

Original Research Article

PSAT1 prompted cell proliferation and inhibited cell apoptosis in multiple myeloma through regulating PI3K/AKT pathway

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Abstract

Purpose: To identify the biological function of phosphoserine aminotransferase 1 (PSAT1) in regulating cell proliferation and apoptosis in multiple myeloma (MM).

Methods: The mRNA and protein levels of PSAT1 were determined using quantitative real-time polymerase chain reaction (PCR) and western blotting, respectively. Cell proliferation was measured using CCK-8 assay.

Results: PSAT1 mRNA and protein expression levels were significantly increased in MM cell lines when compared to control cells. Moreover, downregulation of PSAT1 inhibited MM cell proliferation and induced cell apoptosis, whereas overexpression of PSAT1 promoted MM cell proliferation and suppressed cell apoptosis. Further analysis demonstrated that the underlying mechanism was via regulation of PI3K/AKT pathway.

Conclusion: The results identified a novel role for PSAT1 in the progression of MM, which may provide a therapeutic and a new anticancer target for the therapy of MM.

Keywords: Multiple myeloma, PSAT1, Cell proliferation, PI3K/AKT pathway

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INTRODUCTION

Multiple myeloma (MM) is considered the second most common hematological malignancy worldwide whose clinical manifestations include hypercalcemia, renal insufficiency, anemia, infection and uncontrolled proliferation of monoclonal plasma cells [1]. The incidence of MM is increasing remarkably; accounting for

approximately 10 % of all hematological cancers and 2.1 % of all cancer deaths [2]. Over the past few decades, advances in diagnostic and standard therapeutic techniques, have improved the outcomes and prolonged survival of MM patients [3]. Thus, more molecular biomarkers and diagnostic targets for MM, which may be helpful in MM therapy [4,5].

Phosphoserine aminotransferase 1 is an aminotransferase that participates in serine biosynthesis and is highly expressed in various tissues such as breast and prostate [6,7]. Serine biosynthetic pathway has been shown to be crucial in regulating redox status, cell proliferation, and the biosynthesis of cellular components in several types of tumors, such as breast cancer [8,9] and colorectal carcinoma [10,11]. In addition, PSAT1 has been shown to play a role in tumor cell proliferation, migration and chemo resistance, contributing to tumor progression and poor outcomes [12]. However, its biological functions in the development and progression of MM are rarely known.

In this study, the mRNA and protein expression levels of PSAT1 in MM cell lines were measured and its effects on MM cell proliferation were analyzed. The results identified a novel role of PSAT1 in the progression of MM, which may provide a therapeutic and a new anticancer target for the therapy of MM.

EXPERIMENTAL

Cell lines

Human bone marrow cell line HS-5 was purchased from COBIOER (China), and used as control cells. Multiple myeloma (MM) cell lines LP-1, NCI-H929, and U266 were obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Dulbecco's Modified Eagle's medium containing 10 % fetal bovine serum and penicillin-streptomycin at 37 °C in 5 % CO₂ cell incubator.

RNA extraction and real-time PCR

Total RNA was extracted from MM cell lines using the TRIzol reagent (Vazyme, Nanjing). First-strand cDNA was reverse-transcribed from RNA using a reverse transcriptase kit (Vazyme, Nanjing). Quantitative RT-PCR was performed using the SYBR Green PCR Master Mix (Takara, RR047A). The relative expression of target gene transcripts was calculated using the $2^{-\Delta\Delta Ct}$ method. The sequence of primers for PSAT1 were sense: 5'-GGGTAGGTCCCGTC-3' and antisense: 5'-CCAAAGCCAATTCCA-3'.

Cell proliferation assay

MM cells (1×10^6 cells/mL) were seeded onto 96-well plates and cell proliferation was measured using the Cell Counting Kit-8 (CCK-8, Dojindo). 10ul/well CCK-8 solution was added and cultured for 1 - 4 h. Finally, the optical

density (OD) was read using a microplate reader at 450 nm.

Cell transfection

Vectors containing shPSAT1 or pcDNA3.1-PSAT1, and control vectors were transfected into target cells and seeded into 6-well plates using the LP2000 Transfection Reagent (Thermo Fisher Scientific). The screening and subculture of successfully transfected cells were performed for subsequent analysis.

Western blotting

Cultured cells were harvested, and total protein was extracted in lysis buffer (Beyotime, China), separated by SDS-polyacrylamide gel electrophoresis, and blotted onto PVDF membranes [13]. Primary antibodies used were as follows: PSAT1, Bcl-2(CST), Bax (CST), cleaved caspase-3 and GAPDH, which were obtained from Abcam. All antibodies were diluted in 5 % skim milk. The protein bands were visualized using the Tanon 5200 Western Blot System.

Statistical analysis

The data were analyzed using GraphPad 6.0 software and are presented as mean \pm SEM. Differences between groups were analyzed using unpaired Student's *t* test. *P* < 0.05 was considered statistically significant.

RESULTS

PSAT1 was significantly overexpressed in MM cells

The mRNA and protein expression levels of PSAT1 were measured in MM cell lines (LP-1, NCI-H929, and U266) and control cells by real-time quantitative PCR and western blotting, respectively. Results showed that the expression of PSAT1 mRNA was markedly increased in MM cell lines when compared with control cells. (Figure 1A). Western blotting analysis showed consistent results (Figure 1 B). Especially, in LP-1 cells, PSAT1 mRNA levels were higher than in the other MM cell lines, NCI-H929, and U266, which was thus used in the subsequent analysis.

Downregulation of PSAT1 inhibited proliferation of MM cells

To explore the function of PSAT1 in the development of MM, the recombinant vectors containing control (shNC) or PSAT1 small hairpin RNA (shPSAT1) were transfected into LP-1 cells,

and the transfection efficiency was verified. As expected, PSAT1 was significantly downregulated in LP-1 cells that transfected with shPSAT1 when compared with that transfected with shNC (Figure 2 A). This result was also showed from the western blotting results (Figure 2 B). CCK-8 assay demonstrated that downregulation of PSAT1 inhibited the proliferation of LP-1 cells that transfected with shPSAT1 when compared with that transfected with shNC (Figure 2 C).

To determine the effects of PSAT1 on MM cell apoptosis, the protein levels of Bcl-2, Bax, and cleaved caspase-3(all apoptosis-related proteins) in LP-1 cells were investigated. Downregulation of PSAT1, decreased Bcl-2 expression whereas significantly increased the expression levels of apoptosis-associated proteins, Bax and cleaved caspase-3 in LP-1 cells (Figure 2 D).

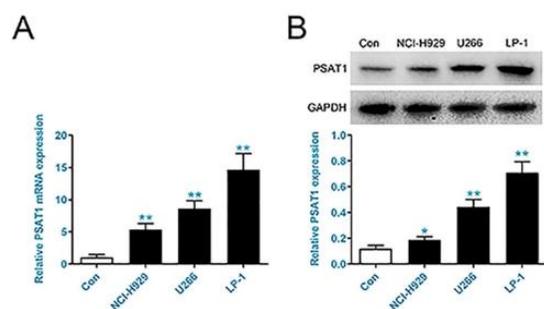


Figure 1: The mRNA and protein expression level of PSAT1 in MM cells. (A) mRNA level of PSAT1 was evaluated in MM cells by qPCR. (B) PSAT1 protein expression level was evaluated in MM cells by western blotting; ** $p < 0.01$

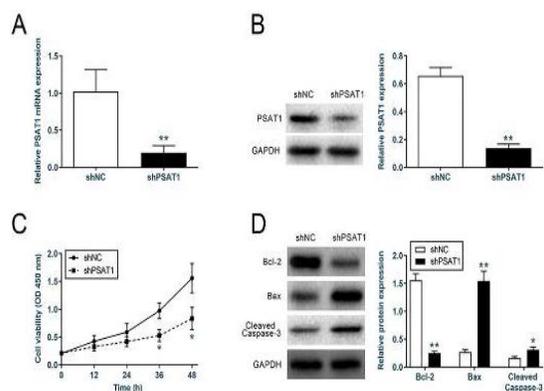


Figure 2: Effect of downregulation of PSAT1 on MM cell proliferation. (A) PSAT1 mRNA levels were determined using qPCR. (B) Protein expression of PSAT1 was measured by western blotting. (C) Cell viability was measured using the CCK-8 assay. (D) Protein expression levels of Bcl-2, Bax and cleaved caspase-3 were measured using western blotting; * $p < 0.05$, ** $p < 0.01$

Upregulation of PSAT1 promotes proliferation of MM cells

The recombinant plasmids, pcDNA3.1-PSAT1 and pcDNA3.1-NC, were transfected into LP-1 cells, and the transfection efficiency was verified by qPCR. The results showed that PSAT1 was significantly upregulated in pcDNA3.1-PSAT1-transfected cells when compared to pcDNA3.1-NC-transfected cells (Figure 3 A). Western blotting results showed similar results (Figure 3 B). Next, the proliferation of LP-1 cells was assessed using CCK-8 assay, and the results revealed that upregulation of PSAT1 enhanced cell proliferation as compared to shNC group (Figure 3 C). Furthermore, the mRNA level of Bcl-2 was increased significantly, whereas the mRNA levels of Bax and cleaved caspase-3 were significantly decreased (Figure 3 D) in LP-1 cells.

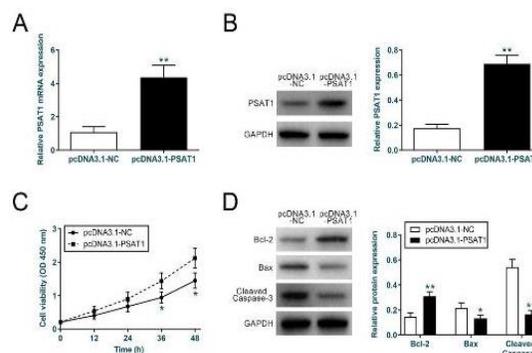


Figure 3: Effects of PSAT1 overexpression on the proliferation of MM cells. (A) The PSAT1 mRNA levels were assessed using qPCR. (B) PSAT1 protein levels were examined by western blotting. (C) Cell viability was measured using CCK-8 assay. (D) The protein expression levels of Bcl-2, Bax, and cleaved caspase-3 were measured by western blotting; * $p < 0.05$, ** $p < 0.01$

PSAT1 modulated the proliferation of LP-1 cells via PI3K/AKT signaling

To further reveal the underlying mechanism, the protein expression levels of p-PI3K and p-AKT were seriously suppressed by shPSAT1 in LP-1 cells, whereas no obvious differences were observed in the protein levels of PI3K and AKT (Figure 4).

As a downstream component of PI3K/AKT pathway, cyclin D1 is a key intracellular mediator of extracellular mitogens and may promote cell proliferation through the modulation of the G1 phase of the cell cycle[14,15]. Thus, the expression of cyclinD1 in shPSAT1-expressing LP-1 cells was examined, and the results

showed that cyclin D1 was downregulated by shPSAT1 (Figure 4).

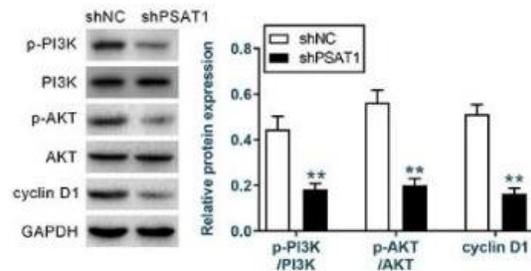


Figure 4: PI3K/AKT/cyclinD1 pathway was regulated by PSAT1. Molecular components of PI3K/AKT/cyclinD1 pathway were measured by western blotting; ** $p < 0.01$

DISCUSSION

Symptomatic plasma cell myeloma, also known as MM, is considered a plasma cell malignancy. Due to the complex clinical symptoms of MM, its treatment is a great challenge. Recent studies have shown that promising treatments for MM are chemotherapeutic agents that can target and kill cancer cells directly. However, it frequently results in the rapid acquisition of drug resistance [16]. Although the pathogenesis of MM remains poorly understood, therapeutic regimens are considered a promising therapeutic strategy.

In this study, it was found that PSAT1 was overexpressed in MM cell. The upregulation of PSAT1 promoted the proliferation of MM cells while downregulation of PSAT1 showed the opposite effect. These results indicate the potential role of PSAT1 in regulating MM cell proliferation. The PI3K/AKT pathway has been found to play a significant role in tumorigenesis and cancer development [17]. Signals from PI3K to the serine/threonine protein kinase AKT mediate cellular processes such as cell cycle, apoptosis, and cell survival via cyclin D1, p21, Bcl-2, and mTOR [18]. During these cellular processes, synthesis of AKT requires PSAT1-related serine biosynthesis. Thus, the protein components of PI3K/AKT pathway were examined in this study using western blotting.

A significant decrease in the expression levels of p-PI3K and p-AKT in shPSAT1-transfected MM cells were observed. These results supported the hypothesis that PSAT1 exerted an important role in the development of MM by PI3K/Akt signaling pathway. In addition, the levels of the cell cycle biomarker, cyclin D1, were also reduced in shPSAT1-transfected LP-1 cells, suggesting limited cell division cycles. However, additional downstream components of PSAT1, such as

JNK/ERK, which are well-known modulators of tumorigenesis [19], have not yet been identified, but this needs to be analyzed in a future study.

CONCLUSION

The results of this study reveal that PSAT1 is significantly upregulated in MM cells and plays a novel role in regulating MM cell proliferation, at least partially via PI3K/AKT/cyclin D1 pathway. Thus, PSAT1 may serve as a molecular biomarker as well as a new potential therapeutic target for MM therapy.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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REFERENCES

- Xu T, Yang W, Chen L, Gao G. What are the implications of cost for myeloma therapy? *Expert Rev Hematol* 2019; 12(12): 1005-1009.
- Barber FD. Multiple Myeloma Early Recognition by Primary Care Nurse Practitioners. *J Nurse Pract*; 2(10): 665-672.
- Kazandjian D. Multiple myeloma epidemiology and survival: A unique malignancy. In: *Semin Oncol*: 2016. Elsevier: 676-681.

4. Levin A, Hari P, Dhakal B. Novel biomarkers in multiple myeloma. *Transl Res* 2018; 201:49-59.
5. Gandolfi S, Laubach JP, Hideshima T, Chauhan D, Anderson KC, Richardson PG. The proteasome and proteasome inhibitors in multiple myeloma. *Cancer Metastasis Rev* 2017; 36(4): 561-584.
6. De Marchi T, Timmermans MA, Sieuwerts AM. Phosphoserine aminotransferase 1 is associated to poor outcome on tamoxifen therapy in recurrent breast cancer. *Sci Rep* 2017; 7(1): 2099.
7. Fu Q, Gao Y, Yang F, Mao T, Sun Z, Wang H, Song B, Li X. Suppression of microRNA-454 impedes the proliferation and invasion of prostate cancer cells by promoting N-myc downstream-regulated gene 2 and inhibiting WNT/ β -catenin signaling. *Biomed Pharmacother* 2018; 97(120-127).
8. Metcalf S, Dougherty S, Krueger T, Hasan N, Biyik-Sit R, Reynolds L, Clem BF. Selective loss of phosphoserine aminotransferase 1 (PSAT1) suppresses migration, invasion, and experimental metastasis in triple negative breast cancer. *Clin Exp Metastasis* 2020; 37(1): 187-197.
9. Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K, Sethumadhavan S, Woo HK, Jang HG, Jha AK et al. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 2011; 476(7360): 346-350.
10. Qian C, Xia Y, Ren Y, Yin Y, Deng A. Identification and validation of PSAT1 as a potential prognostic factor for predicting clinical outcomes in patients with colorectal carcinoma. *Oncol Lett* 2017; 14(6): 8014-8020.
11. Wei Z, Song J, Wang G, Cui X, Zheng J, Tang Y, Chen X, Li J, Cui L, Liu C-Y. Deacetylation of serine hydroxymethyl-transferase 2 by SIRT3 promotes colorectal carcinogenesis. *Nat Commun* 2018; 9(1): 1-16.
12. Gao S, Ge A, Xu S, You Z, Ning S, Zhao Y, Pang D. PSAT1 is regulated by ATF4 and enhances cell proliferation via the GSK3 β /beta-catenin/cyclin D1 signaling pathway in ER-negative breast cancer. *J Exp Clin Oncol* 2017; 36(1): 179.
13. Kurien BT, Scofield RH. Western blotting: an introduction. In: *Western Blotting*. Springer; 2015: 17-30.
14. Guo L, Liang Z, Zheng C, Liu B, Yin Q, Cao Y, Yao J. Leucine affects α -amylase synthesis through PI3K/Akt-mTOR signaling pathways in pancreatic acinar cells of dairy calves. *J Agric Food Chem* 2018; 66(20): 5149-5156.
15. Chen H, Zhou L, Wu X, Li R, Wen J, Sha J, Wen X. The PI3K/AKT pathway in the pathogenesis of prostate cancer. *Front Biosci (Landmark Ed)* 2016; 21(1084-1091).
16. John RR, Malathi N, Ravindran C, Anandan S. Mini review: Multifaceted role played by cyclin D1 in tumor behavior. *Indian J Dent Res* 2017; 28(2): 187.
17. Sherr CJ. G1 phase progression: cycling on cue. *Cell* 1994; 79(4): 551-555.
18. Zhou P, Jiang W, Zhang YJ, Kahn SM, Schieren I, Santella RM, Weinstein IB. Antisense to cyclin D1 inhibits growth and reverses the transformed phenotype of human esophageal cancer cells. *Oncogene* 1995; 11(3): 571-580.
19. Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A, Schmidt EV. Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. *Nature* 1994; 369(6482): 669-671.
20. Musgrove EA, Lee C, Buckley MF, Sutherland RL. Cyclin D1 induction in breast cancer cells shortens G1 and is sufficient for cells arrested in G1 to complete the cell cycle. *P Natl Acad Sci USA* 1994; 91(17): 8022-8026.
21. Tay KC, Tan LT, Chan CK, Hong SL, Chan KG, Yap WH, Pusparajah P, Lee LH, Goh BH. Formononetin: A Review of Its Anticancer Potentials and Mechanisms. *Front Pharmacol* 2019.