitro, which suggests that INPP4B may be a potential therapeutic target for GBC patients. Further research will focus on the mechanisms underlying the potential for targeting INPP4B in GBC treatment.

Abbreviations

AML: acute myeloid leukemia; GBC: gallbladder cancer; IHC: Immunohistochemistry; INPP4B: Inositol polyphosphate 4-phosphatase type II; IRS: immunoreactivity score; OS: overall survival; TSG: tumor suppressor gene.

Declarations

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Authors' contributions

YW and DM: Experimentation and writing of the manuscript. XX and JB: Clinical data collection and analysis. YY and YS: Assist in cell function experiment and clinical data compilation. YL and DS: Concept and design, supervision and writing of the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study obtained approval from the Institute Research Ethics Committee of the Armed Police Corps Hospital of Anhui and written informed consents were obtained by all patients involved.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

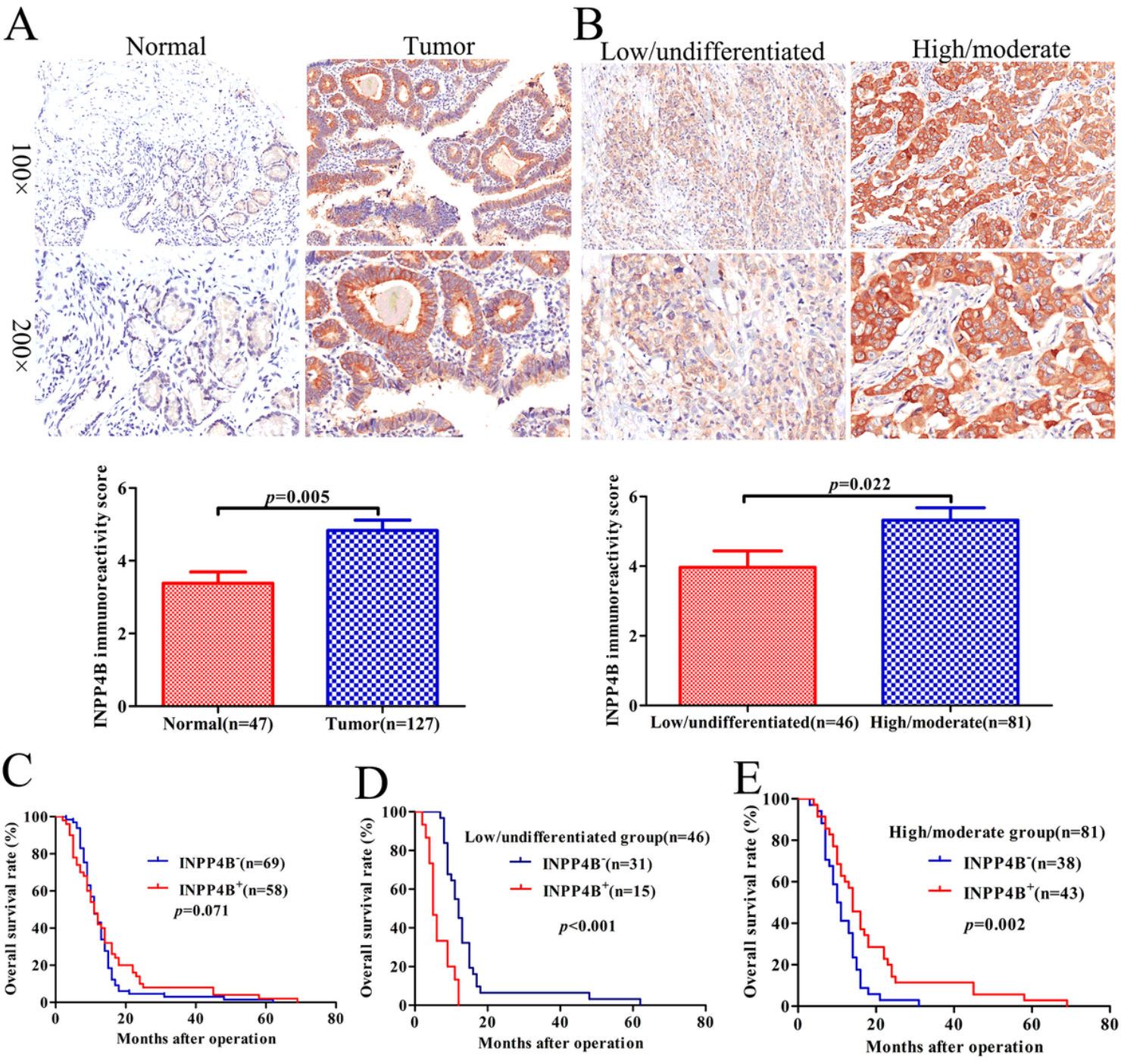

Figure 1

Protein expression levels and prognostic significance of INPP4B in GBC clinical samples. (A) Representative images and immunoreactivity score of INPP4B in tumor tissues and normal tissues. (B) Representative images and immunoreactivity score of INPP4B in low-undifferentiated and high-moderate differentiated of tumor tissues. (C) The overall survival of GBC patients with INPP4B- and INPP4B+. (D) The overall survival of GBC patients in low-undifferentiated group with INPP4B- and INPP4B+. (F) The overall survival of GBC patients in high-moderate differentiated group with INPP4B- and INPP4B+.

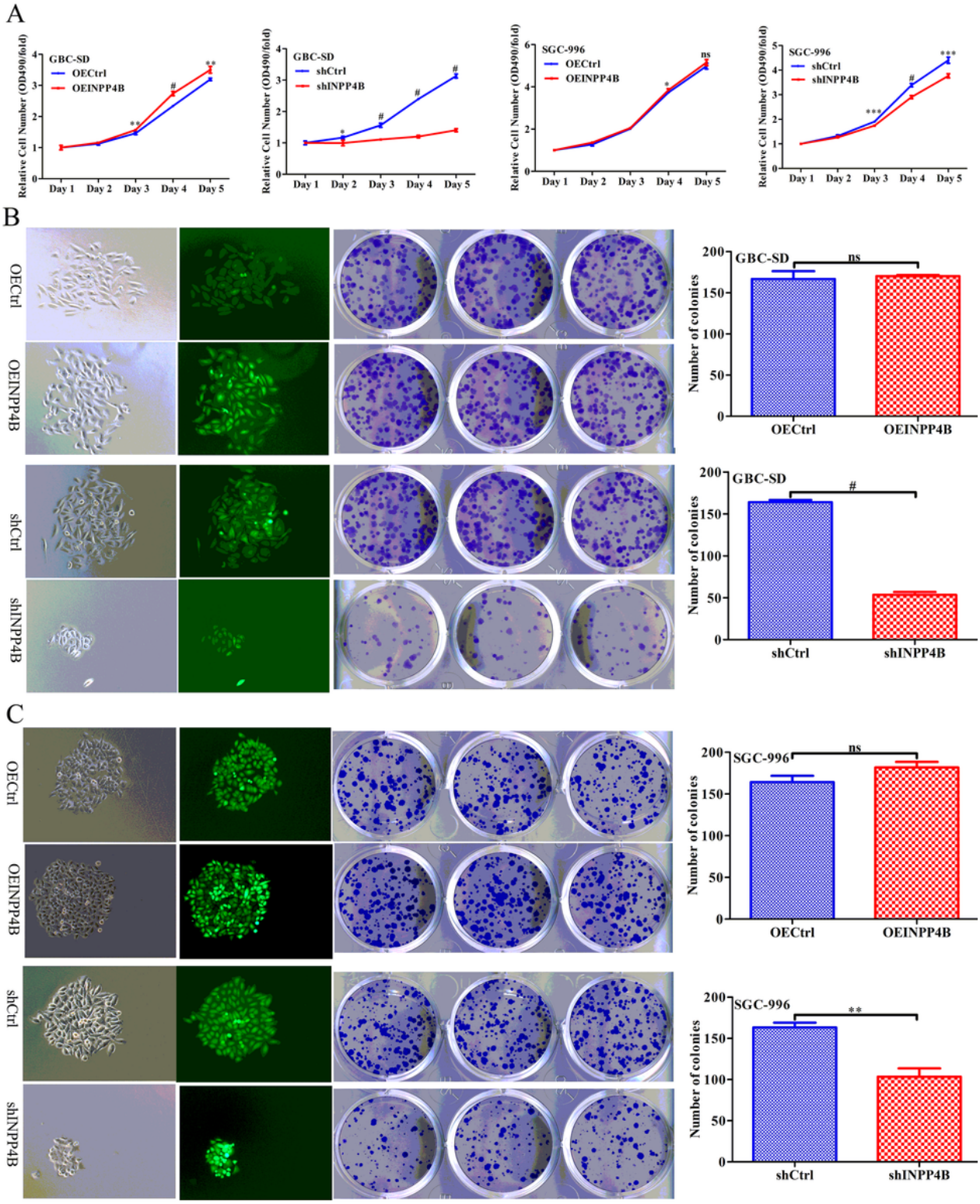

Figure 2

INPP4B controls GBC cell growth in vitro. (A) Proliferation curve for GBC-SD cell and SGC996 cell with INPP4B overexpression, knockdown and negative control. (B, C) Colony formation for GBC-SD cell and SGC996 cell with INPP4B overexpression, knockdown and negative control. *, p<0.05; **, p<0.01; ***, p<0.001; #, p<0.0001; ns, not significant.

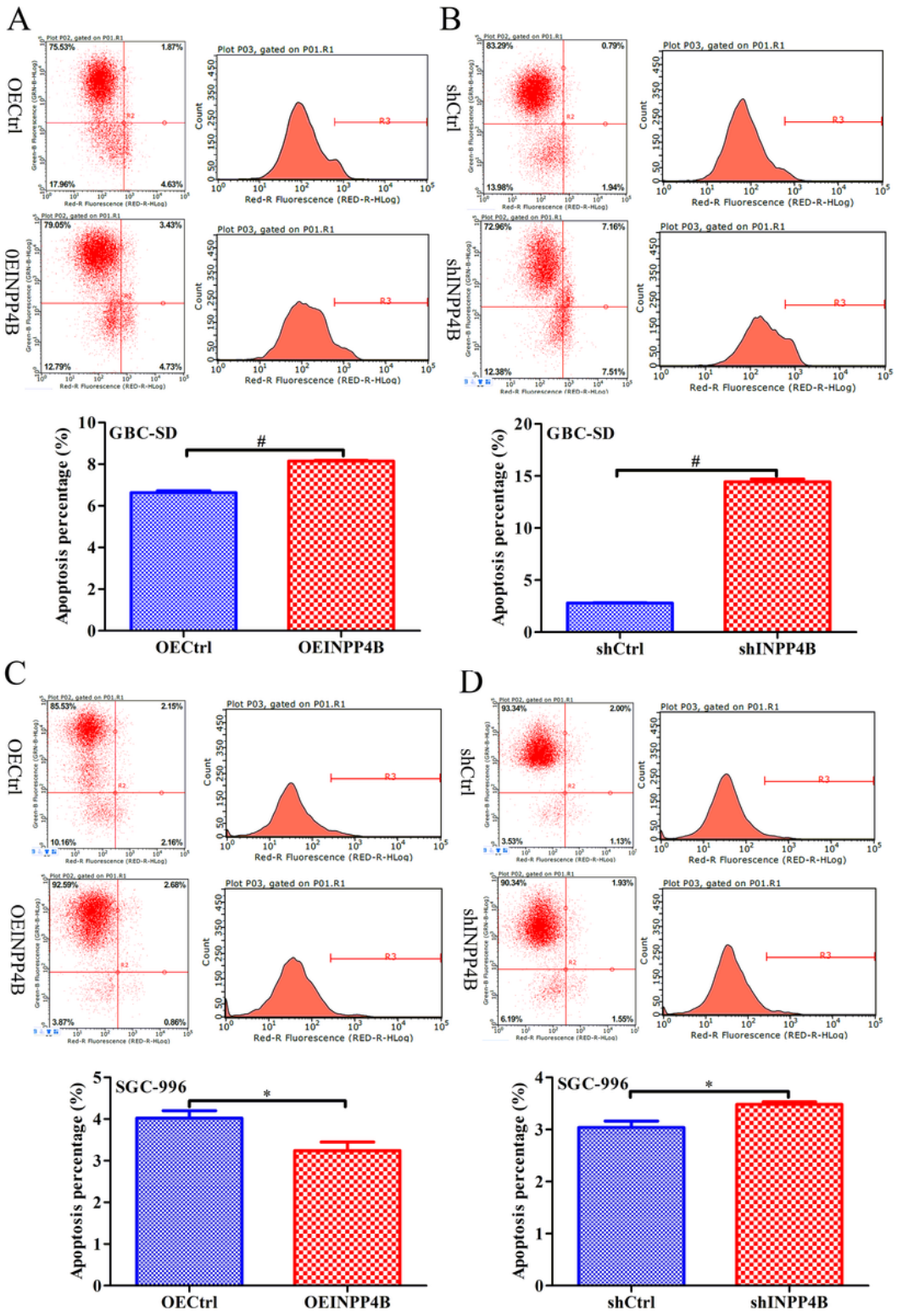

Figure 3

INPP4B controls GBC cells apoptosis in vitro. (A, B) Both INPP4B overexpression and knockdown all significantly promots GBC-SD cell apoptosis. (C) INPP4B overexpression significantly inhibits SGC996 cell apoptosis. (D) INPP4B knockdown significantly induces SGC996 cell apoptosis. *, p<0.05; #, p<0.0001.

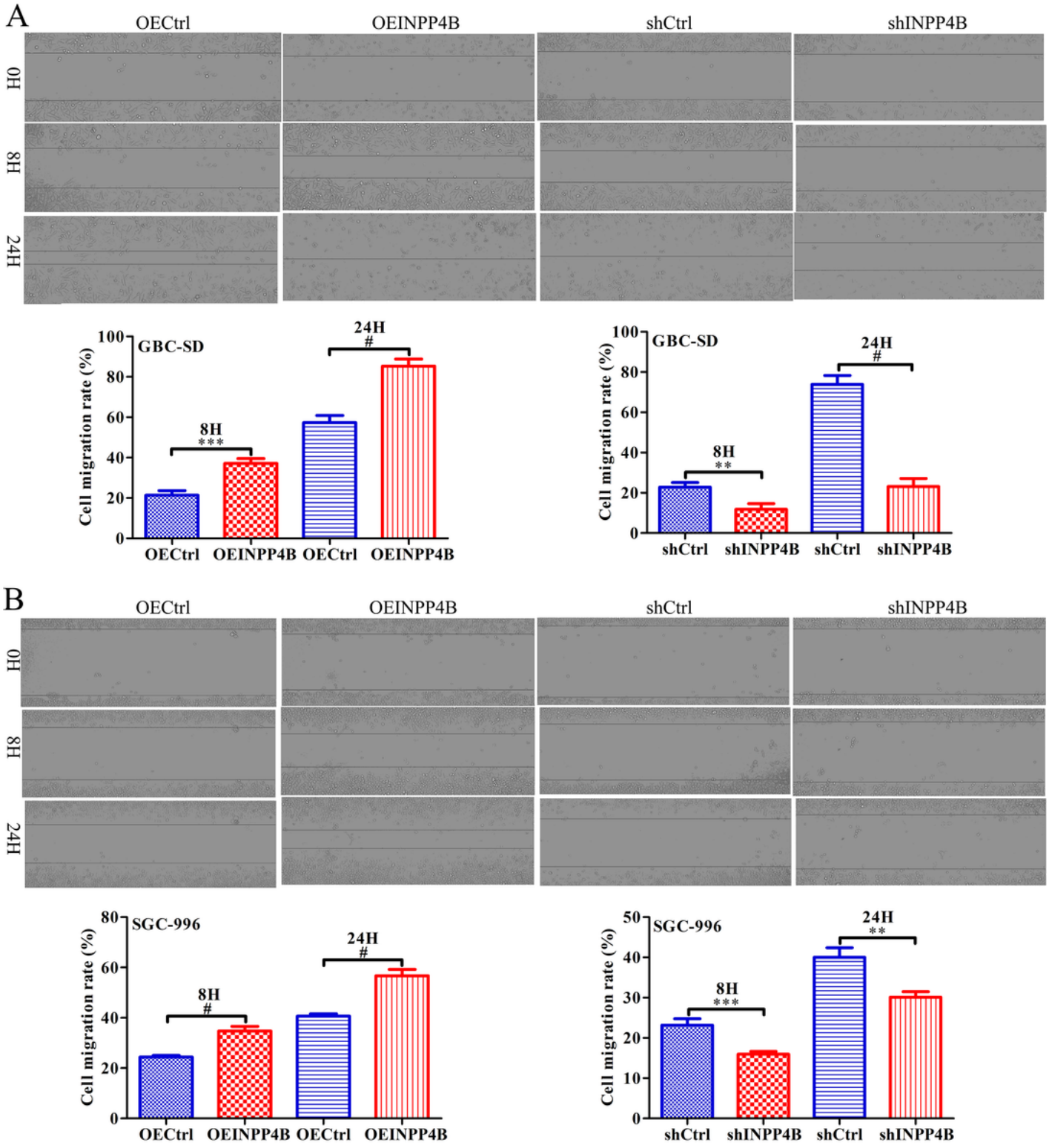

Figure 4

INPP4B regulates GBC cell migration ability in vitro. The migration of GBC-SD cell and SGC-996 cell after different lentivirus infection was detected by scratch wound-healing assays. (A) INPP4B overexpression significantly promots GBC-SD cell migration, while INPP4B knockdown significantly inhibits GBC-SD cell migration. (B) INPP4B overexpression significantly promots SGC996 cell migration, while INPP4B knockdown significantly inhibits SGC996 migration. **, p<0.01; ***, p<0.001; #, p<0.0001; ns, not significant.

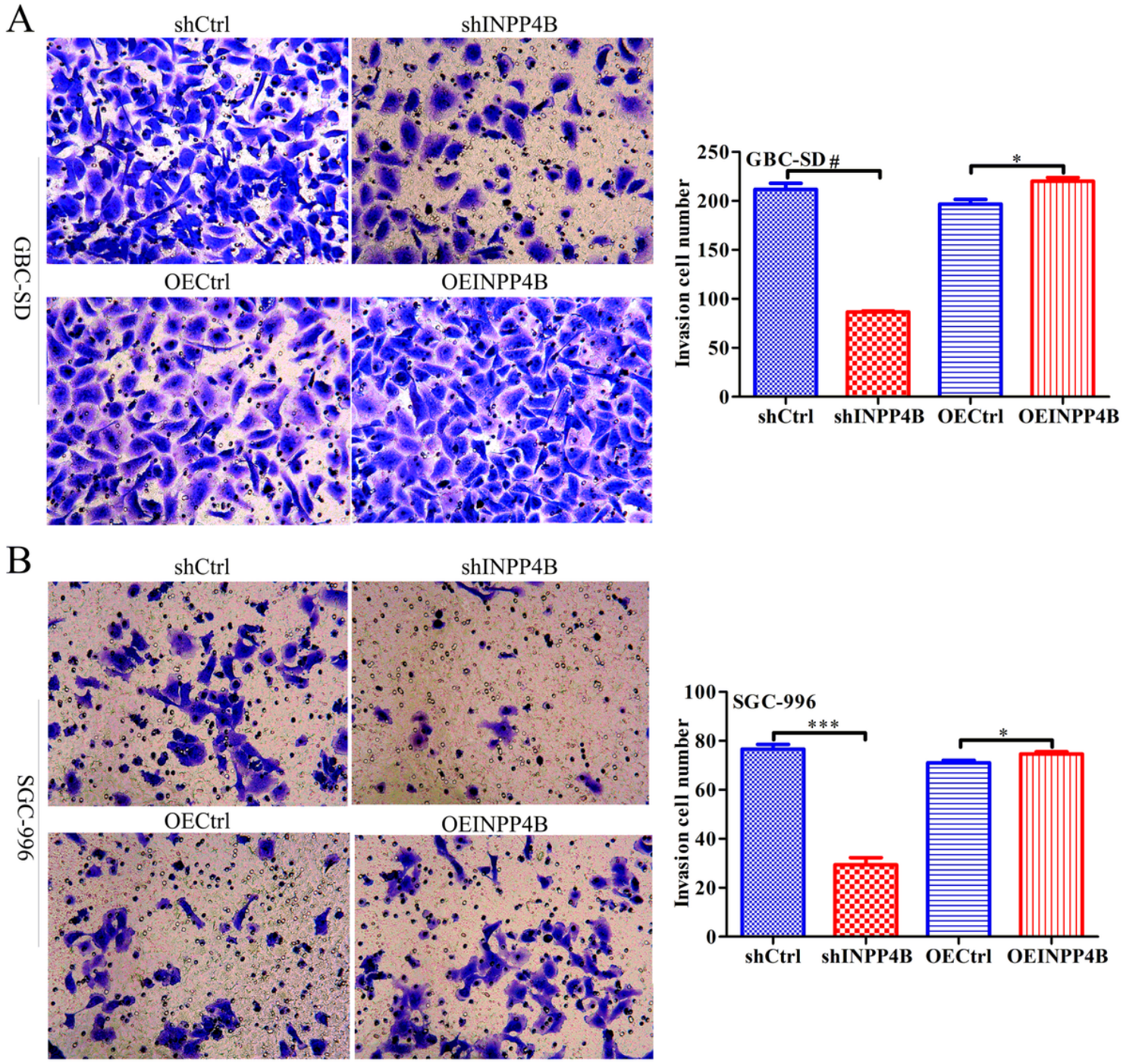

Figure 5

INPP4B regulates GBC cell invasion ability in vitro. The invasion assays of GBC-SD cell and SGC-996 cell after different lentivirus infection was detected by transwell assays. (A) INPP4B overexpression

significantly promots GBC-SD cell invasion, while INPP4B knockdown significantly inhibits GBC-SD cell invasion. (B) INPP4B overexpression significantly promots SGC996 cell invasion, while INPP4B knockdown significantly inhibits SGC996 invasion. *, $p<0.05$; ***, $p<0.001$; #, $p<0.0001$.