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# GFPT1 accelerates immune escape in breast cancer by modifying PD-L1 via O-glycosylation

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## Abstract

**Background** Immune escape is one of the causes of poor prognosis in breast cancer (BC). Glutamine-fructose-6-phosphate transaminase 1 (GFPT1) is the first speed-limiting enzyme of the hexosamine biosynthesis pathway (HBP) and is essential for the progression of BC. Nevertheless, the mechanism of the influence of GFPT1 in BC immune escape is not clear.

**Methods** First, the level of GFPT1 in BC was analyzed by starbase, and GFPT1 expression in BC tissues was measured by qRT-PCR, western blot and IHC. Then, the O-GlcNAc levels were detected by western blot. Thereafter, Co-IP was applied to examine the relationship between GFPT1 and PD-L1. At last, a mouse model was constructed for validation in vivo.

**Results** Firstly, we discovered that GFPT1 was obviously strengthened in BC. Knockdown or introduction of GFPT1 correspondingly degraded and elevated O-GlcNAc levels in cells. Further researches revealed that there was a reciprocal relationship between GFPT1 and PD-L1. Mechanistically, we disclosed that GFPT1 enhanced PD-L1 protein stability through O-glycosylation. More interestingly, GFPT1 accelerated BC cell immune escape via upregulation of O-glycosylation-modified PD-L1. In vivo, silencing of GFPT1 attenuated immune escape of BC cells by reducing PD-L1 levels.

**Conclusion** GFPT1 promoted BC progression and immune escape via O-glycosylation-modified PD-L1. GFPT1 may be a potential target for BC therapy.

**Keywords** Breast cancer, GFPT1, PD-L1, O-glycosylation, Immune escape

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## Introduction

Breast cancer (BC) is a highly heterogeneous malignant tumor, the incidence of which ranks first among female malignant tumors in China and is raising year by year. The age of onset of BC patients in China is also becoming younger, even earlier than that of female patients in developed countries [1]. Traditional surgery, chemotherapy, radiotherapy and targeted therapy are the main treatments for BC, while the complexity of the tumor makes the treatment effect vary among patients, and cancer recurrence or metastasis still exists with the current treatments [2]. Immunotherapy for BC has attracted a great deal of attention as an emerging therapy that markedly improves the treatment outcome of patients. However, immune escape produced during therapy poses a fresh challenge to its clinical effectiveness [3]. Thereby, there is an urgent need to find novel molecular markers to raise patient prognosis.

Metabolic disorders are one of the prominent features of tumorigenesis and development [4]. The hexosamine biosynthetic pathway (HBP) is a vital route in glucose metabolism, which plays a crucial role in stimulating tumorigenesis and accelerating cancer proliferation [5]. Glutamine-fructose-6-phosphate transaminase 1 (GFPT1), the first rate-limiting enzyme in HBP, has received multiplying attention in cancer. It was reported that GFPT1 was aberrantly showed in many malignancies such as BC [6], prostate cancer [7], and pancreatic cancer [8]. At the same time, GFPT1 plays an essential part in tumor development and immune escape. For instance, GFPT1 prohibition in tumor cells enlarged T cell responses and NK cell killing of tumor cells [9]. Facilitated GFPT1 expression helped to defend tumor cells from immune attack by enhancing protein glycosylation and diminishing lymphocyte infiltration [10]. Nevertheless, the potential mechanism of GFPT1 in BC immune escape was unclarity and needs further investigation.

Programmed death ligand 1 (PD-L1), a key immune checkpoint in the immune escape process, raises tumor resistance by binding to its receptor programmed death protein-1 (PD-1), thereby inhibiting T lymphocyte proliferation and inducing tumor immune escape [11]. PD-L1, also called as CD274 or B7-H1, has been implicated in BC, melanoma, lung cancer, endometrial cancer, and many other solid tumors are dramatically heightened [12]. Clinically applied inhibitors hampered PD-1/PD-L1 linkage by depressing PD-L1 expression, sensitizing tumor cells to T cell killing [13]. The use of PD-1/PD-L1 inhibitors clearly improved cancer treatment, whereas the cure rate is not high and the exact mechanism is not clear.

Along with the in-depth study of PD-L1 function and structure, it was disclosed that PD-L1 protein has glycosylation sites [14]. Glycosylation is a post-translational modification of proteins and takes a vital part in regulating protein structure and function. GFPT1 is the speed-limiting enzyme of the HBP route, which engenders uridine diphosphate-N-acetyl-β-glucosamine, a precursor of glycosylation, and is necessary for PD-L1 protein stability. Nevertheless, whether GFPT1 mediates BC immune escape through glycosylated PD-L1 remains unknown.

In summary, the aim of this research was to reveal the mechanism of GFPT1 in BC immune escape. Our research disclosed high expression of GFPT1 in BC tissues and GFPT1 enhanced the level of O-GlcNAc. Further analysis declared that GFPT1 interacted with PD-L1, and GFPT1 facilitated the O-GlcNAc level of PD-L1, improved the PD-L1 protein stability and induced immune escape of BC cells. Our study may provide an effective target for immune escape of BC cells.

## Materials and methods

### Cell culture

Human normal breast epithelial cells (MCF-10A) and BC cells (BT474, MCF-7 , T-47D and MDA-MB-231) were gained from the Chinese Type Culture Conservatory (Wuhan, China). Cells were fostered in DMEM medium (Gibco, USA) increased with 10% FBS, 100 U/ml penicillin and streptomycin, and incubated in a humidified incubator at 37℃ containing 5% $CO_2$. Subsequent experiments were performed when the cells were in good condition.

### Real-time fluorescence quantitative PCR (qRT-PCR)

Total RNA was extracted from BC tissues by the Tissue RNA Purification Kit (EZBioscience). RNA reverse transcription of cDNA was conducted using 4×EZscript Reverse Transcription Premix (EZBioscience). qRT-PCR was carried out via the QuantStudio™ 7 Flex Real-Time Fluorescent Quantitative PCR System. GAPDH was used as an internal control. Primer sequences were as follows.

GFPT1 F: 5'AACTACCATGTTCCTCGAACGA3';
GFPT1 R: 5'CTCCATCAAATCCCACACCAG3'.
PD-L1 F: 5'TGGCATTTGCTGAACGCATTT3';
PD-L1 R: 5'TGCAGCCAGGTCTAATTGTTTT3'.
GAPDH F: 5'GGAGCGAGATCCCTCCAAAAT3';
GAPDH R: 5'GGCTGTTGTCATACTTCTCATGG3'.

### Western blot assay (WB)

40 mg of BC tissue was taken in EP tube, washed with PBS and added with protein lysate to extract total protein. Protein concentration was determined using BCA kit (Beyotime, China). Samples were loaded based on the experimental requirements and the gel electrophoresis was performed in an electrophoresis tank filled with mixed electrophoresis buffer. After that, the protein spots on the gel were transferred to the PVDF membrane. The

PVDF membrane was removed at the end of the transfer and washed for 1 min. The membranes were closed with 5% skimmed milk powder at 37℃ for 2 h. Anti-GFPT1 (1:1000), anti-PD-L1 (1:1000), anti-O-GlcNAc (1:1000), anti-RL2 (1:1000), and anti-GAPDH (1:5000) primary antibody (Proteintech, China) used in this test was mixed and incubated with the membrane at 4℃ overnight. Thereafter, the membranes were washed three times with TBST and incubated with HRP-conjugated goat anti-rabbit secondary antibody (1:10,000, Proteintech, China) diluted in TBST for 2 h at room temperature. After adding the ready-to-use ECL chemiluminescence reagent for color development, the protein signal was visualized with the ECL detection system. Image J was used to analyze the protein grayscale.

#### Immunohistochemistry (IHC)

The specimens were cut into 4 μm sections after tumor tissues were fixed with 10% formaldehyde and embedded in paraffin. The tissue slides were dewaxed with xylene after drying in an oven at 60℃ for 1 h. Next, the sections were placed in citric acid antigen repair solution for antigen repair and then the slides were washed and incubated with 3% $H_2O_2$ for 20 min to quench endogenous peroxidase activity. Then, the slides were closed with 10% goat serum. After 30 min, the closure solution was discarded, primary antibody was added and incubated overnight at 4℃. After the primary antibody incubation, the secondary antibody was added and incubated at 37℃ for 1 h. After incubation, the slides were washed with PBS, followed by the addition of DAB chromogenic solution and re-stained with hematoxylin after completion of chromogenic development. Finally, the sections were dehydrated in 75% alcohol-85% alcohol-anhydrous ethanol-xylene, retrieved and dried. The sections were observed microscopically under a light microscope, and images were acquired and analyzed.

#### Stable knockdown of short hairpin RNA and overexpression plasmids

Cells were transfected with lentiviral vector by Lipofectamine 2000 (Invitrogen). BC cell lines with good growth status were selected and inoculated in 24-well plates at $5\times10^4$ cells per well for 12 h. 0.5 ml of complete medium containing 6 μg/ml Polybrene was increased to each well. The lentivirus was removed and melted in a low temperature environment, and the cells were transfected in plates. After 4 hours of transfection, 0.5 ml of fresh complete medium was added. Continuing transfection for 48 h, the complete medium was changed to 2 μg/ml puromycin and screened continuously for 2 weeks to obtain stably transfected cell lines. MCF-7 and T-47D cells were transfected with GFPT1 overexpression plasmids (Genepharma, China) or control plasmids (Genepharm, China) by Lipofectamine 2000 (Invitrogen).

#### Co-immunoprecipitation (Co-IP)

Co-IP analysis was performed following the instructions of the Pierce Crosslink Magnetic IP/Co-IP Kit (Thermo, USA). Cells were lysed in RIPA buffer and incubated with protein A/G magnetic beads coated with 5 μg of antibody at 4 °C overnight. The antibody-crosslinked magnetic beads were then washed with PBS and eluted with elution buffer. Finally, the eluted complexes were analyzed by WB.

#### Ubiquitination assay

After collecting the cells, 100 μL of lysate containing 1% SDS was added and treated for 10 min, 900 μL of lysate was added to dilute SDS, and the corresponding antibody and Protein G-coupled magnetic beads were added for immunoprecipitation. The latter operation was consistent with WB.

#### Protein stability analysis

Treat with cycloheximide (CHX) at a concentration of 25 μg/mL for 0, 4, 8, 12, and 24 h to inhibit protein synthesis. After incubation, changes in the half-life of PD-L1 protein were measured by immunoblotting analysis.

#### Lactate dehydrogenase (LDH) assay

LDH levels were estimated based on the instructions of the LDH Cytotoxicity Assay Kit (Shanghai, China) to assess cytotoxicity. MCF-7 and T-47D cells were inoculated in 96-well plates. After treatment, the medium in the cells was transferred to a new 96-well plate, incubated with LDH working reagent for 30 min, and tested based on the manufacturer's instructions.

#### Xenograft tumor model

Immunocompetent mice (C57BL/6J female mice, 6 weeks of age) were acquired from Beijing Huafu Kang Biotechnology Company and housed in the SPF laboratory animal room. All animals were used in line with the Institute guidelines, and assays were permitted by the Institute's Animal Use Committee. For subcutaneous inoculation, cells transfected with sh-NC and sh-GFPT1 were resuspended in PBS. Next, cells were injected into 6-week-old mice ($n=6$) at a concentration of $2\times10^7$/ mL. Tumors were measured every 7 days after emergence, and tumor volume was calculated according to the formula (length × width$^2$)/2. After 35 days, mice were anesthetized by intraperitoneal injection of 1% sodium pentobarbital (80 mg/kg) and then euthanized by cervical dislocation. Cervical dislocation is a rapid and painless euthanasia method for mice. Finally, the tumor tissue was stripped,

photographed and weighed. Then, the volume of the mouse transplanted tumor was calculated.

### Statistical analysis

All data were conducted in three independent trials. Data were displayed as mean ± standard deviation, and were analyzed by GraphPad Prism 8.2 using an unpaired t test. $p < 0.05$ was considered statistically significant.

## Results

### GFPT1 expression is raised in BC

To explore the part of GFPT1 in BC development, we first analyzed the GFPT1 mRNA levels in BC tissues and normal breast tissues through the starbase website. The results disclosed that GFPT1 was abnormally heightened in BC tissues (Fig. 1A). Then, qRT-PCR was applied to examine the GFPT1 level in 62 cases of BC tissues collected from our hospital with the corresponding paracancerous tissues. The findings revealed that the GFPT1 mRNA was clearly higher in BC tissues than paraneoplastic tissues (Fig. 1B). The GFPT1 expression in BC tissues and the corresponding paraneoplastic tissues was further verified via WB and immunohistochemistry experiments. The assay results confirmed that the level of GFPT1 in BC tissues was obviously magnified contrast with the paracancerous tissues (Fig. 1C, D). Furthermore, we discovered that GFPT1 expression was elevated in BC cell lines, with MCF-7 and T-47D being the most elevated. Therefore, MCF-7 and T-47D were used for subsequent experiments (Figure S1). The above findings proved that GFPT1 was markedly enlarged in BC tissues. Next, to assess the pathological meaning of GFPT1 in BC tissue, the clinicopathological characteristics of the patients were analyzed. The outcomes showed that GFPT1 was considerably correlated with AJCC stage, T stage and differentiation, and GFPT1 was not noticeably linked to tumor size, age and sex (Table 1).

### GFPT1 alters PD-L1 expression by facilitating O glycosylation

To research the mechanism of GFPT1 in BC, the transfection efficiency of GFPT1 after knockdown or insertion in MCF-7, T-47D cells was first examined by qRT-PCR. The findings declared that GFPT1 was evidently decreased in MCF-7 and T-47D cells after silencing GFPT1, while GFPT1 expression was obviously enhanced in MCF-7 and T-47D after introduction of GFPT1, indicating that the transfection was successful (Fig. 2A, B). Next, the effects of GFPT1 knockdown or overexpression on O-GlcNAc modification level in cells were examined. WB results displayed that the level of O-GlcNAc modification was clearly declined after

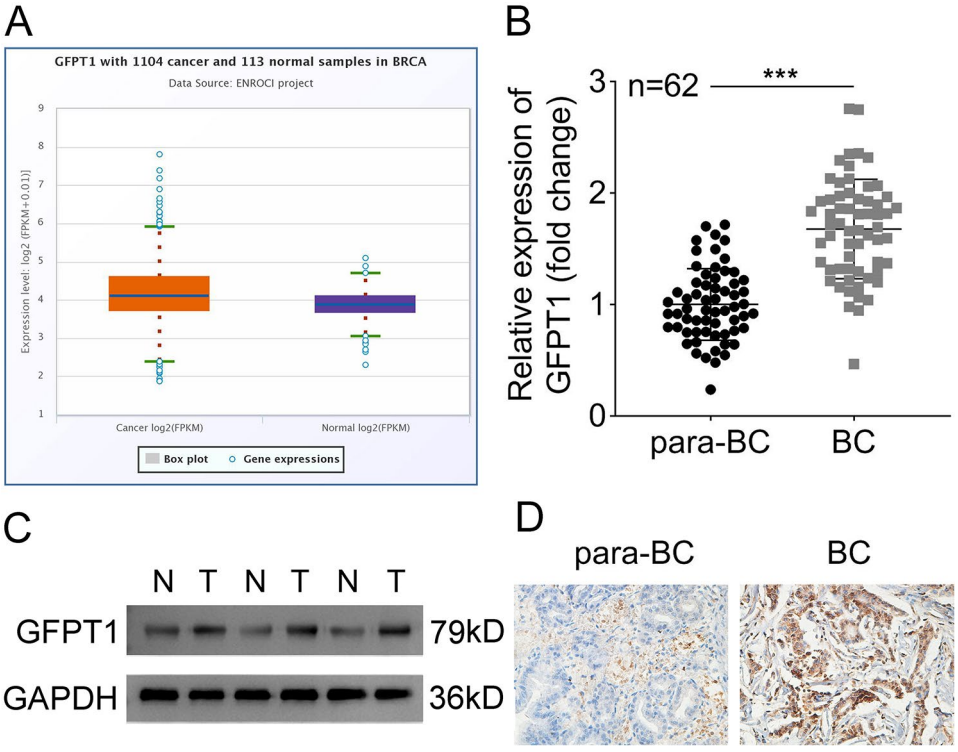


**Fig. 1** Expression of GFPT1 in breast cancer (BC). (**A**) starbase analysis of GFPT1 expression in BC. (**B**) qRT-PCR was used to detect the expression of GFPT1 in paracancer tissues and cancer tissues of BC patients. (**C**) Western blot (WB) was used to measure the GFPT1 protein levels in paracancer tissues and cancer tissues of BC patients. N: Normal; T: Tumor. (**D**) The GFPT1 expression in paracancer tissues and cancer tissues of BC patients were detected by immunohistochemistry (IHC)

**Table 1** Clinicopathologic characteristics of study subjects

| Clinicopathologic characteristics | *n* | Low | High | *p*-value |
|---|---|---|---|---|
| Age (years) | | | | 0.7794 |
| <65 | 28 | 13 | 15 | |
| ≥65 | 34 | 17 | 17 | |
| Sex | | | | 0.9869 |
| Male | 33 | 16 | 17 | |
| Female | 29 | 14 | 15 | |
| AJCC stage | | | | 0.0226* |
| I+II | 30 | 19 | 11 | |
| III+IV | 32 | 11 | 21 | |
| T stage | | | | 0.0228* |
| T1+T2 | 26 | 17 | 9 | |
| T3+T4 | 36 | 13 | 23 | |
| Differentiation | | | | 0.0433* |
| High | 29 | 18 | 11 | |
| Low | 33 | 12 | 21 | |
| Tumor size | | | | 0.6316 |
| <50 mm | 27 | 14 | 13 | |
| ≥50 mm | 35 | 16 | 19 | |

silencing of GFPT1, while the O-GlcNAc modification level was markedly magnified after GFPT1 introduction, implying that overexpression of GFPT1 heightened the level of O-GlcNAc modification in cells (Fig. 2C). Then, the results of Co-IP assay revealed that there was a reciprocal relationship between GFPT1 and PD-L1 (Fig. 2D). To research how GFPT1 regulated the expression of PD-L1 and thus affected the development of BC, we performed WB experiments. The experimental results disclosed that PD-L1 level and RL2 (the O-GlcNAc level of PD-L1) were reduced after GFPT1 silencing, while PD-L1 expression and the O-GlcNAc level of PD-L1 were enlarged after overexpression of GFPT1. It suggested that GFPT1 directly interacted with PD-L1 and changed the level of PD-L1 through O-GlcNAc modification (Fig. 2E). The above outcomes manifested that GFPT1 regulated the O-GlcNAc expression of PD-L1.

### GFPT1 enhances PD-L1 levels and PD-L1 protein stability by heightening O-GlcNAc modification levels

To verify whether GFPT1 affects PD-L1 levels through O-GlcNAc modification, a reversion experiment was carried out. WB results displayed that GFPT1 silencing diminished PD-L1 glycosylation and PD-L1 expression, whereas the addition of Thiamet-G (TMG, O glycosylation promoter) inverted the inhibitory effect of GFPT1 knockdown on PD-L1 glycosylation and PD-L1. It was indicated that GFPT1 affected PD-L1 expression via O-glycosylation (Fig. 3A). Moreover, we discovered that knockdown of GFPT1 decreased the level of ubiquitination modification of PD-L1, which was reversed by the addition of TMG (Figure S5). Next, to study the influence of GFPT1 on PD-L1 protein stability, we added

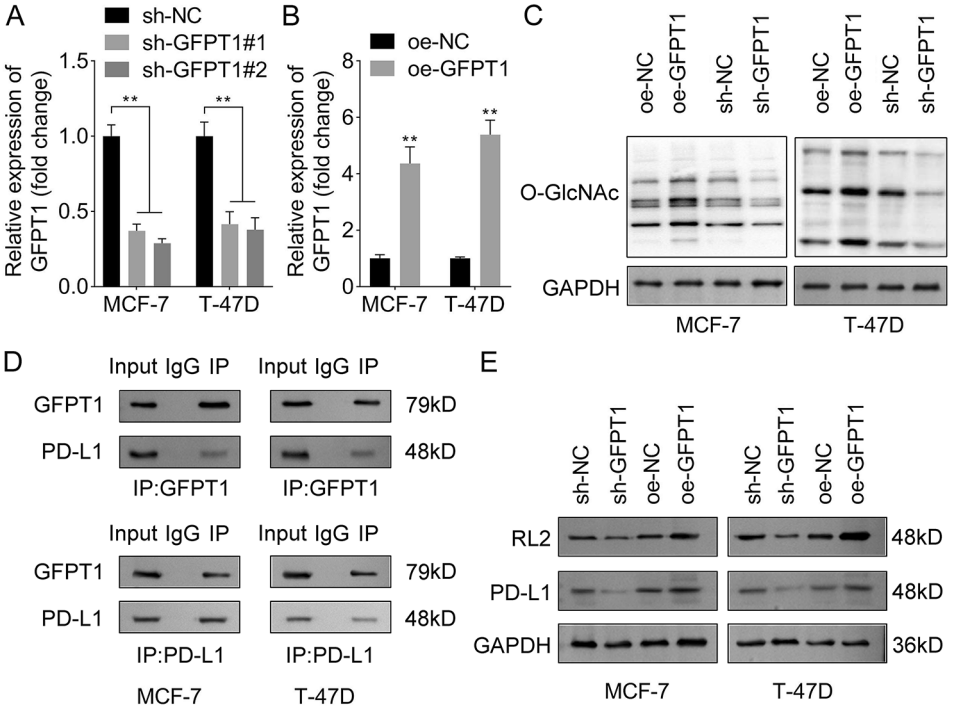


**Fig. 2** GFPT1 binds to PD-L1. (**A, B**) qRT-PCR was used to detect the transfection efficiency of GFPT1 knockdown (**A**) or overexpression (**B**). (**C**) WB was used to measure the protein expression of O-GlcNAc after knockdown or overexpression of GFPT1. (**D**) Co-IP assay was applied to verify the interaction between GFPT1 and PD-L1. (**E**) WB was accustomed to assess the protein expression of RL2 and PD-L1 after knockdown or overexpression of GFPT1 in MCF-7 and T-47D cells

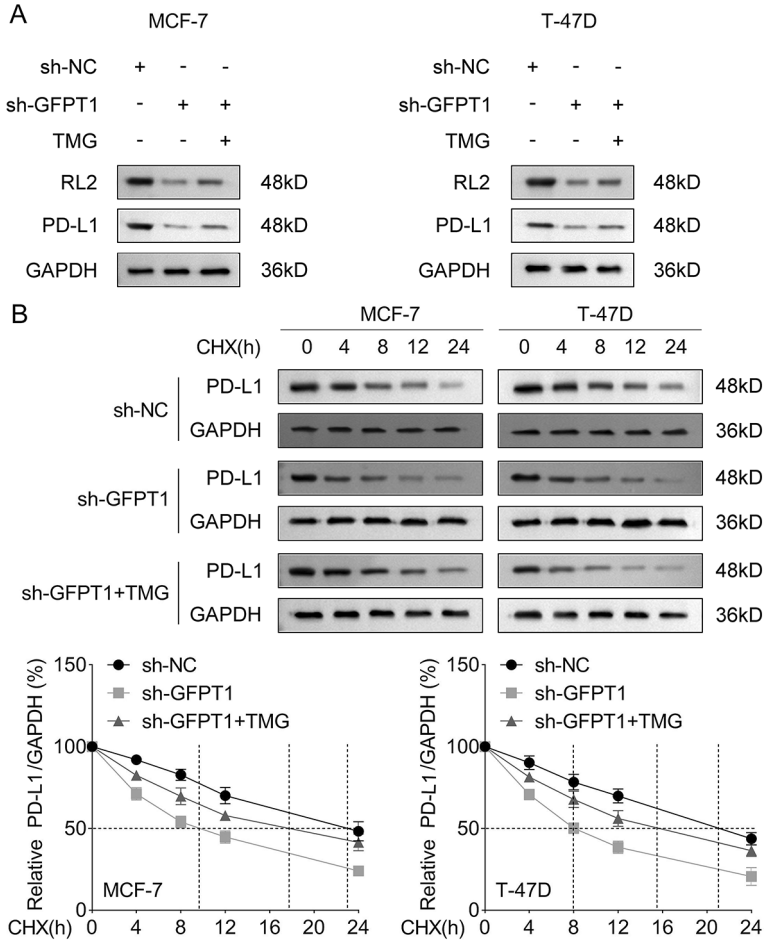


**Fig. 3** GFPT1 enhances PD-L1 levels and PD-L1 protein stability by promoting O-GlcNAc modification levels. (**A**) WB experiments was used to validate the effect of GFPT1 knockdown and TMG addition on RL2 and PD-L1. (**B**) PD-L1 protein level after the addition of cycloheximide CHX at different time points after cell transfection was measured by WB

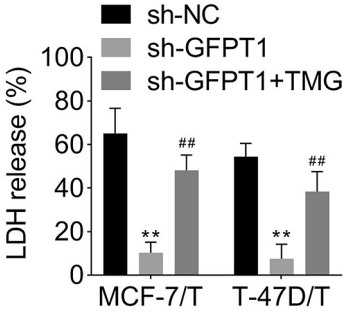


**Fig. 4** Effect of O-glycosylation on BC cells immune escape

CHX to block protein synthesis in BC cells transfected with GFPT1 to observe the effect of GFPT1 on the rate of PD-L1 protein degradation. It was found that the degradation rate of PD-L1 was dramatically strengthened after GFPT1 knockdown, while the addition of TMG rescued this result (Fig. 3B), implying that GFPT1 raised PD-L1 protein stability. The above findings proved that GFPT1 amplified PD-L1 protein stability via O glycosylation.

### GFPT1 elevates BC cell immune escape through accelerating O-glycosylation-modified PD-L1

To study the mechanism of GFPT1 in immune escape, we examined the amount of LDH release in cells. The results disclosed that knockdown of GFPT1 hampered the rate of LDH release, while an increase in TMG returned the inhibitory effect of GFPT1 on LDH release (Fig. 4). The outcomes indicated that GFPT1 facilitated BC cell immune escape through facilitating O-glycosylation-modified PD-L1.

### GFPT1 stimulates immune escape from BC via PD-L1 in vivo

To confirm the impact of GFPT1 on BC tumor growth in vivo, mouse xenograft tumor experiments were performed. Representative pictures of the tumors were shown in Fig. 5A. It was declared that knockdown of GFPT1 caused an evident decline in tumor volume compared to the control group (Fig. 5B). Similarly, the tumor weight after knockdown of GFPT1 was obviously reduced contrast to the control group (Fig. 5C). It was

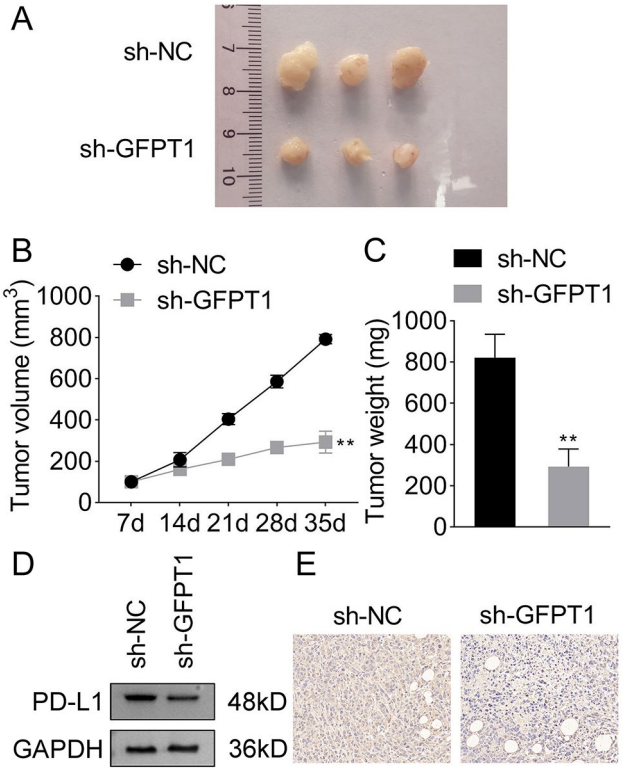


**Fig. 5** GFPT1 promotes tumor growth through PD-L1. (**A**) Representative pictures of tumors. (**B**) Volume change of tumor. (**C**) Weight change of tumor. (**D**) WB assay was used to detect the PD-L1 protein expression after knockdown of GFPT1. (**E**) The PD-L1 expression after knockdown of GFPT1 was measured by IHC

indicated that GFPT1 silencing hampered tumor growth. Next, the impact of GFPT1 knockdown on PD-L1 protein was examined by WB. The outcomes proved that the protein of PD-L1 was clearly depressed after inhibition of GFPT1 (Fig. 5D). The IHC assay further verified that PD-L1 was markedly diminished after GFPT1 silencing (Fig. 5E). Furthermore, we disclosed that overexpression of GFPT1 enhanced tumor volume and weight, which was reversed by oe-GFPT1 and anti-PD-L1 (Figure S7 A-C). WB results discovered that PD-L1 protein expression was elevated after overexpression of GFPT1, whereas PD-L1 protein expression was reduced after oe-GFPT1 and anti-PD-L1 treatment (Figure S7 D). The above findings demonstrated that GFPT1 enhanced BC growth and immune escape via PD-L1 in vivo.

## Discussion

In recent years, people have gradually realized the essential of immunotherapy, and tumor immunology research has become one of the hot spots in oncology research. BC is a highly heterogeneous immunogenic tumor, and there are various immune cells in the immune microenvironment, such as T and B lymphocytes and natural killer cells, with T lymphocytes predominating [15]. Immunotherapy of BC has a longer duration of effect compared to traditional treatment modalities and improves the prognosis of patients. However, due to various factors, some tumor cells evade immune surveillance, which affects the immunotherapeutic efficacy. Some studies have found that GFPT1 is associated with BC immune escape, while the effect of GFPT1 mechanism on BC immune escape is not fully understood.

Reprogrammed metabolism is crucial for tumor development. One of the alterations in tumor cell metabolism is the presence of the Warburg effect, which synergistically raises the HBP activity through increased uptake of glucose and glutamine [16]. Although the glucose involved in the HBP pathway represents only a small fraction of intracellular glucose, the pathway is necessary for the survival of tumor cells. It was found that HBP plays a vital part in post-transcriptional modifications of both tumor suppressor genes and oncogenes, such as maintaining the stability of oncogenic proteins or reducing the stability of tumor suppressor proteins for degradation [17]. This pathway converts glucose to glutamine 6-phosphate to generate UDP-GlcNAc, which is converted to O-GlcNAcylated by OGT and ultimately elevates O-GlcNAc in tumor cells. In recent years, studies have found that O-GlcNAc is obviously enhanced in breast, colon and lung cancers, and it accelerates breast cancer cell genesis, invasion and metabolism [18]. The HBP pathway is one of the central hubs of cellular metabolism. As a speed-limiting enzyme of HBP, GFPT1 was intimately related to altered glycosylation in cancer cells. For example, dysregulation of GFPT1 in hepatocellular carcinoma leads to aberrant glycosylation, which results in tumor development [19]. It has also been shown that GFPT1 is linked to immune escape from cancer. For instance, restriction of GFPT1 in lung cancer cells weakens PD-L1 glycosylation and destabilizes PD-L1 protein thereby enhancing the efficacy of lung cancer immunotherapy [9]. In our research, we disclosed that GFPT1 was dramatically enlarged in BC, and the level of O-GlcNAc modification was clearly reduced after knockdown of GFPT1, while O-GlcNAc modification level was markedly increased after GFPT1 overexpression, indicating that GFPT1 facilitated O-glycosylation.

To investigate the mechanism by which GFPT1 affects BC immune escape through glycosylation, GFPT1 was found to have binding sites to PD-L1 by immunoprecipitation assay. PD-L1 is a target for immunotherapeutic intervention and also regulate the biological behavior of tumor cells and participate in the process of tumorigenesis and development [20]. Although the aberrant elevation of PD-L1 in tumors has been confirmed by clinical studies, the exact mechanism was still not clear. It has been displayed those post-translational modifications of PD-Ll affected protein stability, localization and

protein interactions, which in turn influence the immunosuppressive capacity in the tumor microenvironment [21]. Glycosylation is a common post-translational modification of proteins, and the main types of modifications include N-glycosylation and O-glycosylation. N-Glycosylation refers to the process by which a glycan is attached to the free amino group of asparagine in the protein peptide chain, thereby assembling the glycan chain to the protein. O-Glycosylation, also called O-GlcNA glycosylation, refers to the modification of proteins by linking the glycan to the serine or threonine of the protein peptide chain [22]. PD-L1 extracellular has four glycosylation sites, and the glycan structure of PD-L1 impacts the binding of PD-L1 to PD-1, thereby affecting the anti-tumor effect. In glioma, the PD-L1 co-molecular chaperone FKBP51s also enhanced PD-L1 levels through promoting PD-L1 glycosylation. Inhibition of FKBP51s clearly decreased PD-L1 expression [23]. It was also found that epithelial mesenchymal transition induced high expression of the N-glycosyltransferase STT3 in tumor stem cells through the β-catenin signaling axis, which in turn increases PD-L1 glycosylation, leading to PD-L1 upregulation and tumor immune escape in tumor stem cells [24]. MTHFD2 drives the folate cycle to maintain sufficient uridine related metabolites, including UDP-GlcNAc, which enhanced global O-GlcNAylation of proteins, involving cMYC, thereby raising cMYC stability and PD-L1 transcription [25]. In this study, we disclosed that knockdown of GFPT1 suppressed the O-glycosylation of PD-L1 and PD-L1 level, suggesting that GFPT1 accelerated PD-L1 expression through O-glycosylation. TMG inhibits OGA activity and is a much used promoter in glycosylation, often used to study cellular responses affected by O-glycosylation modifications [26, 27]. Then, a reversion assay was performed to verify that the addition of TMG abrogated the prohibitory effect of GFPT1 knockdown on the O-glycosylation levels of PD-L1 and PD-L1 levels. Subsequently, WB assay after adding CHX at different time points discovered that GFPT1 prolonged the half-life of PD-L1. Studies had shown that LDH mediated tumor immune escape by inhibiting the killing effect of immunity and facilitating the suppressive effect of immunity [28]. The results of LDH release rate proved that knockdown of GFPT1 restrained cellular immune escape, whereas TMG addition abolished this phenomenon. Based on the results of previous experiments, xenograft tumor trials in mice were conducted to further verify that GFPT1 amplified immune escape of BC through PD-L1. The results of this study suggested that GFPT1 was an effective therapeutic target in BC antitumor therapy.

## Conclusion

In conclusion, we found that GFPT1 played a main part in facilitating PD-L1 stability via O-glycosylation. Silencing of GFPT1 reduced the level of glycosylated PD-L1 protein to weaken cellular immune escape. Therefore, targeting GFPT1 may be a potential strategy to counteract PD-L1-mediated immunosuppression in BC.

### Abbreviations

| | |
|---|---|
| BC | Breast cancer |
| GFPT1 | Glutamine-fructose-6-phosphate transaminase 1 |
| HBP | Hexosamine biosynthesis pathway |
| PD-L1 | Programmed death ligand 1 |
| PD-1 | Programmed death protein-1 |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-12811-8.

Supplementary Material 1

Supplementary Material 2

### Acknowledgements

Not applicable.

### Author contributions

W.T. designed the study and drafted the manuscript. Y.G. collected data, processed statistical data, and performed the experiments. S.H. analyzed and interpreted the data. S.W. designed, supervised the study, and revised the manuscript. All authors read and approved the final version of the manuscript.

### Funding

Not applicable.

### Data availability

All data used to support the findings of this study are available from the corresponding author upon request.

## Declarations

### Ethical approval

This study was approved by the Ethics Committee of Shandong University. All methods were performed in accordance with the relevant guidelines and regulations. Animal studies were performed in compliance with the ARRIVE guidelines. All animal study methods were performed in accordance with the relevant guidelines and regulations.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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