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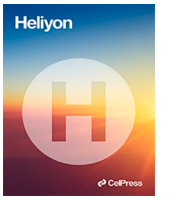

Research article

GABA/baclofen stabilizes PD-L1 and enhances immunotherapy of breast cancer

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ABSTRACT

The programmed death-ligand 1 (PD-L1) on the surface of tumor cells binds to the receptor programmed cell death protein 1 (PD-1) on effector T cells, thereby inhibiting the anti-tumor immune response. Immune checkpoint blockade (ICB) therapy targeting PD-1/PD-L1 has been approved for the treatment of human cancers with lasting clinical benefit. However, many cancer patients did not respond to anti-PD-1/PD-L1 antibody blocking therapy or drugs targeting PD-1/PD-L1. Recent studies have shown that the response to PD-1/PD-L1 blockade may be related to the PD-L1 abundance of tumor cells. Therefore, it is of crucial significance to find drugs to regulate the expression of PD-L1, which can provide new strategies to improve the response rate and efficacy of PD-1/PD-L1 blocking in cancer treatment. Here, we found that GABA and baclofen, upregulates the protein level of PD-L1 by reducing the mRNA and protein levels of *STUB1*, a E3 ubiquitin ligase, thereby decreasing the interaction between STUB1 and PD-L1, and ultimately stabilizing PD-L1. Notably, GABA and baclofen did not affect cell proliferation *in vitro*, while in the treatment of breast cancer in mice, the therapeutic effect of baclofen combined with anti-PD-L1 antibody is significantly better than that of using anti-PD-L1 antibody alone by stimulating tumor infiltration of $CD8^+$ T cells and antitumor immunity. Taken together, we unveiled a previously unappreciated role of GABA/baclofen in stabilizing PD-L1 and enhancing the immunotherapy of breast cancer.

1. Introduction

Immune evasion is one of the most basic characteristics of tumors [1]. To escape immune destruction, the PD-L1-PD-1 pathway is often activated in the tumor microenvironment [2]. In clinical treatment, blockade of PD-L1 or PD-1 has achieved considerable benefits in a variety of human cancers [3,4]. However, there are still many patients who cannot get treatment benefits from it, and the underlying mechanism is still unclear [5,6]. Recent studies have reported that increasing the protein level of PD-L1 is a promising strategy to improve the sensitivity of immunotherapy targeting PD-1 or PD-L1 [7,8]. Atezolizumab is approved by FDA to treat patients with PD-L1 positive breast cancer, which makes it limited in breast cancer treatment. Therefore, searching for drugs that can up regulate PD-L1 level may expand the scope of patients with breast cancer for immunotherapy.

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Because of the excellent therapeutic effect of anti-PD-L1 antibody, the function and regulation of PD-L1 are extensively investigated. It has been reported that CMTM4/6 can suppress STUB1 mediated polyubiquitination of PD-L1 and prevent its lysosomal degradation, thus stabilizing PD-L1, which may improve the effectiveness of current PD-L1/PD-1 blocking therapy [9]. Therefore, broadening our understanding of PD-L1 regulation is of great significance to enhance immunotherapy of cancer.

The major function of γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter in brain [10]. In recent years, studies have reported new functions of GABA, such as GABA metabolism is a regulator of autophagy [11]. However, the relationship between GABA and immunity, especially immunotherapy, is still largely unknown. The GABAB receptor is one of the receptors on the cell membrane that can be activated by GABA. Baclofen is an agonist of GABAB receptor, which is clinically used to treat muscle spasm caused by spinal cord and brain diseases or injuries [12]. Here, we found a new function of baclofen in tumor immunotherapy, which belongs to "conventional drug in new use". Compared with the development of new drugs, it reduces the time and cost of pharmacokinetic and toxicity research, and more importantly, reduces the risk of clinical research failure.

In our study, we demonstrated that GABA up-regulates the expression level of PD-L1. Furthermore, baclofen, an agonist of GABAB receptor, can also increase PD-L1 protein level. Mechanistic research demonstrated that GABA/baclofen decreases the binding of PD-L1 to STUB1, an E3 ubiquitin ligase, by down-regulating the transcription level of *STUB1*, thereby stabilizing PD-L1 and enhancing the therapeutic effect of PD-L1 blocking antibody in mouse model. Collectively, we uncovered a previously unknown GABA/baclofen-STUB1-PD-L1 axis, which regulates PD-L1 stability and provides a new strategy for immunotherapy of breast cancer.

2. Materials and methods

2.1. Cell culture and transfection

MDA-MB-231, HEK293T, H1299 and 4T1 cells were purchased commercially. MDA-MB-231, PETCC140 and HEK293T cells were cultured with DMEM (10% FBS and 1% P/S). H1299 and 4T1 cells were cultured with RPMI 1640. For packaged lentivirus, cells are inoculated into culture dishes, and when the density is 80∼90%, the plasmid (Flag-PD-L1, psPAX2, and pMD2.G in a ratio of 4:3:1) is transfected with EZ-trans (life-iLab). After 8 h of transfection, the medium is replaced, and the medium for 24 and 48 h is collected and then filtered using 0.45 μm filter. The target cells were cultured in a medium with lentivirus for 48 h, and then selected with puromycin. The drugs for treating cells are shown in Table 1.

2.2. Immunohistochemistry

Tumor sections were disaffiliated using deparaffinization buffer. Endogenous peroxidase blocking buffer and citric acid antigen recovery solution were used to eliminate endogenous peroxidase activity and antigen retrieval, respectively. Next, the PBS containing goat serum was used to block the sections for 30 min, and then incubated with the antibodies at 4 °C overnight. After three times of washing with PBS, the sections were incubated with secondary antibodies, and visualized. All antibodies are used as suggested in instructions, including CD8 (HUABIO, 0108-7) and Granzyme B (HUABIO, HA500252).

2.3. Western blot and coimmunoprecipitation

Cells were lysed in Nonidet P-40 lysis buffer containing 1% phosphatase inhibitor and 1% protease inhibitor at 4 °C for 30 min. The cell lysates were heated with SDS-PAGE loading buffer at 100 °C for 10 min and subjected to Western blot. The experimental steps for Western blot refer to our previous work [13]. The antibodies used in this study include PD-L1 (CST, 51296), mouse-PD-L1 (Bioxcell, BE0101), STUB1 (Abcam, ab134064), Cul3 (Abcam, ab75851), ARIH1 (ABclonal, A17123), SPOP (Abcam, ab192233), HA (AbHO, HOA012HA01), Flag (AbHO HO, A012FL01).

2.4. Quantitative real-time PCR

Total RNA was isolated using EZ-press RNA Purication Kit (EZBioscience). cDNA was synthesized from purified RNA using gDNA remover plus 4 × Reverse Transcription Master Mix (EZBioscience). qRT-PCR was performed on the Applied Biosystem 7300 plus Sequence Detection System. The sequences of primers used for qRT-PCR are shown in Table 2.

Table 1
Drugs used to treat cells.

Drug	Source	Category No.
γ-Aminobutyric acid	MCE	HY-N0067
Baclofen	MCE	HY-B0007
Valnoctamide	MCE	HY-121877
Picrotoxin	MCE	HY-101391
IFN-gamma	MCE	HY-P7025A
CGP35348	MCE	HY-103530

2.5. Detection of cell surface PD-L1

Digest tumor cells into single-cell suspension and incubate with anti-mouse CD274 antibody (BioLegend) in the dark for 30 min under ice. Then, wash the cells twice with PBS. Finally, stained cells were analyzed using BD FACSCelesta, and the data was processed with FlowJo.

2.6. Animal experiments

BALB/C female mice (6- to 8-wk-old) were purchased from BiKai Laboratory Animals. 4T1 cells (1×10^5) were injected into the fat pads of the mammary glands of mice. Treatment was given seven days (with a tumor volume of approximately 38 mm^3) after injecting cells into mice. Each mouse was intraperitoneally injected with baclofen (25 mg/kg) per day and anti-PD-L1 antibody (100 μg) every three days. Tumor volume was calculated as follow: length/2 × $width^2$. When the tumor tissue of the mice is about to reach 1.5 cm in any direction, or there are signs of pain, or the presence of the tumor affects the activity and eating of the mouse, the mouse is euthanized.

3. Results

3.1. GABA increases PD-L1 protein level

To find out whether GABA regulate PD-L1 expression, we determined the effect of GABA treatment on PD-L1. Intriguingly, GABA significantly increases PD-L1 protein level in breast cancer cells (Fig. 1A). To make sure of this conclusion, we treated MDA-MB-231 cells with GABA for different times, and found that the PD-L1 level was increased in a time-dependent manner (Fig. 1B). In line with this, when cells were treated with different concentrations of GABA, the expression of PD-L1 increased in a dose-dependent manner (Fig. 1C). Similarly, GABA also increases PD-L1 protein level in H1299 cells (Fig. 1D). Together, GABA treatment significantly increases PD-L1 expression level.

3.2. Baclofen increases PD-L1 protein level

GABA receptors include GABAA and GABAB [14]. To further explore through which receptor GABA up-regulates PD-L1 protein level, several inhibitors or agonists of GABA receptors were used in the following experiments. Notably, baclofen, a GABAB receptor agonist, significantly upregulated PD-L1 protein level (Fig. 2A), whereas valnoctamide, GABAA receptor agonist (Fig. 2B), or picrotoxin, GABAA receptor antagonist (Fig. 2C), has no effect, demonstrating GABA may up-regulate PD-L1 level through GABAB receptor. To further illustrate our point, we used a GABAB receptor antagonist (CGP35348), and found that CGP35348 significantly abolished the upregulation of PD-L1 by GABA and baclofen (Fig. 2D and E). This further demonstrates that GABA and baclofen regulate PD-L1 expression through GABAB receptor. Furthermore, baclofen upregulated PD-L1 level in a time- (Fig. 2F) and dose-dependent manner (Fig. 2G). Similarly, baclofen also upregulated PD-L1 level in H1299 cells (Fig. 2H and I). These data demonstrate that baclofen treatment increases the protein level of PD-L1.

3.3. GABA/baclofen positively regulates the stability of PD-L1

To find out the mechanism by which GABA/baclofen increase the protein level of PD-L1, we examined whether GABA/baclofen regulated the mRNA level of *PD-L1*. Interestingly, neither GABA nor baclofen changed *PD-L1* mRNA level (Fig. 3A and B). An attractive possibility is that GABA/baclofen might affect the stability of PD-L1. To examine this assumption, we screened the expression changes of the genes that have been reported to affect the PD-L1 stability when treated with GABA or baclofen. These include *CMTM4* [9,15], *STT3A* [16], *STT3B* [16], *OUTB1* [17], *CDK4* [18], *CDK6* [18], *Usp8* [19], *STUB1* [9], *Cul3* [18], *SPOP* [18] and *ARIH1* [20]

Table 2
Primers used for qRT-PCR analysis.

Primers	Forward 5′–3′	Reverse 5′–3′
β-actin	GGCATAGAGGTCTTTACGGATGTC	TATTGGCAACGAGCGGTTCC
PD-L1	TGGCATTTGCTGAACGCATTT	TGCAGCCAGGTCTAATTGTTTT
STUB1	AGCAGGGCAATCGTCTGTTCT	CAAGGCCCGGTTGGTGTAATA
Cul3	TGTGGAGAACGTCTACAATTTGG	GCGCCTCTGTCTACGACTT
SPOP	GCCCCGTAGCTGAGAGTTG	ACTCGCAAACACCATTTCAGT
ARIH1	GCCGGACGATGATACCCTG	TCGTAGCGGTAATCCTCCTCC
OUTB1	TCGGTCCTATACAAGGAGTATGC	GGTCTTGCGGATGTACGAGT
Usp8	TTCCATTCAATACTTGGACCTGG	CCAAAGAGCCTTTAGCCAATGT
STT3A	TTGGGACGAATCATTGGAGGA	GTAAGGTGGTACGTGACGATG
STT3B	AGTAGGTGGTACTGTTTACCCAG	AAGTTGGTGCAAGGAACACAC
CDK4	ATGGCTACCTCTCGATATGAGC	CATTGGGGACTCTCACACTCT
CDK6	GCTGACCAGCAGTACGAATG	GCACACATCAAACAACCTGACC
CMTM4	CAAGGTCGCCCAAGTGATCTT	GTGCAGGTTGAGACTGAACATA

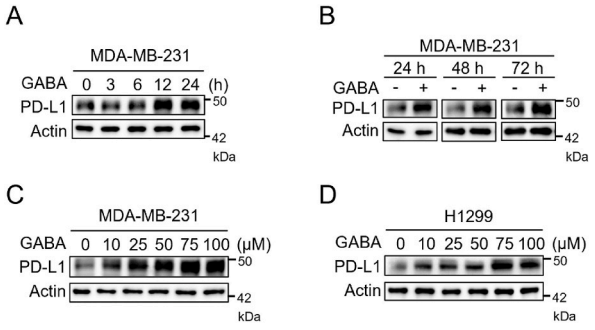

Fig. 1. GABA increases PD-L1 protein level. (**A**) Western blot analysis of PD-L1 protein level in MDA-MB-231 cells treated with or without GABA (100 μM) as indicated for 24 h, 48 h or 72 h. (**B**) Western blot analysis of PD-L1 protein level in MDA-MB-231 cells treated with 100 μM GABA for different time as indicated. (**C** and **D**) Western blot analysis of PD-L1 protein level in MDA-MB-231 cells (C) and H1299 cells (D) treated with different concentrations of GABA as indicated for 48 h.

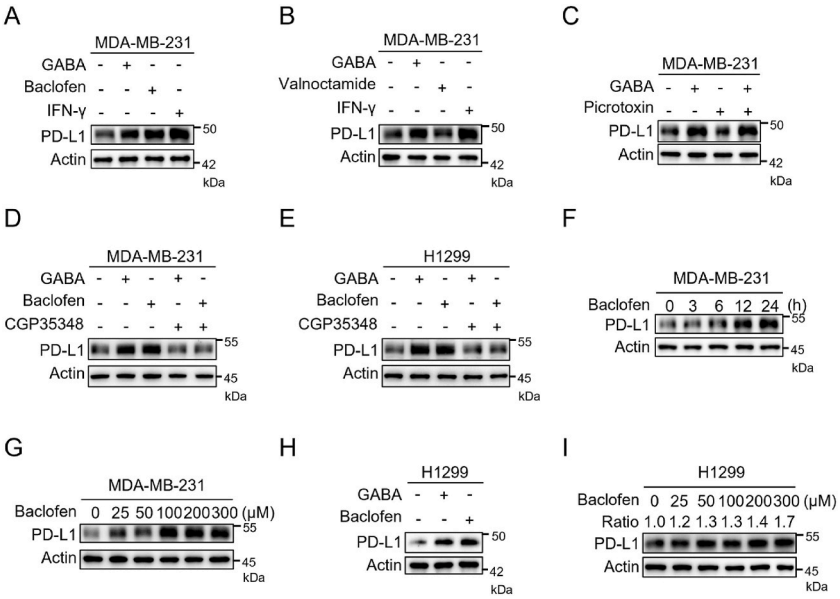

Fig. 2. Baclofen increases PD-L1 protein level. (**A**) Western blot analysis of PD-L1 protein level in MDA-MB-231 cells were incubated with GABA (100 μM, 48 h), baclofen (100 μM, 48 h) or IFN-γ (100 ng/mL, 24 h). IFN-γ serves as a positive control. (**B**) Western blot analysis of PD-L1 protein level in MDA-MB-231 cells were incubated with GABA (100 μM, 48 h), valnoctamide (455 μM, 48 h) or IFN-γ (100 ng/mL, 24 h). IFN-γ serves as a positive control. (**C**) Western blot analysis of PD-L1 protein level in MDA-MB-231 cells were incubated with GABA (100 μM, 48 h), pitcrotoxin (100 μM, 50 h). (**D and E**) Western blot analysis of PD-L1 protein level in MDA-MB-231 and H1299 cells treated with baclofen (100 μM, 48h), GABA (100 μM, 48 h) or CGP35348 (100 μM, 60 h, 12 h earlier than other drugs). (**F**) Western blot analysis of PD-L1 protein level in MDA-MB-231 cells treated with 100 μM baclofen for different time as indicated. (**G**) Western blot analysis of PD-L1 protein level in MDA-MB-231 cells treated with different concentrations of baclofen as indicated for 48 h. (**H**) Western blot analysis of PD-L1 protein level in H1299 cells were incubated with GABA (100 μM, 48 h) or baclofen (100 μM, 48 h). (**I**) Western blot analysis of PD-L1 protein level in H1299 cells treated with different concentrations of baclofen as indicated for 48 h.

(Fig. 3C–M). Notably, GABA/baclofen significantly reduced the transcription levels of *STUB1, Cul3, SPOP* and *ARIH1* (Fig. 3J–M). These data indicate that GABA/baclofen may positively regulate PD-L1 stability.

3.4. GABA/baclofen stabilizes PD-L1 by reducing the binding of PD-L1 to STUB1

In view of the results that GABA/baclofen reduced the mRNA levels of *STUB1, Cul3, SPOP* and *ARIH1* (Fig. 3J–M), we further examined the protein levels of these genes under GABA/baclofen treatment. Interestingly, we found that the protein levels of STUB1, Cul3 and SPOP were significantly reduced, while ARIH1 protein level did not significantly changed (Fig. 4A and B). It has been reported that STUB1 [9], Cul3 [18], SPOP [18] and ARIH1 [20] regulate the stability of PD-L1. Further interaction assay revealed that GABA or baclofen treatment significantly reduced the interaction between PD-L1 and STUB1, while its interaction with Cul3, SPOP or

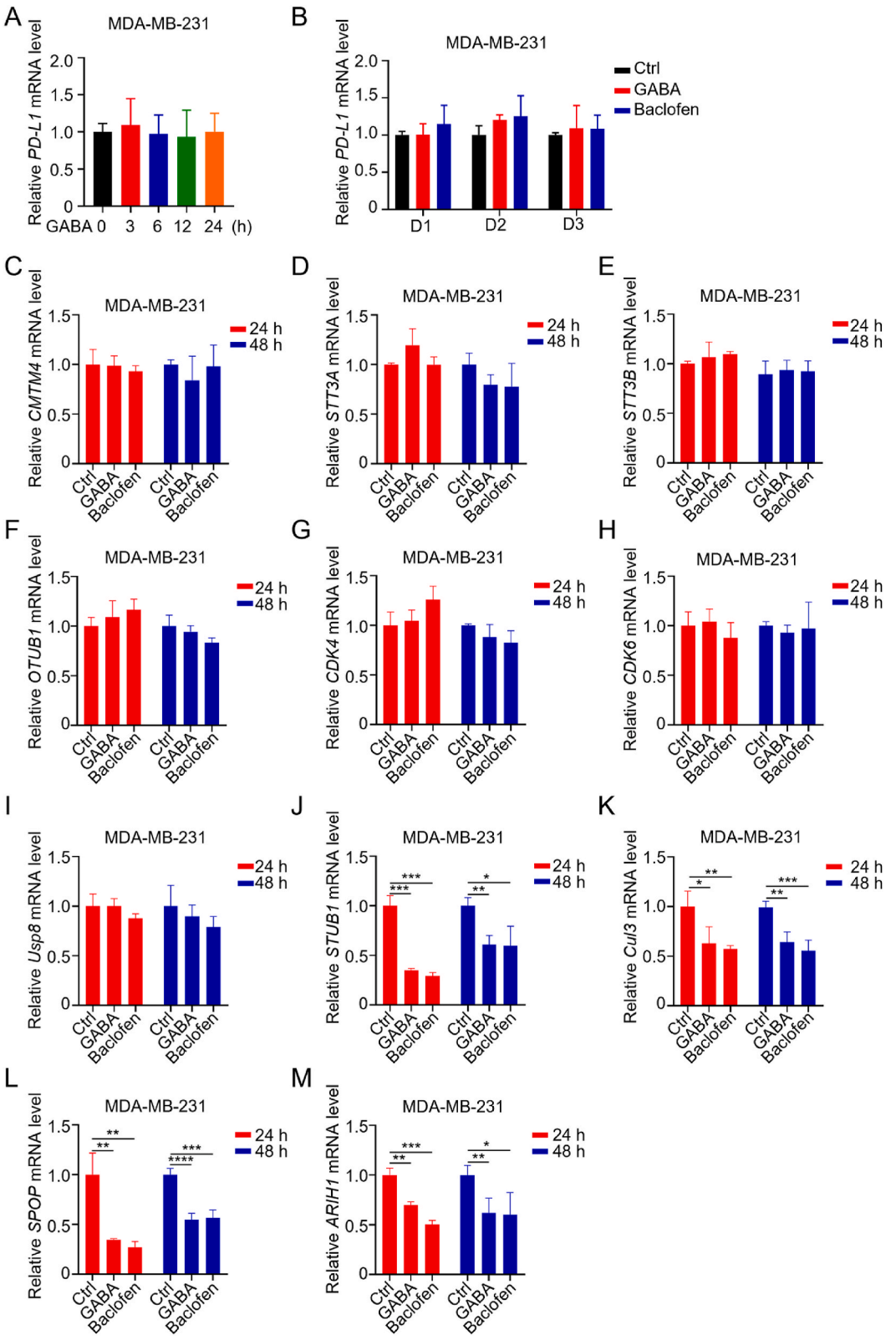

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Fig. 3. GABA/baclofen positively regulates the stability of PD-L1. (**A** and **B**) qRT-PCR analysis of *PD-L1* level in MDA-MB-231 cells treated with GABA (100 μM) or baclofen (100 μM) for different time as indicated. Values are means ± SD from n = 3 independent experiments. (**C**–**M**) qRT-PCR analysis of *CMTM4, STT3A, STT3B, OUTB1, CDK4, CDK6, Usp8, STUB1, Cul3, SPOP* and *ARIH1* levels in MDA-MB-231 cells treated with 100 μM GABA or baclofen for 24 and 48 h. Values are means ± SD from n = 3 independent experiments. Statistical differences were determined by Student's *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

ARIH1 remained unchanged (Fig. 4C). These data demonstrate that STUB1 involves in the process of GABA/baclofen induced stabilization of PD-L1. To confirm this result, we checked the ubiquitination level of PD-L1 upon GABA/baclofen treatment. Result showed that the ubiquitination level of PD-L1 was significantly reduced upon GABA/baclofen treatment (Fig. 4D), which was consistent with the result that GABA/baclofen upregulated PD-L1 protein level. These results imply that GABA/baclofen stabilize PD-L1 by reducing the binding of PD-L1 to STUB1.

3.5. Baclofen enhances immunotherapy for breast cancer in vivo

Recent studies revealed that the efficacy of PD-L1/PD-1 blockade correlates with PD-L1 level in tumor [8,21]. Therefore, GABA/baclofen may affect the efficacy of immunotherapy. We first tested the impact of GABA and baclofen on the proliferation of

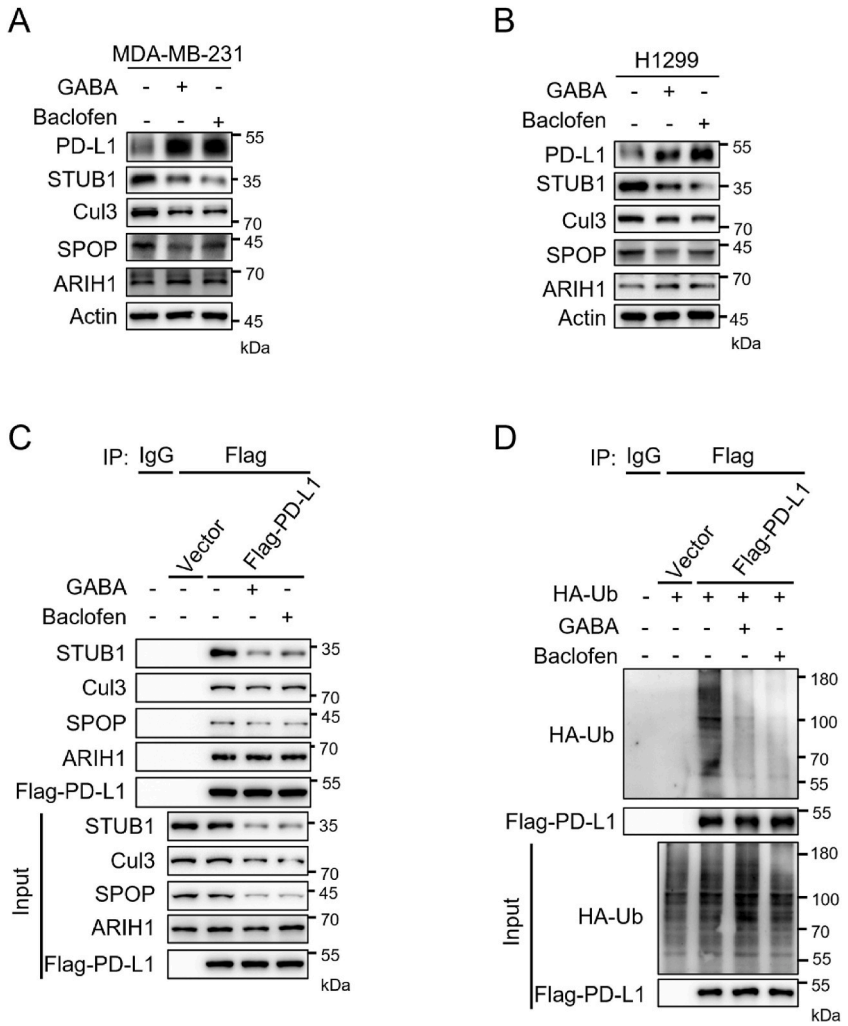

Fig. 4. GABA/baclofen stabilizes PD-L1 by reducing the interaction between PD-L1 and STUB1. (**A** and **B**) Western blot analysis of PD-L1, STUB1, Cul3, SPOP and ARIH1 protein levels in MDA-MB-231 cells (A) and H1299 cells (B) treated with GABA (100 μM) or baclofen (100 μM) for 48 h. (**C**) Assay of PD-L1 interaction with STUB1, Cul3, SPOP and ARIH1 under GABA (100 μM) or baclofen (100 μM) treatment for 48 h in HEK293T cells stably expressing Flag-PD-L1. (**D**) Western blot analysis of PD-L1 ubiquitination level under GABA (100 μM, 48 h) or baclofen (100 μM, 48 h) treatment in HEK293T cells stably expressing Flag-PD-L1. Ubiquitinated PD-L1 proteins were immunoprecipitated with Flag beads and blotted with HA antibody. IgG serves as a negative control.

MDA-MB-231 and PETCC140 cells. Results demonstrated that GABA and baclofen have no effect on the cell proliferation (Fig. 5A and B). We next examined the role of baclofen induced up-regulation of PD-L1 in immunotherapy in mouse model. The tumor bearing mice were treated with baclofen and/or anti-PD-L1 antibody as indicated in Fig. 6A. Intriguingly, the combination of baclofen and anti-PD-L1 antibody markedly repressed tumor progression (Fig. 6B-D). Notably, baclofen significantly enhanced the efficacy of immunotherapy and exhibited a synergetic effect in terms of stimulating tumor infiltration of $CD8^+$ T cells and antitumor immunity (Fig. 6E and F). Moreover, baclofen treatment significantly upregulated PD-L1 levels on the cell membrane surface (Fig. 6G). Collectively, baclofen is a potential drug to enhance the efficacy of immunotherapy.

4. Discussion

Breast cancer is the most common type of tumor in women worldwide [22]. Triple negative breast cancer (TNBC) accounts for about 15~20% of breast cancer [22,23]. Compared with other subtypes, TNBC has the worst prognosis. FDA approved therapeutic drugs targeting PD-L1 can only be used to treat patients with high PD-L1 expression, which greatly limits its application in immunotherapy of breast cancer. Here, we found that GABA/baclofen significantly up-regulated PD-L1 protein level, which indicates that it may be used as an immunomodulatory drug in clinical practice. Baclofen combined with anti-PD-L1 antibody is likely to be used to treat those breast cancer patients with low PD-L1 expression.

GABA is produced by glutamate decarboxylase (GAD) via catalyzing glutamate [24]. Notably, in human or mouse tumor tissues, GABA level and GAD activity are higher than normal tissues, and there is a significant positive correlation between them, which indicates that GABA-ergic system agonists may have a potential regulatory effect on tumor growth [25,26]. In this work, we found a new function of GABA and baclofen in immunotherapy, that is, GABA/baclofen enhances the immunotherapy efficacy by stabilizing the protein level of PD-L1. However, it is still unknown that which signal pathway is responsible for GABA/baclofen induced stabilization of PD-L1, which needs to be thoroughly investigated to clearly demonstrate the underlying mechanism. It is worth noting that studies have shown that baclofen can activate the ERK1/2 signaling pathway [27], but it is unclear whether ERK1/2 is involved in the transcriptional regulation of *STUB1*, *SPOP*, *Cul3*, and *ARIH1* induced by GABA/baclofen. Overall, GABA/baclofen is a highly competitive candidate drug, not only because baclofen significantly enhances the efficacy of immunotherapy, but also because it does not affect the proliferation of MDA-MB-231 and PETCC140 cells (Fig. 5), which may also mean little side effect on the normal breast cells.

The binding of PD-L1-PD-1 suppresses the activation, proliferation and activation of T cells [28]. Therefore, the antagonistic antibody to PD-L1 or PD-1 can be used as an effective immune checkpoint inhibitor (ICIs), which can promote the activation of T cells, recover depleted T cells, and enhance the anti-tumor response mediated by T cells. These drugs have powerful curative effects in some cancer patients, and indeed have completely changed cancer treatment [29]. The anti-PD-L1/PD-1 antibody has become the standard treatment for many types of cancer and has significantly improved the survival rate of patients [29]. GABA/baclofen further enhanced the therapeutic effect of immunotherapy by up-regulating the protein level of PD-L1, which was supported by increased infiltration of $CD8^+$ T cells and expression of granzyme B in tumor (Fig. 6E and F).

It is reported that the stability of PD-L1 protein can be regulated by several PTMs [30]. For example, both glycosylation [31,32] and palmitoylation [33,34] inhibit anti-tumor immunity by stabilizing PD-L1. GSK3β or AMPK regulates the phosphorylation degradation

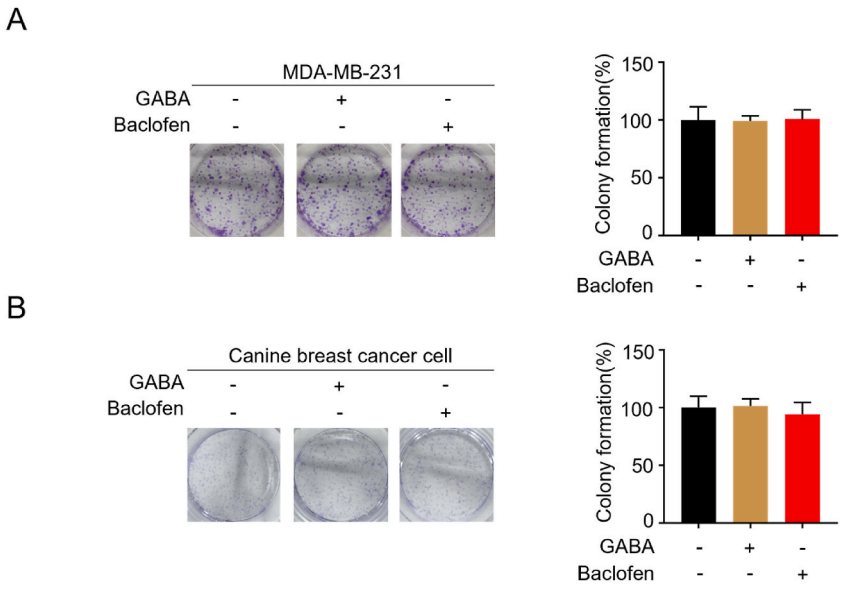

Fig. 5. GABA/baclofen has no effect on the proliferation of human and canine breast cancer cells. (**A** and **B**) The growth of MDA-MB-231 (A) and PETCC140 cells (B) treated with GABA (100 μM) or baclofen (100 μM) by colony formation assay.

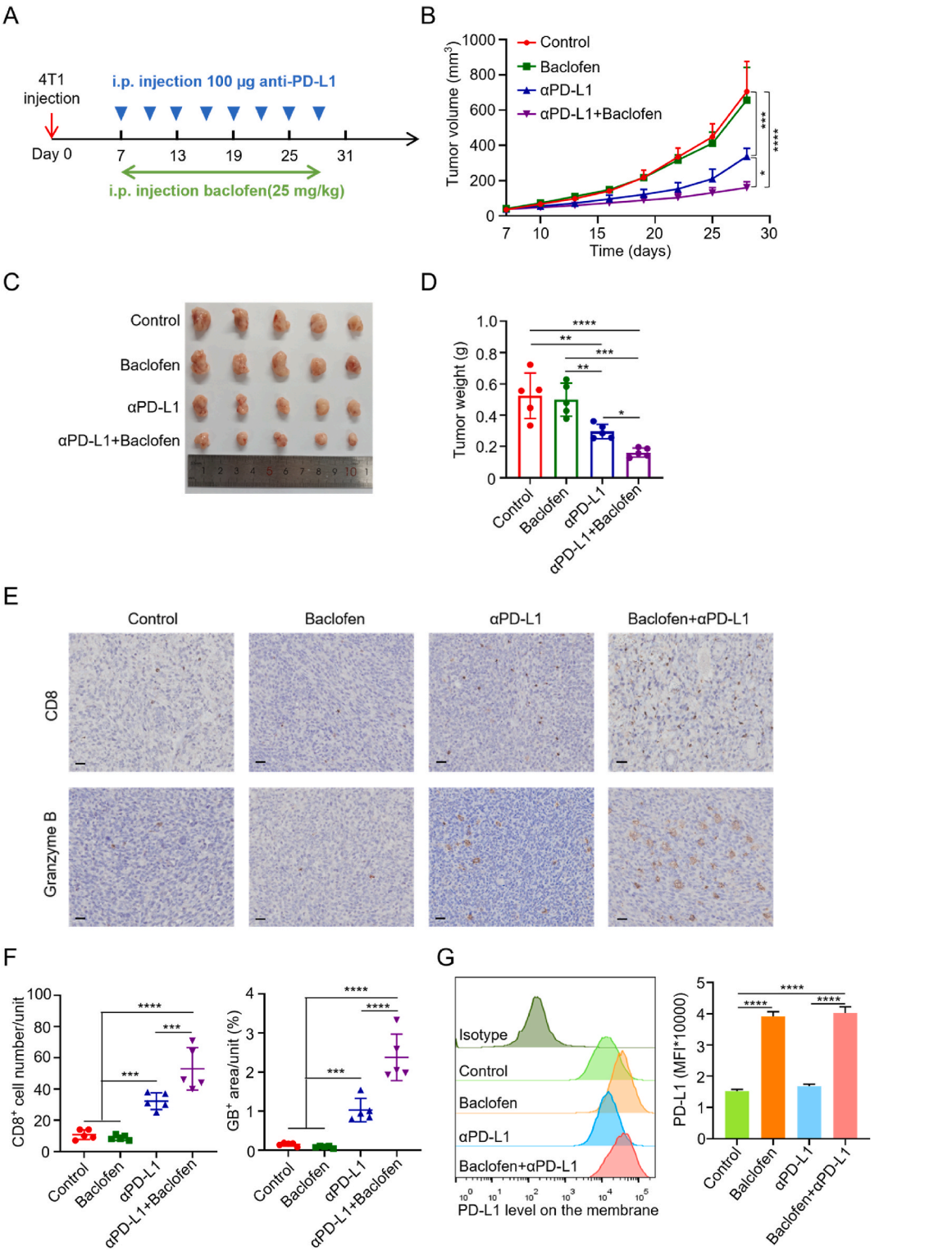

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Fig. 6. Baclofen enhances immunotherapy for breast cancer *in vivo*. (**A**) Schematic representation of the animal experiment process. (**B**) Tumor growth of 4T1 cells in BALB/c mice treated with baclofen or/and anti-PD-L1 antibody was determined. n = 5 mice per group. Statistical differences were determined by ordinary one-way ANOVA. $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (**C**) Representative tumors resected from each group of mice that received different treatment as indicated. (**D**) The weight of tumors resected from each group of mice that received different treatment as indicated was analyzed. Data represent mean ± SD, n = 5 mice per group. Statistical differences were determined by ordinary one-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (**E**) Immunohistochemistry showing $CD8^{+}$ T cell infiltration and granzyme B expression in the 4T1 tumor tissues as indicated (Scale bars, 20 μm). (**F**) Quantifications of images in (E). Data represent mean ± SD from five independent samples of each group. Statistical differences were determined by ordinary one-way ANOVA. $^{***}P < 0.001$, $^{****}P < 0.0001$. (G) Flow cytometry analysis of membrane PD-L1 expression in tumor cells and summarized mean fluorescent intensity (MFI) are shown. Values are means ± SD from n = 3 independent experiments. Statistical differences were determined by one-way ANOVA. $^{****}P < 0.0001$.

of PD-L1 [31,35,36]. Ubiquitylation causes PD-L1 to degrade through proteasome pathway [15,18]. Our data revealed that GABA/-baclofen stabilizes PD-L1 by reducing the binding of PD-L1 to STUB1.

In conclusion, this study established the functional relationship between GABA/baclofen and ICB, revealed an unknown function of baclofen in immunotherapy, and provided a new strategy for clinical treatment of breast cancer. The potential use of GABA/baclofen as an anti-tumor drug is promising, and the intervention of the signal cascade activated by GABA/baclofen may further increase the specificity of cancer treatment.

Ethics statement

The animal experimental protocol of this study was approved by Department of Experimental Animal Science of Fudan University (approval number: 202212004Z).

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Data availability statement

The data related to this study has not been stored in any publicly available repository. All data supporting the findings of this study are available from the corresponding author (Lei Lv, lvlei@fudan.edu.cn) upon request.

CRediT authorship contribution statement

Xue Sun: Writing – original draft. **Mingen Lin:** Software. **Ziyin Tian:** Investigation. **Yan Ma:** Investigation. **Lei Lv:** Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lei lv reports financial support was provided by Nourse Centre for Pet Nutrition.

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28600.

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