TAT-Beclin 1 represses the carcinogenesis of DUSP4-positive PTC by enhancing autophagy

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Received: 20 September 2021 / Accepted: 9 November 2022 / Published online: 6 December 2022
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Abstract

Background DUSP4 is a pro-tumorigenic molecule of papillary thyroid carcinoma (PTC). DUSP4 also exists as an autophagic regulator. Moreover, DUSP4, as a negative regulator of MAPK, can prevent Beclin 1 from participating in autophagic response. This study aimed to explore whether TAT-Beclin 1, a recombinant protein of Beclin 1, could inhibit the tumorigenesis of DUSP4-positive PTC by regulating autophagy.

Methods First, we divided PTC tissues into three groups according to DUSP4 expression levels by immunohistochemical analyses, and evaluated the relationship between autophagic molecules (Beclin 1 and LC3II) and DUSP4 using Western blotting assays. After overexpression of DUSP4 by lentiviral transduction, the in vitro and in vivo roles of TAT-Beclin 1 on DUSP4-overexpressed PTC cells were assessed (including autophagic activity, cell survival and function, and tumor growth). The roles of TAT-Beclin 1 in the survival of DUSP4-silenced PTC cells were also evaluated.

Results Our results showed that the expression levels of autophagic proteins decreased with the increase of DUSP4 expression in PTC tissues. In PTC cells, DUSP4 overexpression-inhibited autophagic activity (including Beclin 1 expression, LC3 conversion rate and LC3-puncta formation) and -promoted cell proliferation and migration were reversed by TAT-Beclin 1 administration. In vivo assays also showed that DUSP4-overexpressed PTC cells had stronger tumorigenic ability and weaker autophagic activity, which was blocked by TAT-Beclin 1 administration.

Conclusion TAT-Beclin 1, as an autophagic promoter, could repress the carcinogenesis of DUSP4-positive PTC, which implies that the use of TAT-Beclin 1 for the PTC patients' treatment might be determined according to the DUSP4 level in their tumors.

Keywords PTC · DUSP4 · TAT-Beclin 1 · Autophagy · Beclin 1

Introduction

The incidence rate of papillary thyroid carcinoma is 70–80% in thyroid carcinoma [1]. The tumors of PTC patients grow slowly, show multifocal, and have a tendency of local lymph node metastasis. However, some PTCs are highly aggressive and easy to dedifferentiate, which eventually develops into poorly differentiated or undifferentiated carcinoma. These above results have quite a negative impact on the survival rate and quality of life of PTC patients. There are lots of biomarkers, which have significant value on predicting the prognosis and therapeutic effect in PTC patients. However, the significance of a large proportion of biomarkers keeps vague in PTCs. Accordingly, it is of great significance to deepen the research on biomarkers related to PTC, on the basis of which the diagnosis and treatment of PTC could be further improved.
Dual specificity phosphatase 4 (DUSP4), a member of the bispecific phosphatase family, negatively regulates the activity of mitogen-activated protein kinase (MAPK) [2]. DUSP4 is considered a biomarker of many malignant tumors. The alteration of DUSP4 expression is related to the occurrence and development of multiple tumors. DUSP4 is regarded as the anti-oncogenic gene of most tumors [2–4], whilst serves as the pro-oncogenic gene of a small number of tumors, including PTC [5–7]. DUSP4 expression level in PTC tissues is significantly higher than that in paracancerous normal tissues [7]. The high expression of DUSP4 is not only associated with lymph node metastasis and extrathyroid infiltration, but also an independent risk factor for lymph node metastasis [7]. Therefore, DUSP4 is considered a significant biomarker of PTC. However, the effect of DUSP4 on the diagnosis and treatment of PTC is still unclear.

Autophagy is a highly conserved cellular homeostasis mechanism. Moderate autophagy serves as a protective mechanism, i.e., protective autophagy, whilst excessive autophagy promotes cell death, i.e., autophagic cell death [8]. The inhibitory effect of autophagic cell death on the survival of tumor cells has been reported in several studies [9–11]. Previous study showed that overexpression of DUSP4 in primary tissue and cell culture models regarding myocardium leads to the impairment of autophagy [12]. The similar result was also reported in the study of hepatocytes [13]. DUSP4 is a negative regulator of MAPK, and can inhibit the activities of c-Jun N-terminal kinase (JNK), p38 and extracellular regulated protein kinase (ERK) [2, 14–16]. JNK is an important autophagic promoter under various stress and pathological conditions [17–20]. JNK can also promote cell death by activating autophagy, which is shown in some malignant tumors [21–23]. Activated JNK is known to dissociate the autophagy molecule Beclin 1 from BCL2-Beclin 1 complex, and free Beclin 1 can activate autophagy after entering autophagy flux [17]. Previous studies have also confirmed that JNK represses tumor growth by Beclin 1-dependent autophagy activation [24, 25]. Remarkably, DUSP4 signaling can inactivate MAPKs including JNK, which results in the decrease of Beclin 1 expression and autophagic activity [13]. The latest research also confirmed that DUSP4 exerts an inhibitory effect on JNK-Beclin 1-autophagy activation signaling pathway [26]. Overall, we hypothesized that DUSP4-positive PTC may be sensitive to the treatment with Beclin 1 recombinant protein, TAT-Beclin 1.

The present study demonstrated that DUSP4-inhibited autophagic responses and promoted proliferation, migration, tumor growth in vivo of PTC cells were all reversed by treatment with TAT-Beclin 1. Therefore, this study provided the first evidence for the application of Beclin 1 recombinant protein in the treatment of PTC by combing the relationship between autophagy and potential biomarker in PTC.

Materials and methods

Clinical tissue specimens

A total of 45 pairs of PTC tissues were obtained with written informed consent via surgical resection at The Second Affiliated Hospital of Hebei Medical University, Shijiazhuang, Hebei, China from May 2016 to February 2020. All tissue specimens were histopathologically examined by three independent pathologists. Fresh tissue specimens were frozen in liquid nitrogen and stored at −80 °C until utilization. All the clinical samples were acquired with informed consent from the participants. The Institutional Review Board of The second hospital of Hebei Medical University reviewed and approved the human studies (2016-R269). The human studies were conducted according to the principles expressed in the Helsinki Declaration.

Cell lines and culture

The PTC cell lines TPC-1 and K1 were from American Type Culture Collection (ATCC). The cells were incubated in RPMI-1640 Medium (Thermo Fisher Scientific; Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific). All cells were kept in humidified air at 37 °C and 5% CO2.

Experimental protocol of TAT-Beclin 1 peptide

TAT-Beclin 1 peptide is a known autophagic inducer [27–29]. The cell permeable TAT-Beclin 1 peptide was obtained from the Peptide Core at the University of Colorado Anschutz Medical Campus. The sequence of the TAT-Beclin 1 peptide was RRRQRRKRRGGYGDHWIH-FTANWV [27]. In in vitro experiments, cells were treated with TAT-Beclin 1 or vehicle (normal saline) at a dose of 10 μM. Tumor-bearing mice were treated with TAT-Beclin 1 or vehicle via intraperitoneal injection (I.P.) at a dose of 20 mg/kg/day for 30 days.

Lentiviral transduction

Recombinant lentiviruses encoding DUSP4 were constructed by homologous recombination between the expression vector (pEX-Puro-Lv105) and cDNA in 293 cells as previously described using the lentivirus construction kit (GeneCopoeia) [30]. The same method was used to construct and package the corresponding control vector. After 2 days, supernatants were collected, and cells were incubated in medium containing lentiviruses at a multiplicity of infection.
(MOI) of 25 for 2 d. The infected cells were selected using puromycin (5 μg/ml). The overexpression efficiency of viral gene was detected using qPCR analysis.

**SiRNA Transfection**

Control siRNAs or siRNAs against human DUSP4 were obtained from Thermo Fisher Scientific. The target sequences were as following:

Control, 5'-CCC ATC CTT CAA CGA GCA T-3',
DUSP4, 5'-CCCAGTACCCTACCAGCAT-3'.

The indicated cells were cultured in 6-well plates and then transfected with siRNAs (100 pmol/well) using RNAiMAX (Thermo Fisher Scientific) in accordance with manufacturer's protocols. For 48 h, the silence efficiency of siRNAs was detected using qPCR analysis.

**Western blotting assays**

The lysates of the indicated cells were prepared from 6-well plates, separated on 10% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes (PVDF, Thermo Fisher Scientific), which were incubated with the specific antibodies against DUSP4, Beclin 1, LC3B and β-actin (Cell Signaling Technology, MA, USA). Horseradish peroxidase (HRP)-linked secondary antibodies were applied to visualize the immunoreactivity under a chemiluminescence system (Omega Lum G, Aplegen, CA, USA).

**Quantitative real-time PCR (qPCR) assays**

The total RNA was extracted and purified by the TRIZOL method. Synthesis of cDNA and qRT-PCR measurements was performed according to the manufacturer’s protocols. The reaction was performed in 25 μL system, and reaction condition was as following: 50 °C for 30 min, 94 °C for 1 min, 57 °C for 1 min, and 72 °C for 7 min. The pre-designed primer sequences for qPCR were as follows:

DUSP4, 5'-CTACATCATGTTGGTCAAC-3' (sense) and
5'-TAGACGATGACCCGAGTA-3' (anti-sense);
GAPDH, 5'-CCTGCTCTACTGGGCTGC-3' (sense) and 5'-GCAGTGGGACACGGAAGGC-3' (anti-sense).

Relative expression was calculated using the 2^-△△Ct method.

**Cell proliferation analysis**

To assess the cell proliferation, cell counting Kit-8 (CCK-8) assays were carried out using the CCK-8 kit (Dojindo, Kumamoto, Japan). For CCK-8 assay, the indicated cells were plated into 96-well plates with 2500 cells/well. Following the indicated time, all cells were incubated with 10 μl CCK-8 reagent. After 2 h incubation, the optical density at 450 nm (OD450) was measured using Varioskan Flash reader (Thermo Fisher Scientific).

**Cell death analysis**

To evaluate the cell death, trypan blue exclusion assays were performed as previously described [19]. The cells failing to exclude the presented blue-dye were defined as the dead cells. The total death rate (%) = number of dead cells/(number of living cells + number of dead cells) × 100%.

**Detection of Caspase3 activity**

The apoptosis level was evaluated by detecting Caspase3 activity using ApoAlert caspase fluorescent assay kit (Clontech, CA, USA). The treated cells on 6-well plates were lysed in 120 μl lysis buffers, and incubated on ice for 10 min. 120 μl reaction buffers containing 12 μl Caspase3 fluorescent substrate (1 mM) were added to each well and incubated for 1 h at 37 °C. The fluorescence intensity was quantified using a fluorescence spectrophotometer (Synergy2, BioTek, VT, USA; excitation at 400 nm and emission at 505 nm).

**Immunofluorescence assays**

The indicated cells were seeded on 6-cm dishes and fixed using 4% paraformaldehyde (PFA). After perforated with 0.5% Triton-100, cells were blocked using 1% bovine serum albumin (BSA) and incubated with the specific antibody against LC3B (Cell Signaling Technology) at 4 °C overnight. Subsequently, cells were stained with fluorochrome-labelled secondary antibody for 30 min and then counterstained with DAPI for 10 min. Ultimately, the cells were observed and counted under the fluorescence microscopy (Olympus IX81, Tokyo, Japan). The cells with more than five LC3-puncta were considered positive cells [31].

**Cell migration assays**

The migratory ability of cells was evaluated by Transwell assay. For Transwell assay, the indicated cells suspended in serum-free DMEM along with 1 mg/ml mitomycin C (aimed to inhibit cell proliferation) were seeded onto the upper chamber of the Transwell. DMEM containing 20% FBS was added into the lower chamber. After 36 h of incubation, the
cells that migrated to the lower surface of the inserts were fixed, stained with 1% crystal violet, and photographed. The migration levels were determined by counting the number of stained cells.

Animal experiments

A total of 30 male athymic BALB/c nude mice (6-week-old; 20–22.5 g) were purchased from Animal center of Gem Pharmatech Co., Ltd (Nanjing, China). LV-DUSP4-transduced and corresponding control K1 cells were inoculated subcutaneously on the ventral side of the right rib at the density of $5 \times 10^6$ cells per mouse (8 mice per group). According to the application of TAT-Beclin 1 peptide and the transduced lentiviruses, the above tumor-bearing mice were divided into four groups: (1) LV-Cont group; (2) LV-Cont + TAT-Beclin 1 group; (3) LV-DUSP4 group; (4) LV-DUSP4 + TAT-Beclin 1 group. The tumor volumes in the four groups of mice were measured every three days to observe the growth of tumors. The shortest diameter (A) and the longest diameter (B) were measured with a caliper to determine the tumor volume. The volume was calculated using the formula $V = (A^2 \times B)/2$. After 30 days, all mice were anesthetized with isoflurane (2%, Inhalation anesthesia), and then sacrificed via cervical dislocation. All mice were considered dead when their hearts and breathings stopped. Their tumors were removed and weighted. Animal experimental protocols were approved by the Institutional Animal Care and Use Committee of The second hospital of Hebei Medical University (2016-R269). The mice were housed in a specific pathogen-free facility with barrier in which the room temperature was 20–30 ℃ and the humidity 60–80% and fed a SPF mouse chow and sterile water.

Immunohistochemistry (IHC) assessment

The tissue sections were prepared, incubated overnight at 4 ℃ with primary DUSP4 antibody (1:100), and then visualized by the PV-9000 DAB detection kit in accordance with manufacturer’s protocols. The sections were observed under the IX81 microscope. DUSP4 staining was graded semi-quantitatively. Staining intensity was graded as 1 (no stain), 2 (weak stain), 3 (clear stain), or 4 (strong stain). The total immunoreactivity score was obtained by multiplying the intensity and abundance (expressed as a fraction).

Statistical analysis

All statistical analyses were performed using GraphPad Prism Software 6. All experiments were repeated at least three times. For comparisons, Wilcoxon rank sum test, one-way ANOVA test or Student’s $t$-test were performed as indicated. Tukey test was used for Post-Hoc Multiple Comparisons. Pearson chi-square test was performed for associations. $P < 0.05$ was considered statistically significant.

Results

The expression of LC3II and Beclin 1 decreased in PTC tissues with higher DUSP4 expression

Firstly, the GEPIA of TCGA database showed that the expression levels of DUSP4 in PTC tissues were significantly higher than that in adjacent tissues (Fig. 1A). According to DUSP4 expression levels, 45 PTC tissues were divided into three groups by the immunohistochemical results (Fig. 1B). It was shown that DUSP4 levels in the three groups increased successively (Fig. 1C). The association analyses regarding clinical data showed that high DUSP4 levels were positively associated with the invasive degree, size and pathological progress (T/N) of PTC tissues (Table 1). As shown in Fig. 1D, E, the protein expression of Beclin 1 and LC3II decreased successively. The results indicated that in PTC tissues, the increase of DUSP4 was accompanied by the decrease of autophagic proteins Beclin 1 and LC3II.

The autophagy inhibited by DUSP4 overexpression was reversed by treatment with TAT-Beclin 1 in PTC cells

Next, we verified the effect of TAT-Beclin 1 on the autophagy of PTC cells regulated by DUSP4 in vitro. As shown in Fig. 2A–C, DUSP4 overexpression decreased the LC3 transformation rate (The ratio of LC3II/LC3I) in PTC cell lines (K1, TPC-1), which was reversed by TAT-Beclin 1 administration. Similarly, DUSP4 overexpression attenuated the LC3-puncta formation in K1 and TPC-1 cells, which was also reversed by TAT-Beclin 1 administration (Fig. 2D–G). These results suggested that TAT-Beclin 1 could compensate the autophagic activity of PTC cells inhibited by DUSP4 overexpression.

The survival and migration promoted by DUSP4 overexpression were reversed by treatment with TAT-Beclin 1 in PTC cells

We documented that TAT-Beclin 1 could block the inhibitory effect of DUSP4 overexpression on the autophagic activity of PTC cells. The effect of TAT-Beclin 1 on
the survival, proliferation and function of DUSP4-regulated PTC cells should be further clarified. As shown in Fig. 3A, F, the total death of K1 and TPC-1 cells inhibited by DUSP4 overexpression was reversed by TAT-Beclin 1 administration. Tumor suppressor protein p53 can play an anti-proliferative function due to its contribution to autophagic death [32, 33]. Accordingly, the detection of p53 protein expression is helpful to confirm the autophagy-related death level of PTC cells. It was observed that under the same intervention, the trend of p53 expression was similar to the total death level in K1 and TPC-1 cells (Fig. 3B, G). As shown in Fig. 3C, H, Caspase3 activity of K1 and TPC-1 cells was also inhibited by DUSP4 overexpression, but its change extent is less than that of total death level. Moreover, TAT-Beclin 1 administration did not affect Caspase3 activity of the two cells upon normal condition and DUSP4 overexpression (Fig. 3C, H).

In addition, the proliferation levels of K1 and TPC-1 cells promoted by DUSP4 overexpression was also reversed by TAT-Beclin 1 administration (Fig. 3D, I). Moreover, the migratory ability of K1 and TPC-1 cells promoted by DUSP4 overexpression was also reversed by TAT-Beclin 1 administration (Fig. 3E, F, J, K). It was suggested that TAT-Beclin 1 could block the survival and migration of PTC cells promoted by DUSP4. Remarkably, DUSP4 knockdown-promoted cell death and p53 expression, and -inhibited the proliferation of K1 and TPC-1 cells were not affected by TAT-Beclin 1 administration (Fig. 4A–H).

The tumor growth of PTC cells promoted by DUSP4 overexpression was reversed by treatment with TAT-Beclin 1

The effect of TAT-Beclin 1 on tumor growth of PTC cells regulated by DUSP4 in vivo should be further elucidated.
The import efficiency of DUSP4-overexpressed K1 cells in xenograft tumors was verified by the results in Fig. 5A. As shown in Fig. 5B, compared with the tumors in LV-Cont group, the tumors formed by DUSP4-overexpressed K1 cells had larger sizes. However, TAT-Beclin 1 administration reversed the increased sizes of xenograft tumors by DUSP4-overexpressed K1 cells (Fig. 5B). In addition, the tumor growth curve formed by DUSP4-overexpressed K1 cells was significantly higher than that in LV-Cont group, which was also reversed by TAT-Beclin 1 administration (Fig. 5C). The alteration trend regarding the weights of removed tumors in each group was similar to the results in Fig. 5B, C (Fig. 5D). These results indicated that TAT-Beclin 1 significantly repressed the tumor growth of PTC cells promoted by DUSP4. Importantly, the protein expression of Beclin 1 and LC3II in the tumors formed by DUSP4-overexpressed K1 cells significantly decreased compared with that in LV-Cont group, which was also reversed by TAT-Beclin 1 administration (Fig. 5E, F). It was suggested that TAT-Beclin 1 could compensate for the inhibited autophagy in the tumors formed by DUSP4-overexpressed PTC cells.

### Discussion

DUSP4 can serve as a potential biomarker for PTC [7]. As a negative regulator of MAPK, DUSP4 can inhibit JNK signaling [2, 16]. JNK is widely considered to be a molecule inducing autophagic death of tumor cells [21–23]. JNK-Beclin 1-autophagy signaling is a classic pathway of JNK-regulated autophagy activation [24, 25], which is also described in PTC-related research [26]. As expected, DUSP4 could inhibit Beclin 1 expression and autophagic activity through inactivating MAPKs including JNK [13], which leaves an intriguing scientific question for PTC research, whether the therapeutic effect of Beclin 1 recombinant protein, TAT-Beclin 1, on PTC is related to DUSP4 expression in tumors. In our study, DUSP4 expression was negatively associated with the expression of Beclin 1 and LC3II in PTC tissues, which implied the inhibitory effect of DUSP4 on Beclin 1 levels and autophagic activity in vivo. The cytoplasm is wrapped in the precursor membrane of autophagosomes to form phagocytic vesicles, which is the biological basis of autophagy activation [34]. In the above process, Class III phosphatidylinositol 3-kinase (PI3KC3) is activated and transformed into phosphatidylinositol-3-phosphate (PtdIns3P) [34]. Beclin 1, an important autophagic regulator, can active PI3KC3, and then promotes the occurrence of autophagy [34]. Previous studies also clarified that Beclin 1 upregulation can lead to the increase of LC3II expression [35–37]. Accordingly, the decrease of Beclin 1 may bridge the high DUSP4 expression and autophagy inhibition. Furthermore, previous study showed that DUSP4 inhibits the dissociation of Beclin 1 from BCL2-Beclin 1 complex by negatively regulating JNK-BCL2 signaling [26]. Therefore, DUSP4 reduces Beclin 1 level by altering protein interaction. Importantly, high DUSP4 levels indicated more obvious growth, aggressiveness and metastasis in PTC tissues, which suggested that inhibition of Beclin 1-dependent autophagy contributes to the poor prognosis of PTC patients. The above results paved the way for further biological detection in vivo and in vitro.

As expected, DUSP4 overexpression reduced the autophagic responses and total death levels of PTC cells, which indicated that DUSP4 inhibits the autophagic death of PTC cells. Remarkably, DUSP4 overexpression also inhibited PTC cell apoptosis, which is consistent with previous

### Table 1

| Characteristics | Case | DUSP4 | P value |
|-----------------|------|-------|---------|
|                 |      | L     | M      | H      |
| Total           | 45   | 15    | 15     | 15     |
| Gender          |      |       |        |        |
| Male            | 12   | 3     | 5      | 4      |
| Female          | 33   | 12    | 10     | 11     |
| Age             |      |       |        |        |
| ≥ 65            | 20   | 6     | 8      | 6      |
| < 65            | 25   | 9     | 7      | 9      |
| Degree of invasion |      |       |        |        |
| Intrathyroid    | 22   | 11    | 8      | 3      |
| Extrathyroid    | 23   | 4     | 7      | 12     |
| PTC diameter    |      |       |        |        |
| ≥ 3.0 cm        | 27   | 5     | 9      | 13     |
| < 3.0 cm        | 18   | 10    | 6      | 2      |
| Pathological stage (T) |      |       |        |        |
| T2              | 24   | 12    | 8      | 4      |
| T3-pT4          | 21   | 3     | 7      | 11     |
| Pathological stage (N) |      |       |        |        |
| N0              | 32   | 14    | 13     | 5      |
| N1              | 13   | 1     | 2      | 10     |
| Outcome         |      |       |        |        |
| Persisted/recurrent | 2    | 2     | 0      | 0      |
| Death           | 1    | 1     | 0      | 0      |
| Cured           | 42   | 12    | 15     | 15     |

*P value was acquired by Pearson chi-square test
*P < 0.05
Previous studies showed that JNK activation can also lead to the enhancement of Bax-dependent apoptosis [38, 39]. However, the reduction in total death levels caused by DUSP4 overexpression was much more obvious than the reduction in apoptosis caused by DUSP4 overexpression, suggesting that DUSP4-regulated PTC cell death is composed of different death forms, which further confirms reports. Previous studies showed that JNK activation can also lead to the enhancement of Bax-dependent apoptosis [38, 39]. However, the reduction in total death levels caused by DUSP4 overexpression was much more obvious than
the negative regulation of DUSP4 in the autophagic death of PTC cells. As a tumor suppressor molecule, p53 contributes to autophagic cell death and apoptosis [32, 33, 40, 41]. Therefore, DUSP4 maintains the survival of PTC cells through inhibiting two forms of death, which is shown by the decline in p53 expression level. The above inference was also verified by the enhancement of proliferative and migratory ability of PTC cells by DUSP4 overexpression. However, treatment with TAT-Beclin 1 not only restored the autophagic activity and total death inhibited by DUSP4 overexpression, but also suppressed the proliferation and migration promoted by DUSP4 overexpression in PTC cells. These results indicated that DUSP4 signal attenuates autophagic cell death by the inhibition of Beclin 1 expression, which enhances the survival and function of PTC cells. Therefore, the application of TAT-Beclin 1 can significantly promote the autophagic death, whereby inhibiting the survival and function in DUSP4-overexpressed PTC cells. It was observed that the application of TAT-Beclin 1 could not affect the total death levels and proliferation in DUSP4-silenced PTC cells, indicating that due to the upregulation of autophagy, DUSP4 knockdown does not leave any space for TAT-Beclin 1 administration, which verified the above theory in reverse. Notably, TAT-Beclin 1 administration had no effects on PTC cell apoptosis under normal condition and DUSP4 overexpression. Previous literatures showed that the enhanced interaction between BCL2 and Beclin 1 can lead to the attenuated combination of BCL2 and Bax, which promotes apoptosis [42, 43]. Nevertheless, Beclin 1-dependent autophagy has anti-apoptotic property [37, 44, 45]. Accordingly, we conjecture that in this experimental system, Beclin 1 overexpression caused by TAT-Beclin 1 did not affect the apoptosis level of PTC cells due to the above counteraction. Overall, our in vitro assays further

![Fig. 3](Image)

The survival and migration promoted by DUSP4 overexpression were reversed by treatment with TAT-Beclin 1 in PTC cells. A, F The total death levels of lentiviruses-transduced K1 and TPC-1 cells treated with TAT-Beclin 1 for 24 h were evaluated by trypan blue staining. B, G p53 protein expression in K1 and TPC-1 cells treated as in A, F was detected by Western blotting. C, H The apoptosis of treated K1 and TPC-1 cells was measured by detecting Caspase3 activity. D, I The proliferation of treated K1 and TPC-1 cells was evaluated by CCK-8 assays. (E, F, J, K) The migration of treated K1 and TPC-1 cells was assessed by Transwell assays. Scale bar, 100 μm. Results are presented as mean ± SEM from three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 by one-way ANOVA test.

![Fig. 4](Image)

The role of TAT-Beclin 1 on DUSP4-silenced PTC cells. A, E mRNA levels of DUSP4 in K1 and TPC-1 cells transfected with the indicated siRNAs for 48 h. B, F The total death levels of siRNAs-transfected K1 and TPC-1 cells treated with TAT-Beclin 1 for 24 h were assessed by trypan blue staining. C, G p53 protein expression in K1 and TPC-1 cells treated as in B, F was detected by Western blotting. D, H The proliferation of treated K1 and TPC-1 cells was measured by CCK-8 assays. Results are presented as mean ± SEM from three independent experiments. ns, not statistically significant. ***P < 0.001 by one-way ANOVA test.
confirmed that TAT-Beclin 1, as a promoter of Beclin 1-dependent autophagy, can specifically repress PTC tumorigenesis caused by DUSP4 overexpression. In vivo assays also showed that PTC cells with overexpressed DUSP4 could form larger tumors, which was accompanied by lower expression of Beclin 1 and LC3II in tumors. Nevertheless, the in vivo effects of DUSP4 overexpression could be blocked by the application of TAT-Beclin 1, which further confirmed the repressive effect of TAT-Beclin 1 on PTC with high DUSP4 levels. The current working model regarding our study is described in Fig. 6.

**Conclusion**

Our study confirmed that TAT-Beclin 1 can effectively repress the carcinogenesis of DUSP4-positive PTC in vivo and in vitro, which is attributed to its ability to block the inhibitory effect of DUSP4 on the autophagic cell death of PTC. Based on these data, the prevention and treatment of PTC may be further improved in the future, i.e., in the treatment of PTC, the addition of TAT-Beclin 1 can be selected according to DUSP4 expression.
Author contributions NH and LLZ were responsible for conception and writing of the manuscript. LLZ, YMS and YHT performed the experimental work. All authors contributed to substantial discussion of content, reviewing and revising the manuscript before submission.

Funding This work was supported by The Natural Science Foundation of Hebei Province (Grant No. H2018206180).

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

Declarations

Conflict of interest All authors declare that they have no conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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