Negative feedback loop between ZBTB7A and TGF-β in breast cancer

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Abstract. Zinc finger and BTB domain containing 7A (ZBTB7A) is aberrantly expressed in breast cancer, but the involvement of ZBTB7A in breast cancer remains controversial. Transforming growth factor-β (TGF-β) is a pleiotropic cytokine which promotes breast cancer metastasis. ZBTB7A and TGF-β are important factors in tumor development. However, the association between ZBTB7A and TGF-β in breast cancer remains unknown. The results of the present study revealed that TGF-β1 induced the expression of ZBTB7A via the phosphoinositide 3-kinase-protein kinase B signaling pathway in human breast cancer cells, and ZBTB7A inhibited the expression of TGF-β1 through indirectly suppressing the promoter activity of TGF-β1. Furthermore, no significant correlation between the expression of ZBTB7A and TGF-β1 were identified in breast cancer tissues using tissue microarray assay and human cancer genomics analysis. These results have identified a negative feedback loop between ZBTB7A and TGF-β signaling, suggesting ZBTB7A as a potential modulator of breast cancer metastasis. Thus, the results of the present study suggested that ZBTB7A is a potential prognostic biomarker for breast cancer.

Introduction

Zinc finger and BTB domain containing 7A (ZBTB7A), also known as FBI, LRF or OCZF, is a member of the poxvirus and zinc finger/broad-complex, tramtrack and bric-à-brac and Kruppel family of transcriptional repressors, which serve essential functions in cell transformation and malignancy (1). Previous studies have revealed that ZBTB7A is implicated in the pathogenesis of several types of cancer, including non-small cell lung and ovarian carcinomas, gliomas, and T-cell and B-cell lymphomas (2-5). ZBTB7A is considered to be an oncogene, but a previous study indicated that it may also serve as a tumor suppressor in certain types of cancer, including prostate cancer and melanoma (6,7). Although ZBTB7A is overexpressed in human breast cancer (8), the involvement of ZBTB7A in breast cancer remains unclear.

Transforming growth factor-β (TGF-β) is a ubiquitous cytokine known to possess pleiotropic but context-dependent effects on cell growth, differentiation and immune modulation (9-11). TGF-β has three isoforms in mammals: TGF-β1, -β2 and -β3, of which TGF-β1 is the most active, accounting for 90% of body cell lines (12). Regarding the pathogenesis of breast cancer, TGF-β1 exhibits a tumor-inhibiting effect in the early stage, but as the tumor develops, this inhibition is often reversed thereby promoting the development of tumors (13). It was revealed that TGF-β1 facilitates the metastasis of tumor cells by affecting the tumor microenvironment, enhancing invasion and suppressing immune cell function (14). The epithelial-mesenchymal transition (EMT) is the primary way by which cells obtain tumor invasion and metastasis ability, and TGF-β1 is an essential factor known to induce the EMT of cells (15-18). The association between TGF-β1 and tumor development makes TGF-β1 a potential target for cancer therapy aimed at inhibiting tumor metastasis.

ZBTB7A and TGF-β1 are important factors in tumor development. Furthermore, previous studies have suggested that ZBTB7A is involved in the fine-tuning of TGF-β1 expression in atherosclerosis (19), and that TGF-β1 suppresses ZBTB7A expression in human bladder cancer cells (20). Therefore, the present study aimed to determine the association between ZBTB7A and TGF-β1 in breast cancer cells and tissue.

Materials and methods

Cell culture. 293T and human breast cancer MCF-7 cells (American Type Culture Collection, Manassas, VA, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-activated fetal bovine
Plasmid construction. The ZBTB7A-expressing plasmid was generated through inserting the encoding region into a pcDNA3.1 expression vector (Invitrogen; Thermo Fisher Scientific, Inc.) at the site of the HindIII restriction enzyme. The ZBTB7A primers used were as follows: Forward, 5'-AGCATGTAATCCCGACACAC-3' and reverse, 5'-GCTAAGGCTTTTGAGGCCATGGA-3'. TGF-β1 promoter was subcloned into the pGL4.10 basic plasmid (Promega Corporation, Madison, WI, USA) at the KpnI and HindIII restriction enzyme sites to drive luciferase expression. The amplification primers used were as follows: TGF-β1 PP1 (702 bp) forward, 5'-TGGTTGAACACACATGC-3' and reverse, 5'-GTGGTCTGGCAGAGAGC-3'; TGF-β2 PP2 (512 bp) forward, 5'-GGTGGTACCGCTAGATACAGAC-3' and reverse, 5'-GTTAAAGGTTGGTCCGCGAGG-3'.

Transient transfection and luciferase activity assay. Transient gene delivery was performed to assess the effect of ZBTB7A on TGF-β1 promoter activity in 293T and MCF-7 cells, as described previously (21,22). Briefly, 1x10^5 cells were mixed with TGF-β1 promoter constructs (0.75 µg), ZBTB7A expression vector (0.75 µg) and Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) Cells transfected with a pcDNA3.1 expression vector, rather than ZBTB7A, were used as negative control. Cell extracts were prepared 48 h following transfection using a 1X lysis buffer (Promega Corporation), and a 10 µl aliquot of the supernatant was mixed with 50 µl Luciferase Assay reagent (Promega Corporation) and analyzed using a microplate luminometer. Luciferase activity was normalized using a Renilla luciferase internal control.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and semi-quantitative RT-PCR. For RT-qPCR, total RNA was extracted from 2x10^5 293T or MCF-7 cells as indicated using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Isolated RNA was reverse transcribed using the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The following primers were used: ZBTB7A forward, 5'-CTTCCGT TCCCGGACCACCAGCA-3' and reverse, 5'-ATCTGGCCGT TCCAGGAGTGCG-3'; β-actin forward, 5'-ATCTGGCACC CACACC-3' and reverse, 5'-GCATTACTCCTGCTT-3'. The expected PCR products of ZBTB7A and β-actin were 361 and 837 bp, respectively. The PCR products were then visualized on 1.5% Tris-borate-EDTA (TBE) agarose gels stained with ethidium bromide.

Western blot analysis. A total of 2x10^5 MCF-7 cells were first starved in serum-free DMEM for 24 h at 37°C and stimulated with 20 ng/ml TGF-β1 (Merck KGaA, Darmstadt, Germany) at 37°C for different amounts of time (0-6 h). Subsequently, the cells were harvested by centrifugation at 500 x g for 10 min at room temperature, and total protein was extracted using lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China). Protein concentration was evaluated using a BCA Protein Assay kit (cat. no. P0012; Beyotime Institute of Biotechnology) according to the manufacturer's protocol, and 30 µg total protein per lane was separated using 10% SDS-PAGE and transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% non-fat dry milk for 1 h at room temperature, followed by incubation with various primary antibodies at 4°C overnight. The primary antibodies were against ZBTB7A (cat. no. 175918; dilution, 1:1,000; Abcam, Cambridge, MA, USA), β-actin (cat. no. 17001; dilution, 1:5,000; Cell Signaling Technology, Inc., Danvers, MA, USA). Loading variations were normalized against β-actin, which was identified using the anti-β-actin antibody (cat. no. 4970; dilution, 1:1,000; Cell Signaling Technology, Inc.). Membranes were subsequently washed three times with PBST (pH 7.4). The membranes were then incubated for 1 h at room temperature with horse-radish peroxidase (HRP)-conjugated secondary antibodies (cat. no. AP-132P; dilution, 1:5,000; Merck Millipore, Darmstadt, Germany). Subsequently, the membranes were incubated with Enhanced Chemiluminescence Western Blotting Detection Reagent (Beijing ComWin Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions, and the blots were visualized by exposure to X-ray films in the dark. Densitometric analyses were conducted using Scion Image software (version 4.1; Scion Corporation, Frederick, MD, USA), normalizing against β-actin.

Immunofluorescent staining. MCF-7 cells were washed twice with PBS. Following fixation with 4% paraformaldehyde for 20 min at room temperature, cells were permeabilized with 0.2% Triton X-100 for 30 min at room temperature. Immunofluorescent staining was performed as described previously (24). DNA was stained using DAPI (Sigma-Aldrich; Merck KGaA). The ZBTB7A (cat. no. 175918; Abcam) and TGF-β1 (cat. no. 130348; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) antibodies were used at a dilution of 1:300 and incubated for 2 h at room temperature. Following this, they were washed three times with PBS, and then incubated for 1 h at room temperature with fluorescein isothiocyanate-conjugated secondary antibodies (cat. no. BA1101; dilution, 1:100; Boster Biological Technology Co., Ltd., Wuhan, China). Cells were examined using a confocal laser scanning microscope and
images were produced using the QImaging system (QCAPTURE PRO 7; Surrey, BC, Canada) using a 10X oil-immersion objective (magnification, x100).

**Electrophoretic mobility shift assay (EMSA).** Nuclear extract was extracted from MCF-7 cells using a Nuclear Extraction kit (Applygen Technologies, Inc., Beijing, China). Biotin-labeled TGF-β1 probes were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The sequences were as follows: Probe 1 wild-type, 5'-CTGGTTTGGCGGGGAGA-3', and mutant, 5'-CTGTCTGCAAAACGGAGC-3'; probe 2 wild-type, 5'-TTCCTGTTGGCGGMCAGGGCGGT-3' and mutant, 5'-TTCCTCCTGAAAACCCAAAAACGGGT-3'. EMSA was performed using a LightShift Chemiluminescent EMSA kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Briefly, biotin-labeled probes (100 or 200 fm) and 20 µg nuclear extract prepared from the MCF-7 cells were incubated for 20 min at room temperature. Free probes were separated from DNA-protein complexes using electrophoresis on a native 6% polyacrylamide gel in 0.5X TBE buffer. Following electrophoresis, the DNA was transferred to a positively charged nylon membrane, cross-linked and detected using the chemiluminescence reagent. IgG (cat. no. A7016; Beyotime Institute of Biotechnology) was used as a negative control of ZBTB7A.

**LY294002 inhibition experiment.** MCF-7 cells with or without pretreatment with 10 µM or 25 µM LY294002 (Calbiochem, Merck Millipore, Darmstadt, Germany), a PI3K inhibitor, for 0.5 h were cultured in the presence of 20 ng/ml TGF-β1 for 6 h at 37°C. Cells were harvested, and ZBTB7A mRNA expression was analyzed using semi-quantitative RT-PCR, and ZBTB7A protein expression was determined using immunofluorescence assays (magnification, x100) and western blotting.

**Tissue microarray and human cancer genomics analysis.** A tissue microarray (BR2086; US Biomax, Inc., Rockville, MD, USA) consisting of 185 breast cancer samples was used. These samples were histologically interpretable and were analyzed for the correlation between ZBTB7A and TGF-β1. Immunohistochemical staining was performed as detailed in our previous study (25). The tissues were blocked with 1% bovine serum albumin (Roche Diagnostics, Basel, Switzerland) at room temperature for 1 h. Rabbit polyclonal antibody directed against ZBTB7A (cat. no. 175918; dilution; 1:50; Abcam) and mouse monoclonal antibody against TGF-β1 (cat. no. 130348; dilution; 1:25; Santa Cruz Biotechnology, Inc.) were used. In addition, ZBTB7A mRNA data for breast cancer were downloaded from the Bittner breast data set on Oncomine (www.oncomine.org; Thermo Fisher Scientific, Inc.). This dataset was used for correlation analysis between ZBTB7A and TGF-β1 expression levels. The correlation analysis was performed using GraphPad Prism software (version 5.0; GraphPad Software, Inc., La Jolla, CA, USA). Spearman’s rank correlation coefficients were generated to determine the degree of the correlation.

**Statistical analysis.** All experiments were performed ≥3 times, and the results were expressed as the mean ± standard deviation unless otherwise stated. GraphPad Prism software (version 5.0) was used for statistical analysis. Comparisons between two groups were performed using the two-tailed Student’s t-test. Comparisons among multiple groups were performed using one-way analysis of variance with post hoc intergroup comparisons using the Tukey test. Spearman’s rank-correlation coefficients were used to assess the correlation between ZBTB7A and TGF-β1 expression levels. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**TGF-β1 induces the expression of ZBTB7A in MCF-7 cells.** First, the effect of TGF-β1 on the expression of ZBTB7A was evaluated in MCF7 cells. RT-qPCR analysis results demonstrated that the ZBTB7A mRNA expression levels were significantly upregulated following TGF-β1 treatment, in a time-dependent manner, compared with untreated cells (Fig. 1A). Similarly, western blot analysis and immunofluorescence assay results revealed that ZBTB7A protein expression levels were significantly increased following TGF-β1 treatment, in a time-dependent manner, compared with untreated cells (Fig. 1B and C). These data supported the notion that TGF-β1 induces the expression of ZBTB7A in MCF-7 cells.

**TGF-β1 induces the expression of ZBTB7A via the phosphoinositide 3-kinase (PI3K)-Akt signaling pathway.** The PI3K-Akt signaling pathway is an important pathway in terms of tumor proliferation, invasion, and metastasis, and it is known to be a non-classical pathway which involves TGF-β signaling. Therefore, the involvement of the PI3K-Akt signaling pathway in TGF-β1-induced ZBTB7A expression was determined. TGF-β1 was demonstrated to increase the expression of phosphorylated Akt without altering the expression of total Akt (Fig. 2A). Furthermore, when TGF-β1 treatment was combined with the PI3K inhibitor LY294002, the mRNA and protein expression levels of ZBTB7A decreased compared with TGF-β1 treatment alone (Fig. 2B-D). These results indicated that the PI3K-Akt signaling pathway may, at least in part, be responsible for TGF-β1-induced ZBTB7A expression in breast cancer cells.

**ZBTB7A inhibited the expression of TGF-β1 in 293T and MCF-7 cells.** To determine the effect of ZBTB7A on TGF-β1 expression, 293T and MCF7 cells were transfected with the ZBTB7A expression plasmid (Fig. 3A). As presented in Fig. 3B and C, the ectopic expression of ZBTB7A in 293T and MCF7 cells led to significant downregulation of TGF-β1 mRNA and protein expression compared with the control cells. These results indicated that ZBTB7A is a modulator of TGF-β1 expression and signaling in 293T and MCF7 cells.

**ZBTB7A suppresses the promoter activity of TGF-β1 indirectly.** The mechanism by which ZBTB7A regulates the expression of TGF-β1 was further investigated. The promoter region of TGF-β1, containing 702 bp upstream of the transcription start site, was cloned and used to drive the expression of the luciferase reporter in 293T and MCF-7 cells (PGL4.10-TGF-β1 PP1, -702+1). Deletion of the S′ end of the TGF-β1 PP1 promoter generated the 512 bp fragment upstream of the transcription start site (PGL4.10-TGF-β1 PP2, -512+1).
Figure 1. TGF-β1 induces the expression of ZBTB7A in MCF-7 cells. (A) MCF-7 cells were treated with 20 ng/ml TGF-β1 for the indicated time points (0-6 h). RNA was extracted and subjected to reverse transcription-quantitative polymerase chain reaction analysis. ZBTB7A mRNA expression was normalized using β-actin as a control. ZBTB7A mRNA expression was significantly increased following ≥1 h of TGF-β1 treatment. (B) Representative western blot analysis using a primary antibody directed against ZBTB7A. Changes in ZBTB7A protein expression were quantitated and normalized to β-actin. (C) Representative images from the immunofluorescence assay (magnification, x100). The mean fluorescence intensity of ZBTB7A was quantitated using a confocal laser scanning microscope. ZBTB7A protein expression was significantly increased following ≥1 h TGF-β1 treatment. Data are presented as the mean ± standard deviation following three independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. 0 h. TGF-β1, transforming growth factor-β1; ZBTB7A, zinc finger and BTB domain containing 7A.

Figure 2. TGF-β1 induces the expression of ZBTB7A via the PI3K-Akt signaling pathway. (A) MCF-7 cells were treated with 20 ng/ml TGF-β1 for 0-6 h, and then cells were harvested and the indicated proteins were analyzed using western blotting. MCF-7 cells with or without pretreatment with 10 μM or 25 μM LY294002, a PI3K inhibitor, for 0.5 h were cultured with 20 ng/ml TGF-β1 for 6 h. Cells were harvested, and ZBTB7A mRNA expression was analyzed using (B) semi-quantitative RT-PCR, and ZBTB7A protein expression was determined using (C) immunofluorescence assays (magnification, x100) and (D) western blotting. TGF-β1, transforming growth factor-β1; ZBTB7A, zinc finger and BTB domain containing 7A; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; p-, phosphorylated.
The luciferase assay demonstrated that ZBTB7A significantly inhibited the promoter activities of TGF-β1 PP1 and TGF-β1 PP2, suggesting that the -512-+1 fragment of the TGF-β1 promoter is an important regulation site for ZBTB7A in 293T and MCF-7 cells (Fig. 4A). ZBTB7A is a transcriptional factor, so it was hypothesized that ZBTB7A may suppress TGF-β1 promoter activity through directly binding to the promoter of TGF-β1 PP2. To confirm this hypothesis, two probes within the -512-+1 fragment of TGF-β1 promoter sequence were utilized for EMSA. Unexpectedly, no specific shifted band with the anti-ZBTB7A antibody was observed in the EMSA assay (Fig. 4B). This suggested that ZBTB7A suppressed the promoter activity of TGF-β1 in an indirect manner, leading to the downregulation of TGF-β1 in MCF-7 cells.

Correlation between ZBTB7A and TGF-β1 expression in breast cancer tissue. To further identify the association between ZBTB7A and TGF-β1 expression in breast cancer, a tissue microarray (BR2086), consisting of 185 breast cancer cases, was used. No significant correlation (r=-0.077; P=0.296; Table I) was identified between the expression of ZBTB7A and TGF-β1 upon analysis of the human breast cancer tissue microarray, regardless of breast cancer types. To determine the association between ZBTB7A and TGF-β1 expression, the mining Bittner breast data set on Oncomine was also used. In agreement with the results of the tissue microarray, no significant correlation (r=-0.001; P=0.982) was identified between the mRNA expression levels of ZBTB7A and TGF-β1 in breast cancer tissue (335 cases; data not shown). These data
Figure 4. ZBTB7A suppresses the promoter activity of TGF-β1 indirectly. (A) ZBTB7A inhibited the promoter activity of TGF-β1. MCF-7 and 293T cells were co-transfected with plasmids of ZBTB7A, PGL4.10-TGF-β1 PP1 or PGL4.10-TGF-β1 PP2, and Renilla. Subsequently, the cells were harvested and subjected to a dual-luciferase reporter assay. (B) Direct binding analysis between ZBTB7A and promoter sequences of TGF-β1 in MCF-7 cells. An electrophoretic mobility shift assay was performed. IgG was used as a negative control. Data are presented as the mean ± standard deviation following three independent experiments. *P<0.001, with comparisons indicated by lines. ZBTB7A, zinc finger and BTB domain containing 7A; TGF-β1, transforming growth factor-β1; IgG, immunoglobulin G; Comp-probe, unlabeled competitive probe; mProbe, unlabeled mutant probe; Ab, antibody.

Table I. Correlation between ZBTB7A and TGF-β1 expression in breast cancer.

| Variable          | n (%) | Spearman's R | P-value |
|-------------------|-------|--------------|---------|
| ZBTB7A expression |       |              |         |
| TGF-β1 expression | 104   | -0.077       | 0.296   |
| -                 | 99    | 4.8          | 95.2    |
| +                 | 74    | 8.6          | 91.4    |

ZBTB7A, zinc finger and BTB domain containing 7A; TGF-β1, transforming growth factor-β1.

suggested that there was no significant correlation between the expression of ZBTB7A and TGF-β1 in breast cancer tissue.

Discussion

Embryonic fibroblasts (MEF) from ZBTB7A-null mice are resistant to cellular or viral oncogene-induced carcinogenic transformation. On the other hand, ectopic expression of ZBTB7A makes MEF cells susceptible to oncogenic transformation (26). These results suggest an important function for ZBTB7A in carcinogenesis. ZBTB7A is considered to be an oncogenic transcription factor in multiple types of cancer, including in colorectal and oral cancer (27,28). However, previous studies have revealed that ZBTB7A may also act as a tumor suppressor through the transcriptional repression of glycolysis in colon cancer (29), suppression of melanoma cell adhesion molecule in melanoma (7), and inhibition of a SRY-box 9-dependent pathway for tumor invasion and the bypassing of cellular senescence in prostate cancer (6). Therefore, ZBTB7A has dual but context-dependent effects on tumor formation and development. ZBTB7A has been demonstrated to be aberrantly expressed in breast cancer tissue (8). It is thus important to elucidate whether the elevated expression of ZBTB7A in breast cancer functions as an oncogene, or as a compensatory tumor suppressor gene.

Initial studies on TGF-β concerned its ability to induce malignant behavior in normal fibroblasts (30), which led to the idea that TGF-β was an essential promoting factor in tumorigenesis. However, TGF-β was later demonstrated to possess profound growth suppressive effects on numerous cell types, including epithelial cells (31). Thus, TGF-β possesses
pleiotropic but context-dependent effects on tumor transformation. In the early stage of breast cancer, TGF-β suppresses tumor formation, but in the late stage of breast cancer it promotes tumor development (13). Notably, TGF-β signaling was demonstrated to be activated in bone metastases from patients with breast cancer (32). Abnormal upregulation of TGF-β is positively correlated with the progression, angiogenesis and metastasis of breast cancer, resulting in adverse clinical outcomes in the late stage of the disease (33). TGF-β1 is known as the predominant isoform in breast tissue and cells, thus, TGF-β1 was selected for the present study.

ZBTB7A and TGF-β1 possess dual, but context-dependent effects on tumor formation and development. Furthermore, ZBTB7A has been demonstrated to be a potential transcriptional regulator of TGF-β1 expression in human atherosclerotic arteries (19). In addition, TGF-β1 suppresses ZBTB7A expression in human bladder cancer cells (20). In the present study, the association between ZBTB7A and TGF-β1 was investigated in breast cancer. First, the effect of TGF-β1 on ZBTB7A expression was evaluated, which revealed that TGF-β1 significantly induced the expression of ZBTB7A through the PI3K-Akt signaling pathway in MCF-7 cells. TGF-β1 has been reported to suppress ZBTB7A expression in human bladder cancer cells, which is inconsistent with the results of the present study in breast cancer cells. The inconsistency may be explained by the context-dependent function of TGF-β1 and ZBTB7A in different tumor stages and tumor types, as described in previous studies (2-7,9-11). Next, the effect of ZBTB7A on TGF-β1 expression was evaluated, and it was revealed that the ectopic expression of ZBTB7A led to significant downregulation of TGF-β1 mRNA and protein expression levels in MCF-7 and 293T cells. The potential mechanisms of ZBTB7A-induced TGF-β1 expression were further evaluated, and ZBTB7A was demonstrated to inhibit the promoter activity of TGF-β1 PPI (-702+1 fragment) and TGF-β1 PP2 (-512+1 fragment), suggesting that ZBTB7A regulates the transcription of TGF-β1 through the fragment of -512+1 within TGF-β1 promoter sequences. However, the EMSA assay confirmed that ZBTB7A did not bind to the TGF-β1 sequences. However, the EMSA assay confirmed that ZBTB7A may suppress the promoter activity of TGF-β1 indirectly.

Taken together, the results of the present study suggested a negative feedback loop between ZBTB7A and TGF-β1 in breast cancer cells. To further confirm the results obtained from the cell model, a tissue microarray with 185 breast cancer samples was used, and the association between ZBTB7A and TGF-β1 expression was assessed. No significant correlation between the expression of ZBTB7A and TGF-β1 in breast cancer tissue was identified from the clinical data. The results of the tissue microarray were further confirmed using the Bittner breast data set in Oncomine, with 335 breast cancer cases. The results obtained from clinical analysis supported the existence of the negative feedback loop between ZBTB7A and TGF-β1 in breast cancer, resulting in the balance and homeostasis of ZBTB7A and TGF-β1 expression.

In conclusion, to the best of our knowledge, this is the first study to reveal a negative feedback loop mechanism between ZBTB7A and TGF-β1 in breast cancer, which may aid in understanding the pleiotropic functions of ZBTB7A in the TGF-β1 signaling network and in breast cancer progression.

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References

1. Maeda T, Hobbis RM, Merghoub T, Guernah I, Zelent A, Cordon-Cardo C, Teruya-Feldstein J and Pandolfi PP: Role of the proto-oncogene Pokemon in cellular transformation and ARF repression. Nature 433: 278-285, 2005.
2. Apostolopoulou K, Pateras IS, Evangelou K, Tsantoulis PK, Liontos M, Kittas C, Tiniakos DG, Kotsinas A, Cordon-Cardo C and Gorgoulis VG: Gene amplification is a relatively frequent event leading to ZBTB7A (Pokemon) overexpression in non-small cell lung cancer. J Pathol 213: 294-302, 2007.
3. Jiang L, Siu MK, Wong OG, Tam KF, Lam EW, Ngan HY, Le XF, Wong ES, Chan HY and Cheung AN: Overexpression of proto-oncogene FBL1 activates membrane type 1-matrix metalloproteinase in association with adverse outcome in ovarian carcinomas. Mol Cancer 9: 318, 2010.
4. Maeda T, Merghoub T, Hobbis RM, Dong L, Maeda M, Zakrzewski J, van den Brink MR, Zelent A, Shigematsu H, Akashi K et al: Regulation of B versus T lymphoid lineage fate decision by the proto-oncogene LRF. Science 316: 860-866, 2007.
5. Rovin RA and Winn RC: Pokemon expression in malignant glioma: An application of bioinformatics methods. Neurosurg Focus 19: E8, 2005.
6. Wang G, Lunardi A, Zhang J, Chen Z, Ala U, Webster KA, Tay Y, Gonzalez-Billalabettia E, Egie A, Shaffer DR, et al: Zbtb7a suppresses prostate cancer through repression of a Sox9-dependent pathway for cellular senescence bypass and tumor invasion. Nat Genet 45: 739-746, 2013.
7. Liu XS, Genet MD, Haines JE, Mehanna EK, Wu S, Chen HI, Chen Y, Qureshi AA, Han J, Chen X, et al: ZBTB7A suppresses melanoma metastasis by transcriptionally repressing MCAM. Mol Cancer Res 13: 1206-1217, 2015.
8. Aggarwal A, Hunter WJ III, Aggarwal H, Davey MS, Murphy RF and Agrawal DK: Expression of transforming growth factor beta (TGF-beta) signaling axis molecules and E-cadherin in colorectal cancer. Tumour Biol 33: 1005-1014, 2012.
9. Lampropoulos P, Zizi-Sermetzoglou A, Rizos S, Kostakis A, Nikiteas N and Papavassiliou AG: Prognostic significance of transforming growth factor beta (TGF-beta) signaling axis molecules in human breast carcinoma and other cancers. Exp Mol Pathol 89: 140-148, 2010.
10. Day J, Zlotogora J, Brin-Uriel Y, Paltiel A, Agger T, Elidan U, et al: ZBTB7A and PPIA are the most significantly overexpressed genes in MCF7 cells. Proc Natl Acad Sci USA 107: 17575-17579, 2010.
11. Hu S, Wang XL, Wang XW, Gao W, Hwang D, Li C, et al: Pokemon regulates EMT and miR-200 expression in breast cancer cells. Mol Cancer 13: 126, 2014.
12. Lampropoulos P, Zizi-Sermetzoglou A, Zotos S, Kostakis A, Nikiteas N and Papavassiliou AG: Prognostic significance of transforming growth factor beta (TGF-beta) signaling axis molecules and E- cadherin in colorectal cancer. Tumour Biol 33: 1005-1014, 2012.
13. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victoria GD, Shen Y, Du J, Rubtsov YP, Rudensky AS, et al: TGF-beta-induced Foxp3 inhibits T(H)17 cell fate by antagonizing RORgammat function. Nature 453: 236-240, 2008.
14. Luo S, Kleemann GA, Atrash JM, Shaw WM and Murphy CT: TGF-β1 and insulin signaling regulate reproductive aging via oocyte and germline quality maintenance. Cell 143: 299-312, 2010.
15. Flanders KC and Wakefield LM: Transforming growth factor-beta and the mammary gland: the role of the receptor. Breast Cancer Res Treat 49: 219-225, 1999.
16. Derycke V and Akhurst RJ: Differentiation plasticity regulated by TGF-beta family proteins in development and disease. Nat Cell Biol 9: 1000-1004, 2007.
17. Johansson J, Tabor V, Wikell A, Jalkanen S and Fuze J: TGF-β1-induced epithelial-mesenchymal transition promotes monocyte/macrophage properties in breast cancer cells. Front Oncol 5: 3, 2015.
18. Lv ZD, Kong B, Li JG, Qu HL, Wang XG, Cao WH, Liu XY, Wang Y, Yang ZC, Xu HM and Wang HB: Transforming growth factor-beta 1 enhances the invasiveness of breast cancer cells by inducing a Smad2-dependent epithelial-to-mesenchymal transition. Oncol Rep 29: 219-225, 2013.
Walsh LA and Damjanovski S: IGF-1 increases invasive potential of MCF-7 breast cancer cells and induces activation of latent TGF-β1 resulting in epithelial to mesenchymal transition. Cell Commun Signal 9: 10, 2011.

Deng B, Yang X, Liu J, He F, Zhu Z and Zhang C: Focal adhesion kinase mediates TGF-β1-induced renal tubular epithelial-to-mesenchymal transition in vitro. Mol Cell Biochem 340: 21-29, 2010.

Dhaouadi N, Li JY, Feugier P, Gustin MP, Dab H, Kacem K, Bricca G and Cerutti C: Computational identification of potential transcriptional regulators of TGF-β1 in human atherosclerotic arteries. Genomics 103: 357-370, 2014.

Li W, Kidiyoor A, Hu Y, Guo C, Liu M, Yao X, Zhang Y, Peng B and Zheng J: Evaluation of transforming growth factor-β1 suppress Pokemon/epithelial-mesenchymal transition expression in human bladder cancer cells. Tumour Biol 36: 1155-1162, 2015.

Zu X, Yu L, Sun Q, Liu F, Wang J, Xie Z, Wang Y, Xu W and Jiang Y: SP1 enhances Zbtb7A gene expression via direct binding to GC box in HePG2 cells. BMC Res Notes 2: 175, 2009.

Maeda T, Hobbs RM and Pandolfi PP: The transcription factor Pokemon: A new key player in cancer pathogenesis. Cancer Res 65: 8575-8578, 2005.

Zhao Y, Yao YH, Li L, An WF, Chen HZ, Sun LP, Kang HX, Wang S and Hu XR: Pokemon enhances proliferation, cell cycle progression and anti-apoptosis activity of colorectal cancer independently of p14ARF-MDM2-p53 pathway. Med Oncol 31: 288, 2014.

Sartini D, Lo Murio L, Morganti S, Pozzi V, Di Ruscio G, Rocchetti R, Rubini C, Santarelli A and Emanuelli M: Pokemon proto-oncogene in oral cancer: potential role in the early phase of tumorigenesis. Oral Dis 21: 462-469, 2015.

Liu XS, Haines JE, Mehanna EK, Genet MD, Ben-Sahra I, Asara JM, Manning BD and Yuan ZM: ZBTB7A acts as a tumor suppressor through the transcriptional repression of glycolysis. Genes Dev 28: 1917-1928, 2014.

Sanchez-Capelo A: Dual role for TGF-beta1 in apoptosis. Cytokine Growth Factor Rev 16: 15-34, 2005.