BMC Cancer

RESEARCH

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TOB1 inhibits the gastric cancer progression by focal adhesion pathway and ERK pathway based on transcriptional and metabolic sequencing

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Abstract

Gastric cancer is one of the most malignant digestive tract tumors worldwide and its progression is associated with gene expression and metabolic alteration. We revealed that the gastric cancer patients with lower expression level of TOB1 exhibited poorer overall survivals according to the data in Kaplan–Meier Plotter. The unphosphorylated TOB1 protein which is effective expressed lower in gastric cancer cells. The gastric cancer cells with TOB1 gene depletion performed higher abilities of proliferation, migration and invasion and lower ability of apoptosis in vitro. The TOB1 gene depletion also promoted the tumorigenesis of gastric cancer cells in vivo. The gastric cancer cells with TOB1 gene overexpression had the converse behaviors. The transcriptional and metabolic sequencing was performed. The analyzation results showed that genes correlate-expressed with TOB1 gene were enriched in the pathways related to ERK pathway, including focal adhesion pathway, which was verified using real-time quantitative PCR. After inhibiting ERK pathway, the proliferation, colony formation and migration abilities were reduced in gastric cancer cells with low phosphorylated TOB1 protein expression level. Moreover, Pearson correlation analysis was adopted to further analyze the correlation of enriched metabolic products and differentially expressed genes. The expression of Choline, UDP-N-acetylglucosamine, Adenosine and GMP were related to the function of TOB1. This study demonstrates the genes and metabolites related to focal adhesion pathway and ERK pathway are the potential diagnosis and therapeutic targets to gastric cancer with TOB1 depletion.

Keywords TOB1, Gastric cancer, Focal adhesion pathway, ERK pathway, Metabolites

Introduction

According to the cancer statistics in 2023, gastric cancer is still the fifth digestive system cancer-related death cause worldwide [1, 2]. The study of development of molecular mechanisms in gastric cancer helps its diagnosis and intervention.

Focal adhesion is adhesive contact between cells, as well as cells and extracellular matrix. The focal adhesion connects cells to the matrix by biochemical and physical signals, relaying on the interaction of the transmembrane protein with the corresponding extracellular

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ligand and the various intracellular proteins connected to the actin cytoskeleton [3, 4, 5]. Focal adhesion is relevant to cell migration, cell polarization, metastatic cancer formation and other cellular and pathological events [6]. There is a bidirectional transmitting signal across the cell membrane, leading to a direct physical connection between the cells and the extracellular matrix due to focal adhesion [7]. In the focal adhesion pathway, extracellular matrix transmits biochemical signals through transmembrane protein complex ITGA/ITGB. Focal adhesion kinase 1 (FAK1), a member of the FAK subfamily, is a cytoplasmic protein. FAK can activate the phosphorylation function of PI3K and then the downstream molecular signaling pathways are activated [8]. Extracellular matrix is mainly composed of collagen, non-collagen, proteoglycans, elastin and aminoglycans. Collagen is the major component of the extracellular matrix providing connective tissue with the ability to resist the external force [9, 10].

The protein product of transducer of ErbB-2.1 (TOB1) is a member of the anti-proliferative family [11]. TOB1 is activated in a non-phosphorylated form and inactivated by phosphorylation [12]. There are accumulating evidences that has shown the function of TOB1 is a tumor suppressor. It has been verified that TOB1 was downregulated in esophageal squamous cell carcinoma leading to malignant progression and poor prognosis [13]. TOB1 was also meaningful for predicting prognosis in Papillary thyroid cancer [14]. However, the expression and biological role of TOB1 in gastric cancer has not been well understood. And the involved molecular pathways and metabolic contents have not been elucidated fully.

In this study, we identified the function of TOB1 in gastric cancer cells by knocking down and overexpressing TOB1 gene. To further study the molecular mechanism and involved metabolites, we performed transcriptional and metabolic sequencing. Our study provides potential diagnosis biomarkers and intervention targets for gastric cancer.

Materials and methods

Cell lines and cell culture

The gastric cancer cell lines KATOIII, SNU-16 and AGS were purchased from ATCC. The gastric cancer cell line HGC-27 was purchased from Shanghai Institutes for Biological Sciences. The gastric mucosal epithelial cell line GES-1 was a gift from Laboratory of medical genetics, China Medical University. MKN1 and MKN45 cell lines were gifts from Sun Yat-sen University. HGC-27 cells were transfected with plasmid GV102 carrying RNA interfere sequence AGTATTCTAACCAGCAATT or AACAAGGTTGCACGTACTT to knockdown TOB1 gene. TOB1 gene was overexpressed in AGS cells with plasmid GV358-TOB1. HGC-27, MKN45 and SUN-16 cells were cultured using RPMI 1640 medium with 10% fetal bovine serum (Gibco). AGS cells were cultured using F-12 K medium with 10% fetal bovine serum. MKN1 cells were cultured using DMEM medium with 10% fetal bovine serum.

The TOB1 gene overexpression vector was GV358 (Shanghai Genechem Co.,Ltd.). The TOB1 gene silencing vector was GV102 carrying RNAi sequence 1-AGTATT CTAACCAGCAATT or RNAi sequence 2-AACAAG GTTGCACGTACTT (Shanghai Genechem Co.,Ltd.). The ERK pathway inhibitor SCH772984 (Selleck, CAS#942,183–80-4) with a concentration of 10 μmol/L was used for 36 h.

Cell proliferation assay

The proliferation of the studied gastric cancer cells was detected using CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega). All the performance followed the instruction. The absorbance was measured at 492 nm in each well.

Colony formation assay

For colony formation assay, 200 infected gastric cancer cells were cultured and maintained in six-well plates for 14 days. Colonies were fixed with methanol and stained with Giemsa (Sigma-Aldrich, USA) for 30 min. After being washed with PBS for three times, the number of colonies with more than 30 cells was counted.

Wound healing assay

A 10-μl pipette tip was used to scratch the cell monolayer. Photographs were taken immediately, 24 h and 48 h after wounding. Each assay was repeated for three times at least.

Transwell invasion assay

Corning 8.0-mm Transwell inserts (8-μm pore size, 24-well plate) and BD BioCoat™ Matrigel™ Invasion Chambers (Corning Incorporated Life Sciences, Tewksbury, MA, USA) were used to perform the Transwell cell invasion assays. The assays were done according to the manufacturer's instructions.

Animal studies

The animal experiments were approved by the Ethics Committee of Harbin Medical University (HMUIRB20170037) and performed according to the Guidelines of Laboratory Animal Usage of Harbin Medical University. Five four-week-old BALB/c nude female mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and assigned randomly into TOB1 silencing HGC-27 group and the control group. Around

1×10^7 cells were collected and suspended in 200 μl PBS and were injected into the subcutaneous of each BALB/c nude female mouse. The tumor volumes were measured every three days.

After tumor cells inoculation, the skin of BALB/c nude female mice at the injection site was observed daily for inflammatory reaction and subcutaneous tumor formation. The time of tumor formation was recorded. The long diameter (a) and short diameter (b) of tumors were measured with Vernier calipers every 3 days after tumor formation in BALB/c nude female mice. The tumor volume (V) [$V=1/2ab^2$ mm^3] was calculated to draw the growth curve. After four weeks, the BALB/c nude female mice were killed by cervical dislocation. The tumor tissue was dissected and stained by Hematoxylin–Eosin (HE) before being observed.

Western blot

RIPA lysis buffer (C1053-3, Applygen) was used to resuspend the studied cells. BCA protein assay (P1511-5, Applygen) was performed to exam the concentrations of the extracted protein. The primary antibodies include anti-TOB1 antibody (ab168947, Abcam), anti-TOB1 (phospho S164) antibody (ab78915, Abcam), anti-β actin antibody (Zhong Shan Gold Bridge, Beijing), anti-caspase-3 antibody (ab32351, Abcam). The secondary antibodies include anti-IgG mouse antibody (58,802, Cell Signaling Technology), anti-IgG rabbit antibody (93,702, Cell Signaling Technology).

Real-time quantitative PCR assay

The DNA extraction kit (QIAmpDNA mini Kit, Qiagen) was used to extract DNA following the instruction book. TRIzol Reagent was used to extract RNA. Transcriptor First strand cDNA Synthesis Kit (Roche) was used to reverse transcription of total RNA into cDNA. The related primers are listed in Supplementary Table 1.

RNA-sequencing and data analyzation

The RNA sequencing was performed by Novogene Technology Co., LTD, Beijing. The RNA of TOB1 silencing HGC-27 cells and HGC-27 control cells was extracted as above. RNA quality inspection and quantification were performed. In a brief, the RNA sequencing libraries were constructed using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) according to the manufacturer's recommendations. The schedule of RNA sequencing data analyzation was performed as previous [15].

Untargeted metabolic profiling and data analyzation

The Untargeted metabolic profiling was performed by Novogene Technology Co., LTD, Beijing. LC–MS/MS analyzation were performed. The metabolites were annotated through KEGG database, HMDB database and Lipidmaps database. Principal components analysis (PCA) and Partial least squares discriminant analysis (PLS-DA) were performed at metaX.

Survival analyzation

The survival analyzation was performed using Kaplan–Meier Plotter at the website of http://kmplot.com/analysis/index.php?p=service&cancer=gastric. The Affy ID 202704_at (TOB1) was valid and 875 gastric cancer cases were analyzed.

Gene expression correlation analyzation

The gene expression correlation analyzation was performed using GEPIA2 (http://gepia2.cancer-pku.cn/#index). The Stomach adenocarcinoma from TCGA tumor was selected. The non-log scale was used for calculation. The log-scale axis was used for visualization. Pearson correlation analyzation was done.

Pearson correlation analyzation between genes in focal adhesion pathway and differentially expression metabolites

R 3.6.1 (Corr.test) was used to calculate Pearson correlation coefficient between genes and metabolites. The linear relationship between genes and metabolites was ranged -1 to 1. The greater positive correlation, the number was tended to 1. The greater negative correlation, the number was tended to -1. The significantly correlation between genes and metabolites was analyzed and P value < 0.05 indicated statistically significant.

Statistical analyzation

SPSS 15.0 software was used for statistical analysis, and values were expressed as mean ± standard deviation (bar chart). Independent samples t test, ANOVA (Dunnett's Multiple Comparison Test) and Chi-square test were used. The mark * indicates $P<0.05$, ** indicates $P<0.01$ and *** indicates $P<0.001$, respectively representing the degree of statistical difference.

Results

Low level of TOB1 is related to poor survival of gastric *cancer* and phosphorylated TOB1 is highly expressed in gastric *cancer* cells

Our previous study found the phosphorylation of TOB1 inactivated its biological function. The phosphorylated TOB1 level was significantly higher in gastric cancer tissues than that in normal gastric tissues. Gastric cancer patients with elevation of nuclear TOB1 phosphorylation had poorer overall survival [16]. Moreover, 875 gastric cancer cases in Kaplan–Meier Plotter were analyzed. The

result showed gastric cancer patients with lower TOB1 expression exhibited the poorer overall survival (Fig. 1A).

The amplification and expression levels of TOB1 were examined in several gastric cancer cell lines HGC-27, AGS, MKN1, MKN45, SNU-16, KATOIII and gastric mucosal epithelial cell line GES-1. The result showed that the copy number of TOB1 gene was higher in most gastric cancer cells (Fig. 1B). The mRNA expression level was also elevated in gastric cancer cells except AGS (Fig. 1C). For the expression level of total TOB1 protein, it was no significant difference in most gastric cancer cells. However, the phosphorylated TOB1 proteins were higher in most of the examined gastric cancer cells (Fig. 1D, Supplementary Fig. 1A). The further analyzation showed the proportion of the unphosphorylated TOB1 was low relative to the total TOB1 protein in most gastric cancer cells (Fig. 1E).

TOB1 regulates proliferation, apoptosis, migration, invasion in vitro and tumorigenesis in vivo of gastric *cancer* cells

To explore the biological function of TOB1 in gastric cancer cells. TOB1 gene was knocked-down in gastric cancer cell line HGC-27 (Fig. 2A-B, Supplementary Fig. 1B) or was overexpressed in gastric cancer cell line AGS (Fig. 2C-D, Supplementary Fig. 1C).

The proliferation of TOB1 silencing HGC-27 cells and TOB1 overexpressing AGS cells were examined using MTS assay. The proliferation ability was promoted in TOB1 depleted HGC-27 cells, while it was attenuated in TOB1 overexpressing AGS cells (Fig. 2E-F). Similarly, the colony formation assay results showed that the size and number of colonies were increased after silencing TOB1 and was reduced after overexpressing TOB1 (Fig. 2G-H).

In addition, the expression of apoptosis marker caspase-3 was examined in TOB1 knocked-down HGC-27 cells and TOB1 overexpressing AGS cells. The results indicated that silencing TOB1 could inhibit the apoptosis of gastric cancer cells and TOB1 overexpression led to the activation of apoptosis (Fig. 3A-B).

The migration and invasion assays demonstrated that TOB1 deficiency promoted the migration (Fig. 3C) and invasion (Fig. 3E) in gastric cancer cells, while overexpression of TOB1 could attenuate the migration (Fig. 3D) and invasion (Fig. 3F).

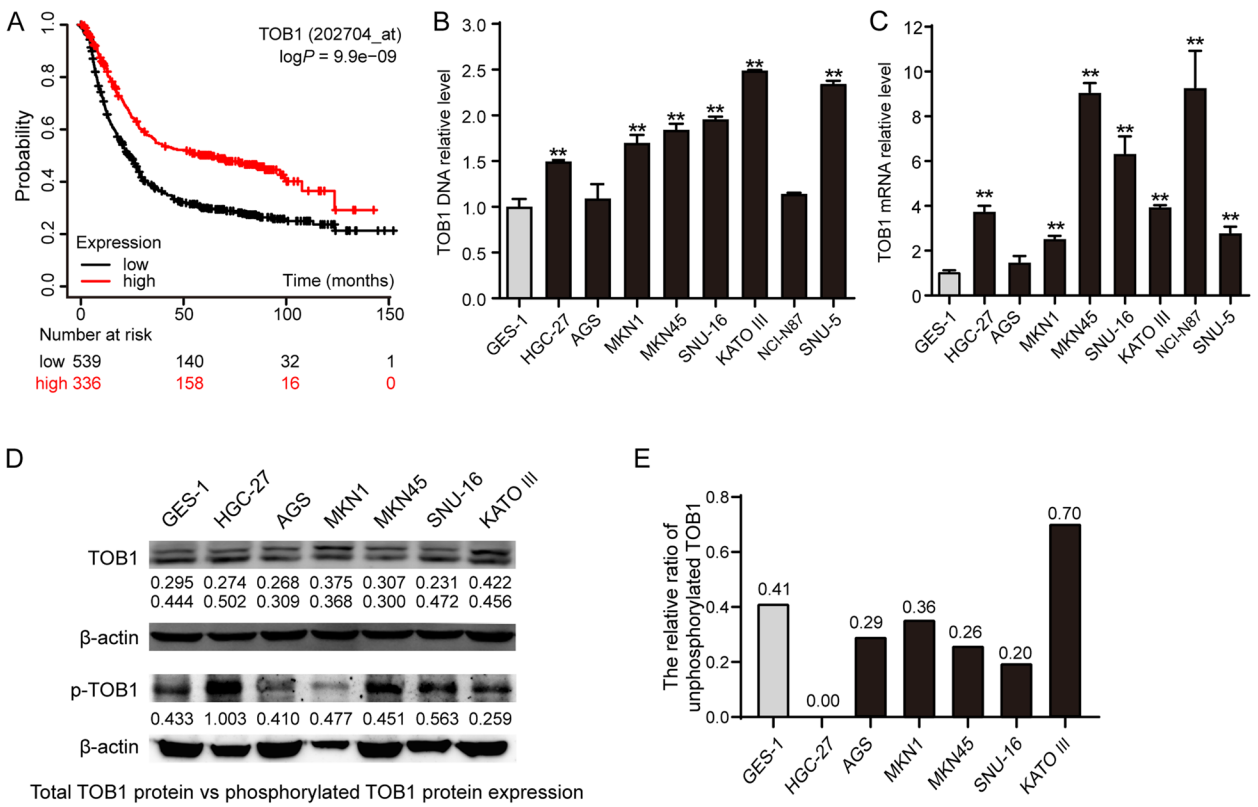

Fig. 1 The low expression of effective TOB1 is in gastric cancer leads to poor survival. (**A**) TOB1 expression level is related to survival of gastric cancer. (**B**) The TOB1 amplification level in gastric cancer cell lines and gastric cell line. (**C**) The TOB1 mRNA expression level in gastric cancer cell lines and gastric cell line. (**D**) The total and phosphorylated TOB1 protein expression level in gastric cancer cell lines and gastric cell lines. (**E**) The relative ratio of unphosphorylated TOB1 to total TOB1

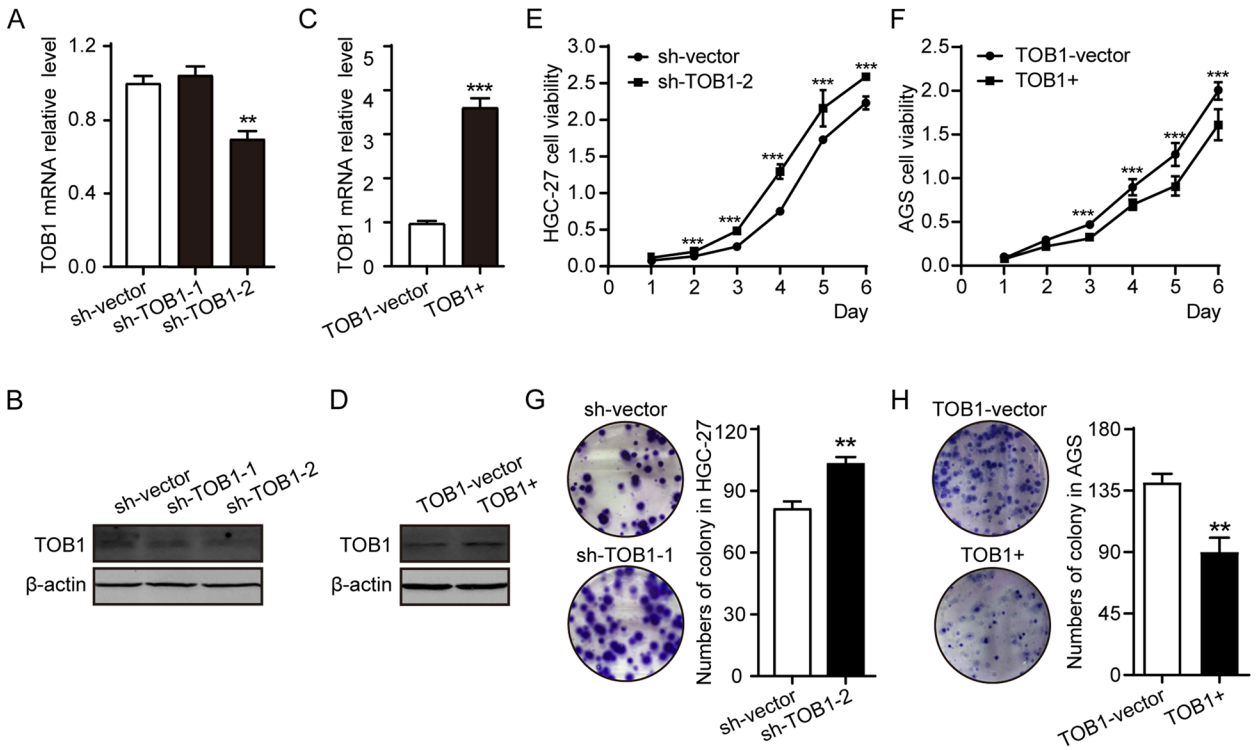

Fig. 2 TOB1 regulates proliferation and colony formation in vitro of gastric cancer cells. (**A-B**) The expression level of TOB1 in TOB1 depleted HGC-27 cells. (**C-D**) The expression level of TOB1 in TOB1 overexpressed AGS cells. (**E–F**) The proliferation abilities of gastric cancer cells after interfering TOB1 expression. (**G-H**) The colony formation abilities of gastric cancer cells after interfering TOB1 expression

To study the tumorigenesis of gastric cancer cells after silencing TOB1 in vivo, the subcutaneous tumorigenesis model was applied. The tumor volumes of TOB1-silencing HGC-27 cells were significantly larger than the control group (Fig. 3G, Table 1). The HE assays demonstrated that the tissues from the nude mice were gastric tumor (Fig. 3H).

Analyzation of TOB1 regulated genes and the involved pathways in gastric *cancer* cells

To further study the mechanism of TOB1 regulating the biological behaviors of gastric cancer cells, the transcriptional sequencing was performed in TOB1 silencing HGC-27 cells. According to the transcriptional sequencing results, there were 381 differentially expressed mRNAs in TOB1 silencing HGC-27 cells including 188 up-regulated mRNAs and 193 down-regulated mRNAs (Supplementary Fig. 2A). GO and KEGG enrichment analysis were performed based on the 381 differential expression mRNA (Supplementary Fig. 2B-C).

After TOB1 silencing, the dominant differentially expressed genes were enriched in the focal adhesion pathway (four up-regulated genes TLN2, PPP1R12C, THBS1, CAV3 and eight down-regulated genes COL9A3, COL1A2, COL6A6, TNC, VAV3, LAMC3, LAMA4, LAMA5), cAMP signaling pathway (one up-regulated gene GRIN3A and six down-regulated genes RYR2, GRIA4, FOS, CAMK2A, GRIA3, VAV3), the extracellular matrix receptor interaction pathway (three up-regulated genes TNC, THBS1, DAG1 and six down-regulated genes FREM2, COL9A3, COL1A2, LAMC3, LAMA4, COL6A6), proteoglycans in the cancer related pathway (three up-regulated genes MMP2, PPP1R12C, MYC and six down-regulated genes WNT6, LUM, CAV3, CAMK2A, DCN, VAV3) and cGMP-PKG signaling pathway (one down-regulated gene GUCY1B1) (Supplementary Table 2).

The dominant differentially expressed genes COL1A2 and LAMA5 were verified and downregulated in TOB1 silencing HGC-27 cells significantly (Fig. 4A). Moreover, the expression of TOB1 was positively correlated with the expression of COL1A2, LAMA5, FOS and COL12A1 while negatively correlated with COL21A1 and PPP1R12C expression in gastric cancer tissues (Fig. 4B). The above genes were related to TOB1 and participated the focal adhesion pathway, indicating TOB1 was involved in regulating the focal adhesion pathway.

Interestingly, most of the dominant differentially expressed genes enriched pathways (cAMP signaling pathway, proteoglycans in the cancer related pathway,

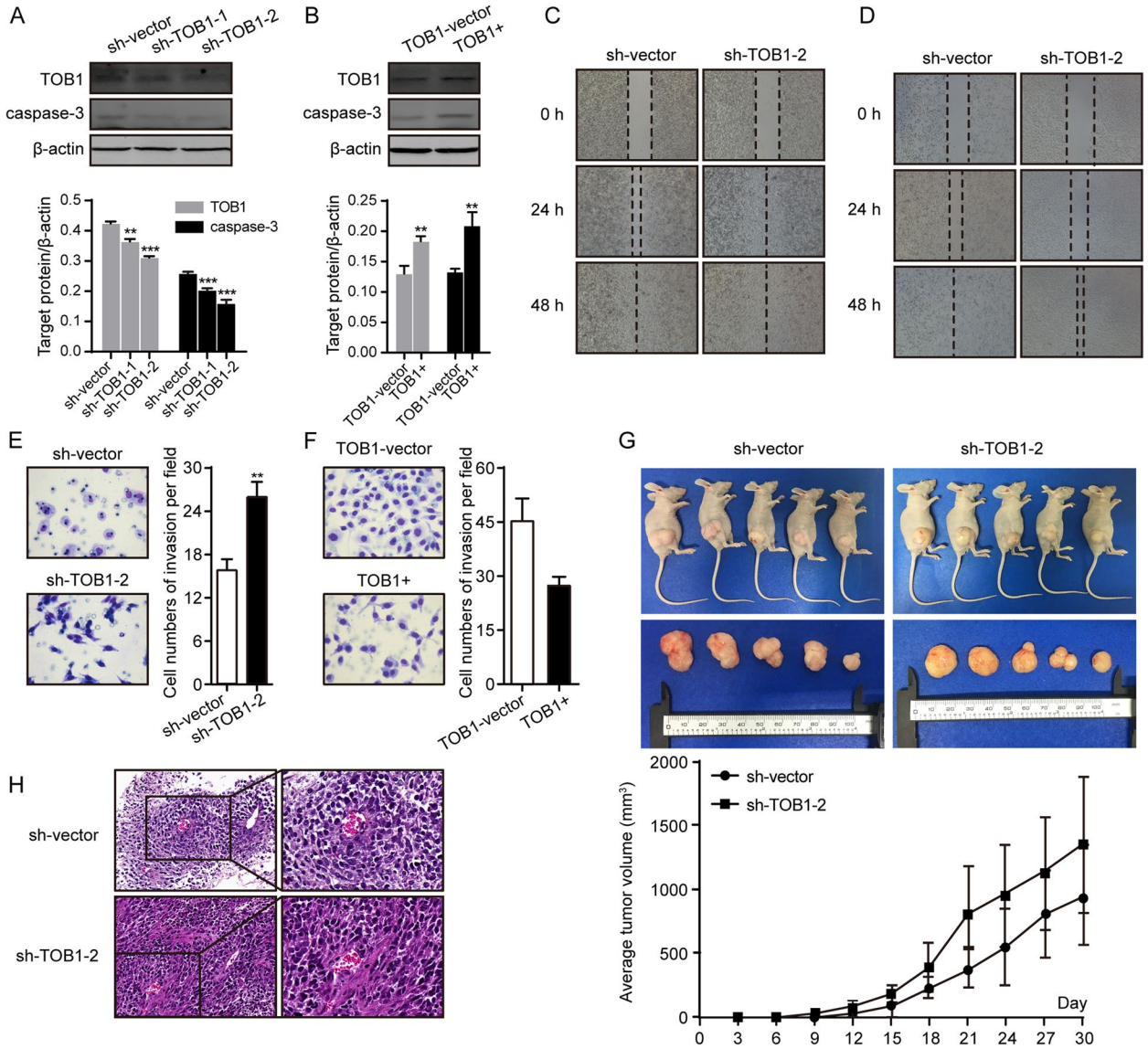

Fig. 3 TOB1 regulates apoptosis, migration, invasion in vitro and tumorigenesis in vivo of gastric cancer cells. (**A-B**) The apoptosis of gastric cancer cells after interfering TOB1 expression. (**C-D**) The migration abilities of gastric cancer cells after interfering TOB1 expression. (**E–F**) The invasion abilities of gastric cancer cells after interfering TOB1 expression. (**G-H**) The tumorigenesis of gastric cancer cells after depletion of TOB1

Table 1 Comparison of tumorigenesis between nude mice transplanted with gastric cancer cells ($\overline{X} \pm S$)

	HGC-27-vector ($n=5$)	HGC-27 with TOB1 depletion ($n=5$)	*P* value
tumorigenesis time (day)	12.40 ± 0.40	9.40 ± 0.40	< 0.001
Volume (mm^3)	949.7 ± 64.88	1356 ± 136.5	0.04
Weight (g)	0.978 ± 0.075	1.410 ± 0.123	0.02

cGMP-PKG signaling pathway, focal adhesion pathway) are pointing to the ERK pathway. SCH772984 was used to inhibit the ERK pathway in MKN1 cells with low phosphorylated TOB1 protein expression level. After inhibiting ERK pathway, the proliferation (Fig. 4C), colony formation (Fig. 4D), wound healing (Fig. 4E) and migration (Fig. 4F) abilities of MKN1 cells were decreased. In addition, the expression level

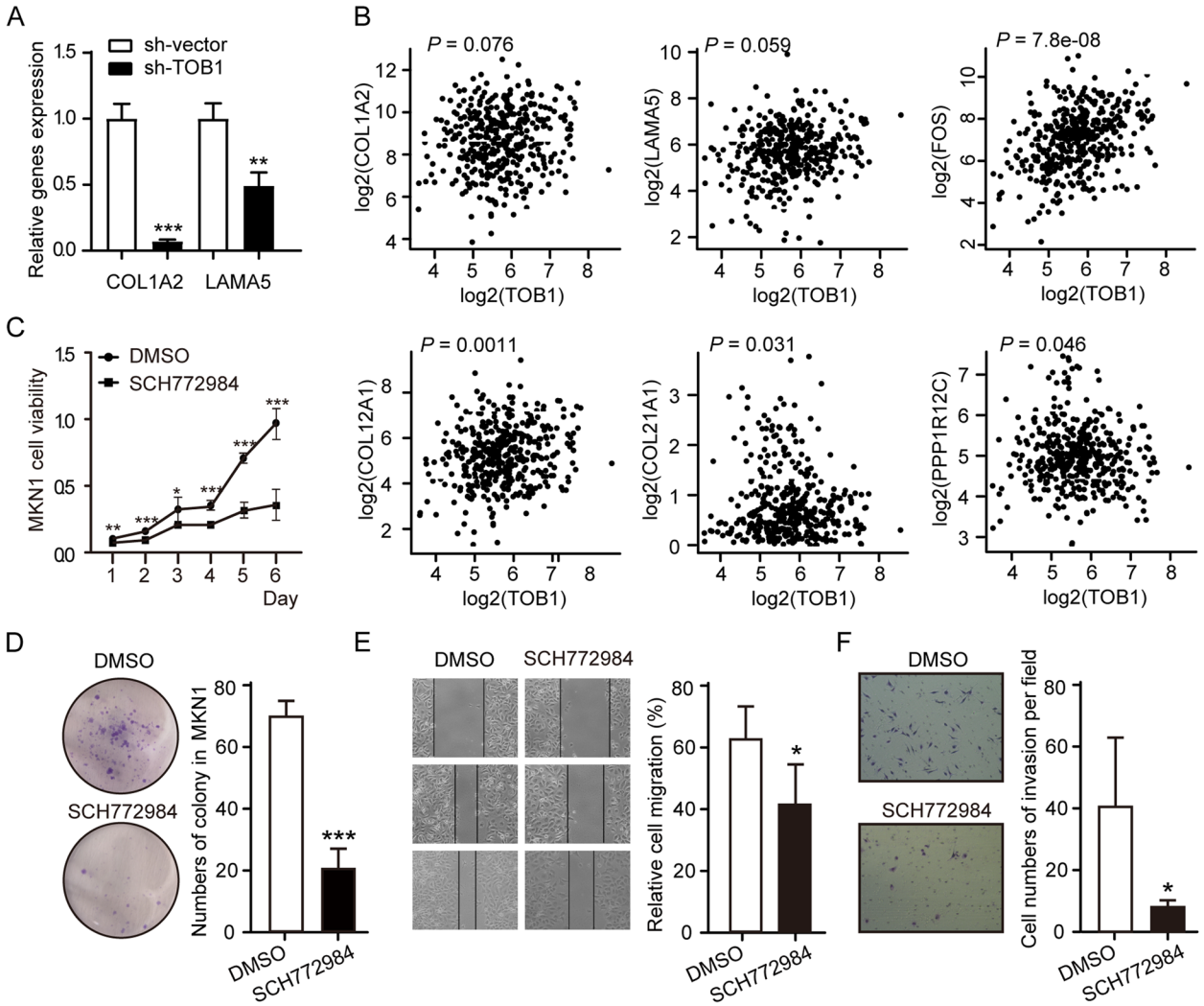

Fig. 4 Analyzation of TOB1 regulated genes and the involved pathways in gastric cancer cells. (**A**) The expression level of COL1A2 and LAMA5 was coordinated with the transcriptional sequencing analyzation results. (**B**) The expression correlation between TOB1 and differential expressed genes enriched in the focal adhesion pathway and ERK pathway. (**C**) The proliferation ability of gastric cancer cells with low phosphorylated TOB1 protein level after inhibition of ERK pathway. (**D**) The colony formation ability of gastric cancer cells with low phosphorylated TOB1 protein level after inhibition of ERK pathway. (**E**) The wound healing ability of gastric cancer cells with low phosphorylated TOB1 protein level after inhibition of ERK pathway. (**F**) The migration ability of gastric cancer cells with low phosphorylated TOB1 protein level after inhibition of ERK pathway

of phosphorylated TOB1 protein were examined after treating with SCH772984 in a concentration of 1 μM, 2 μM, 5 μM, 10 μM and 20 μM, respectively. It was also examined after using SCH772984 for 12 h and 24 h. The results showed that the phosphorylation level of TOB1 was not affected by ERK inhibitor SCH772984 in the way of concentration or time (Supplementary Fig. 3).

TOB1 silencing influence metabolic products involved in focal adhesion pathway and ERK pathway

To further study the TOB1 regulating metabolic mechanism, the metabolic sequencing was performed using LC–MS/MS in TOB1 silencing HGC-27 cells and the wild type cells. Supplementary Fig. 4 indicated the established analysis model is reliable.

To further analyze the differential metabolic phenotypes, a model of metabolomic expression level and sample types was established using partial least squares discrimination analysis (PLS-DA). The model access parameters indicated the established model is reliable (Supplementary Fig. 5). The metabolic sequencing results showed that significant changes of metabolic phenotypes occurred in HGC-27 cells after TOB1 silencing (Supplementary Fig. 6A, Supplementary Table 3 and 4).

To explore the potential metabolic pathways regulated by TOB1, KEGG enrichment analysis was performed based on the 30 negative and 60 positive metabolites differentially expressed (Supplementary Fig. 6B). The enriched metabolites and the involved pathways are listed in Supplementary Table 5.

The correlation of enriched metabolic products with the differentially expressed genes was analyzed. The results showed that the expression level of UDP-N-acetylglucosamine and COL1A2 (Pearson's R = 0.98, $P < 0.001$), LAMA5 (Pearson's R = 0.84, $P < 0.05$) were significantly correlated. The expression level of Choline and LAMA5 (Pearson's R = 0.85, $P < 0.05$) were significantly correlated. The expression level of Adenosine and COL1A2 (Pearson's R = 0.95, $P < 0.01$), LAMA5 (Pearson's R = 0.94, $P < 0.01$) were significantly correlated. The expression level of GMP and COL1A2 (Pearson's R = 0.91, $P < 0.05$), LAMA5 (Pearson's R = 0.93, $P < 0.01$) were significantly correlated (Fig. 5A, Supplementary Table 6).

Interestingly, the expression of tumor suppressor gene PTEN and the downstream gene GSK3B were correlated with TOB1 expression in gastric cancer significantly (Fig. 5B). As known, PTEN is vital in the focal adhesion pathway. PTEN also participates in insulin resistance pathway which is correlated with the negative metabolite UDP-N-acetylglucosamine. PTEN is also related to Choline metabolism in the cancer related pathway, the positive metabolites Choline and cGMP-PKG signaling pathway as well as the positive metabolites Adenosine and GMP.

Taken together, TOB1 silencing activates the focal adhesion pathway and ERK pathway and is relevant to the decreased expression of Choline, UDP-N-acetylglucosamine, Adenosine and GMP.

Discussion

According to our previous study, TOB1 is very important in the progression of gastric cancer [16, 17, 18]. Although the expression level of TOB1 is higher in most gastric cancer cells, the high level of phosphorylation of TOB1 in gastric cancer cells leads to the functional inactivation of TOB1 [19]. Gastric cancer patients with higher level of nuclear phosphorylation TOB1 displayed poorer survival rates [16]. Moreover, it is suggested that SNPs including rs12601477 rs34700818, rs4626 and rs61482741) on TOB1 gene are crucial in the occurrence and development of gastric cancer in the Chinese Han population of northeast China [20].

We knocked down TOB1 expression in HGC-27 with a TOB1 expressing highly and overexpressed TOB1 in AGS with a low expression level of TOB1. The common tumor characteristics were tested in gastric cancer cells after knocking-down or overexpressing TOB1 in vitro and in vivo. The results indicated that TOB1 is a vital gene regulating the progression of gastric cancer.

To further study the involved molecular pathways and related metabolic events of TOB1 in gastric cancer cells, we performed transcriptomic and metabolic sequencing. The differentially expressed genes were enriched in several pathways including focal adhesion pathway. It's interesting that most of those pathways are pointing to ERK pathway. In our research, the expression of COL1A2 and LAMA5 was suppressed after TOB1 silencing in gastric cancer cells. Moreover, according to the expression data in GEPIA, the expression of COL1A2, LAMA5, COL12A1, COL21A1 and PPP1R12C which were involved in the focal adhesion pathway was correlated with TOB1 in gastric cancer tissues. After inhibiting ERK pathway in gastric cancer cells with low function of TOB1 protein, the progression of gastric cancer cells were reduced.

COL1A2 gene, COL12A1 gene and COL21A1 gene encode the pro-alpha2 chain of type I collagen [21], the alpha chain of type XII collagen and Type XXI collagen respectively which belong to the collagen family [22, 23]. LAMA5 gene encodes laminin alpha chain, a family of extracellular matrix glycoproteins [24]. PPP1R12C gene encodes a subunit of myosin phosphatase which regulates the catalytic activity of protein phosphatase 1 delta [25].

As the main structural protein of extracellular matrix, collagen is an important part of tumor microenvironment and crucial in tumor development. On one hand, collagen express highly in various cancers and is a biological marker of cell differentiation and invasion. Collagen can promote the migration and invasion of tumor cells by interacting with adhesion molecules or other extracellular components [26]. On the other hand, clinical studies have shown that collagen may in some cases slow the development of tumor cells [27]. Therefore, the interaction between collagen and other components of the extracellular matrix has a dual effect on tumor development.

We further analyzed the correlation of enriched metabolic products with COL1A2 and LAMA5 which were the differentially expressed genes in the focal adhesion pathway. The results showed that the expression levels of UDP-N-acetylglucosamine, Choline, Adenosine and GMP were significantly correlated with TOB1. In addition, PTEN was down-regulated in the gastric cancer cells with TOB1 silencing.

In the normal cells, TOB1 is a tumor suppressor. The extracellular matrix transmits signals through ITGBA/B located in the cell membrane. Then the PI3K is regulated by a series of signal transduction.

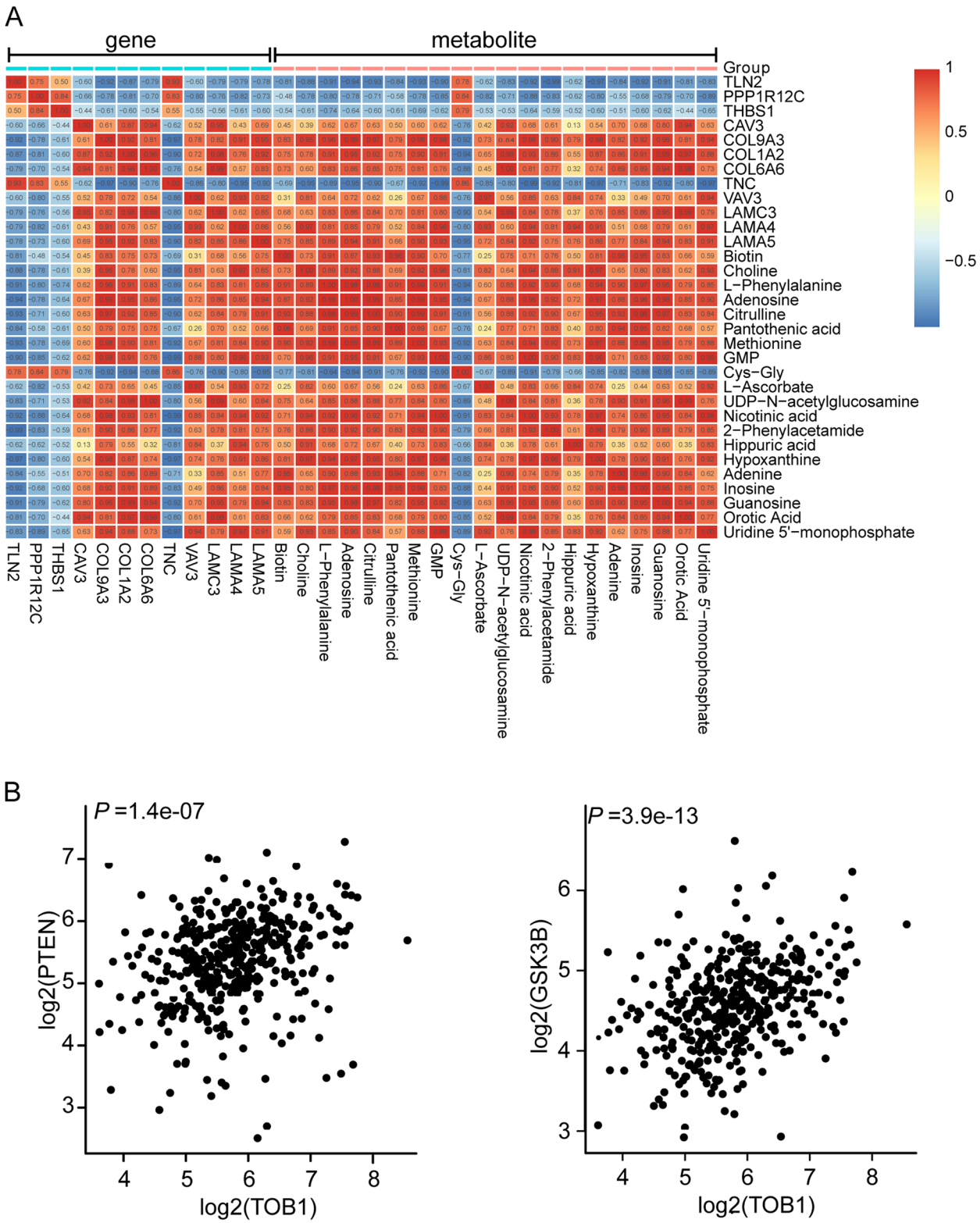

Fig. 5 TOB1 silencing influences metabolic products related to the focal adhesion pathway. (**A**) The Pearson correlation analysis of TOB1-regulating genes in focal adhesion pathway and metabolites in gastric cancer cells. (**B**) The expression of PTEN and GSK3B are significantly related to TOB1 in gastric cancer

UDP-N-acetylglucosamine inhibits PI3K by glycosylation. PI3K can add phosphate group to PIP2 and convert it into PIP3. This reaction is reversible and high expression of PTEN can dephosphorylate PIP3. PI3K also can activate CHK which phosphorylates Choline. In the normal cells with functional TOB1, GSK-3β can phosphorylate β-catenin and the phosphorylated β-catenin is degraded by ubiquitin mediated protein degradation (Fig. 6A).

On the contrary, in the gastric cancer cell with TOB1 silencing, the extracellular matrix signal is abnormal. The amount of UDP-N-acetylglucosamine is much low, leading to the inhibition loss of PI3K. Moreover, the expression level of PTEN is low in the gastric cancer cells with

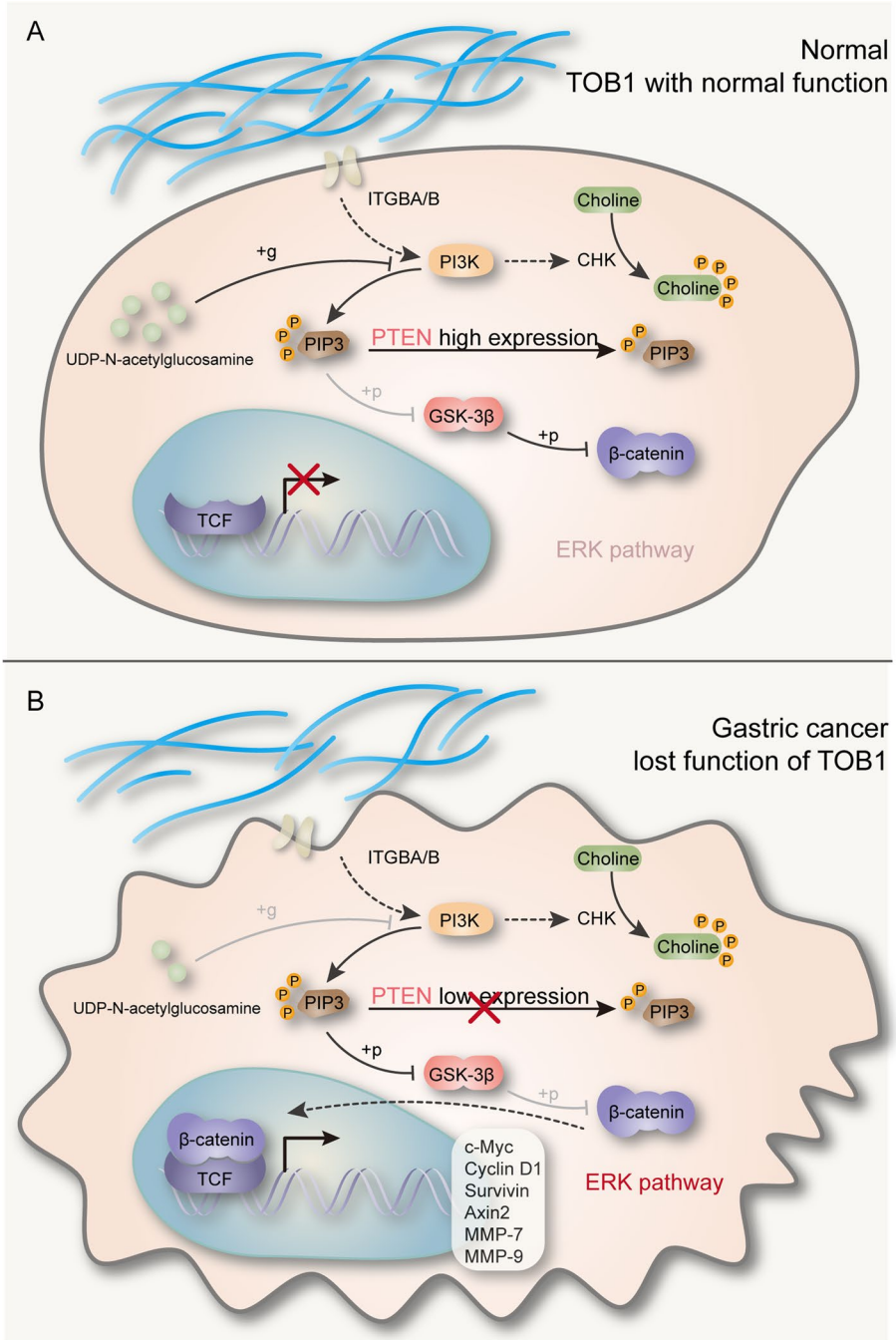

Fig. 6 Schematic representation of the molecular mechanisms underlying TOB1 regulated gastric cancer progression. (**A**) The molecular mechanism in normal gastric cells with TOB1 normal function. (**B**) The molecular mechanism in gastric cancer cells with TOB1 losing function

TOB1 silencing and PIP3 cannot be dephosphorylated. PIP3 activates Akt and phosphorylate GSK-3β. The phosphorylated GSK-3β is inactivated and β-catenin cannot be degraded. Then β-catenin enters nuclei and together with TCF to initiate transcription of downstream genes. In addition, ERK pathway can be activated in the gastric cancer cell with TOB1 silencing (Fig. 6B). According to a previous report [28], TOB1 repressed proliferation by inhibiting β-catenin signaling pathway in gastric cancer. Overexpression of TOB1 could significantly reduce the cell viability and reduce the expression of β-catenin and its target genes in gastric cancer cell lines. In contrast, knockdown of TOB1 significantly increased the survival rate and increased β-catenin expression level and its target genes by increasing the phosphorylation of Akt and GSK3β. The luciferase activity assay result showed that down-regulation of TOB1 expression increased the transcriptional activity of β-catenin significantly in AGS cells.

In conclusion, TOB1 inhibits the progression of gastric cancer through regulating focal adhesion pathway and ERK pathway. Genes involved in focal adhesion pathway and ERK pathway and the related metabolites such as UDP-N-acetylglucosamine, Choline, Adenosine and GMP can be potential biomarkers for the diagnosis and prognosis of gastric cancer.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-12894-3.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.
Supplementary Material 7.

Acknowledgements
We thank Professor Chunshui Zhou for providing language help.

Authors' contributions
Jingcui Yu and Songbin Fu contributed to initial study concept and experimental design. Hongjie He, Mingming Chen, Yuanyuan Wang, Yawen Li, Dong Wang, Kexian Dong, Mansha Jia, Xiangning Meng and Wenjing Sun performed experiments and data analysis. Kexian Dong drafted the manuscript. Mingming Chen, Dong Wang and Kexian Dong performed the sequencing and bioinformatics analyses. Hongjie He, Kexian Dong, Mingming Chen, Yuanyuan Wang, Yawen Li and Dong Wang prepared all the figures and tables. Jingcui Yu obtained funding. All authors discussed the results and commented on the manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by the National Natural Science Foundation of China (81372174) and the Open Project Program of Key Laboratory of Preservation of Human Genetic Resources and Disease Control in China (Harbin Medical University), Ministry of Education (LPHGRDC2020-006).

Availability of data and materials
The datasets generated during the current study are available in the NCBI repository, PRJNA1152717 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1152717).

Declarations

Ethics approval and consent to participate
The animal experiments were approved by the Ethics Committee of Harbin Medical University (HMUIRB20170037) and performed according to the Guidelines of Laboratory Animal Usage of Harbin Medical University.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 1 April 2024 Accepted: 3 September 2024
Published online: 11 September 2024

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