

Short Communication

Effect of SSAT on the Proliferation and Apoptosis of Prostate Cancer Cells *via* Regulation of Akt, GSK-3 β and β -catenin Expression

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ABSTRACT

To investigate the effect of spermidine/spermine-N(1)-acetyltransferase (SSAT) on the proliferation and apoptosis of prostate cancer LNCaP cells under hypoxic conditions. Prostate cancer LNCaP cells were transfected with the eukaryotic expression plasmid pcDNA3.1 (D)/V5-His-SSAT, and cultured at 1% oxygen concentration. Western blotting was used to detect expression of SSAT, Akt, GSK-3 β and β -catenin proteins; and flow cytometry was used to detect cell apoptosis rate and mortality rate. SSAT was successfully transferred into prostate cancer LNCaP cells. Compared to the blank cell group and the blank vector group, the expression of Akt, GSK-3 β and β -catenin proteins in the SSAT transfection group was significantly down-regulated ($P < 0.05$), the cell proliferation was significantly inhibited ($P < 0.05$), and both apoptosis rate and mortality rate increased ($P < 0.05$). Transfection of SSAT can reduce the proliferation and increase the apoptosis and necrosis rate of prostate cancer LNCaP cells by inhibiting the expression of Akt, GSK-3 β and β -catenin proteins.

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Authors' Contribution

XZ conceived the study, data collection and analysis and wrote the manuscript. EL and SH conceived the study, data collection. ZZ reviewed and edited the manuscript.

Key words

Spermidine/spermine-N(1)-acetyltransferase (SSAT), Akt, GSK-3 β , β -catenin, prostate cancer

Prostate cancer is a malignant tumor seriously threatening the health of men, and its pathogenic mechanism is not yet clear (Azal *et al.*, 2020; Masiuk *et al.*, 2020). It is an important task to study the molecular mechanism of prostate cancer cell proliferation, migration and invasion in developing anti-cancer therapy. In the research of cancer treatment, it is found that the metabolic function of polyamines, a class of cationic small molecule compounds, is disturbed in cancer patients, so polyamines have become attractive targets for therapeutic intervention. Spermidine/spermine-N(1)-acetyltransferase (SSAT) is the first rate-limiting enzyme in the catabolic pathway of polyamine metabolism. As SSAT strictly controls the intracellular polyamine level, it becomes a focus in the research of the relationship between polyamines and tumors (Wang *et al.*, 2017; Li *et al.*, 2019). At present, the effect of SSAT on the proliferation, migration and invasion of prostate cancer cells and its molecular mechanism have not been fully understood.

Serine/threonine protein kinase B (PKB, also known as Akt), is an important target kinase downstream of phosphatidylinositol-3-kinase (PI3K) (Shi *et al.* 2020). More and more studies have proved that PI3K/Akt

signaling pathway plays a major role in the proliferation and metabolism of various cell types (Ji *et al.*, 2020; Li *et al.*, 2020). PI3K can recruit and activate the downstream factor Akt via changing its conformational and exposing its phosphorylation site. Next, GSK-3 β is phosphorylated and loses its ability to bind to β -catenin, resulting in the activation of the Wnt signaling pathway (Kashyap *et al.*, 2013).

To investigate whether SSAT can participate in the molecular biological behaviors of prostate cancer by inhibiting the expression of Akt, GSK-3 β and β -catenin, SSAT gene was transfected into prostate cancer cells in this study to observe the effect of SSAT on Akt, GSK-3 β and β -catenin proteins and its effect on the regulation of Akt, GSK-3 β and β -catenin in the growth of prostate cancer.

Materials and methods

Human prostate cancer LNCaP cell line was cultured with McCoy's 5A medium containing 10% fetal bovine serum, and the medium was changed regularly for passage. The medium was changed before cells were seeded and transfected with X-tremeGENE HP transfection reagent. After that, the cells were cultured at 37 °C, 1% O₂, 94% N₂, and 5% CO₂ for 48 h. They were divided into the blank cell group, the blank vector group, and the SSAT transfection group. Liposomes were added without transfection of Akt siRNA in the blank vector group. SSAT was transfected

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in the SSAT group, and no intervention was given in the blank cell group.

Western blotting was used to detect the expression of SSAT, Akt, GSK-3 β and β -catenin proteins. The total protein in each group of cells was extracted with RIPA lysate, centrifuged, and the supernatant was transferred to a new centrifuge tube. After the protein concentration was determined by the BCA method and adjusted in consistency, the loading buffer was added in proportion and boiled for 5 min. After polyacrylamide gel electrophoresis was performed in the electrophoresis buffer, the proteins were transferred to the PVDF membrane, blocked with TBST containing 5% skim milk powder for 1 h, and added with 1: 5000 V5, Akt, GSK-3 β and β -catenin primary antibodies to incubate overnight at 4°C. Then, they were washed 3 times with TBST, incubated with corresponding secondary antibodies for 1 h, and washed with TBST 4 times for color development and luminescence.

Cells in the logarithmic growth phase were taken from each group for detecting the cell proliferation activity using MTT colorimetric method (Präbst *et al.*, 2017). The absorbance value (OD_{490}) was measured at the 490 nm wavelength of the microplate reader, and the cell proliferation was observed. The experiment was repeated 3 times.

The cells in each group were cultured under hypoxia for 48 h, and the cell mortality rate and apoptosis rate were detected by flow cytometry. The experiment was repeated 3 times.

SPSS 25.0 software package was used for analysis. Measurement data were expressed as the mean \pm SD. One-way analysis of variance, followed by LSD-t test, was used to compare each variable for differences among the groups. The $P < 0.05$ was considered statistically significant.

Results and discussion

The total protein in each group of cells cultured for 48 h was extracted, and the expression of SSAT proteins with V5 tag was detected by western blotting. The experimental results showed that SSAT was successfully transferred into prostate cancer LNCaP cells, while no exogenous SSAT proteins were expressed in the blank cell group and the blank vector group. After 48 h of cell transfection and hypoxic culture, the expression of Akt, GSK-3 β and β -catenin proteins in the SSAT transfection group decreased significantly, and there were significant differences compared to the blank cell group and the blank vector group ($P < 0.05$, Fig. 1).

It was observed after 24 h of cell culture that the cells in the SSAT transfection group were less viable compared to the blank cell group and the blank vector group. The differences were significant at 72 h of culture ($P < 0.05$, Fig.

2). MTT assay results showed that the proliferation was reduced in prostate cancer cells transfected with SSAT, and SSAT exerted an inhibitory effect on the proliferation of prostate cancer LNCaP cells. Sun *et al.* (2008) found that SSAT has an inhibitory effect on the growth of colorectal cancer cells.

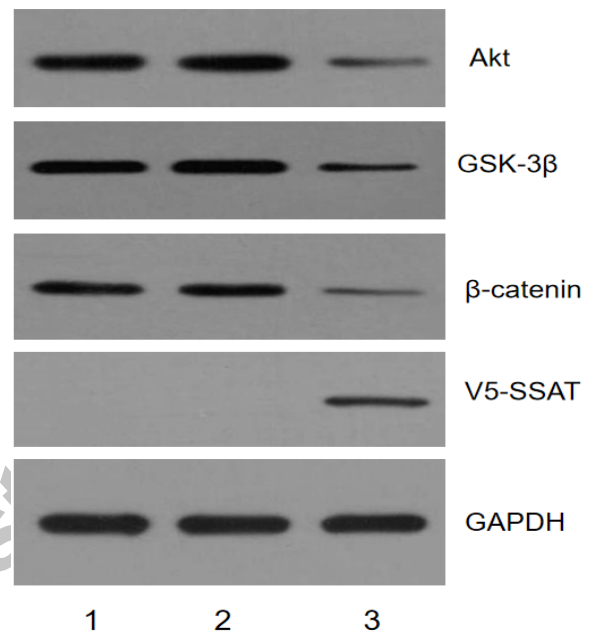


Fig. 1. Western blotting results of SSAT, Akt, GSK-3 β and β -catenin protein expression in each group (n=3). 1, Blank cell group; 2, Blank vector group; 3, SSAT transfection group.

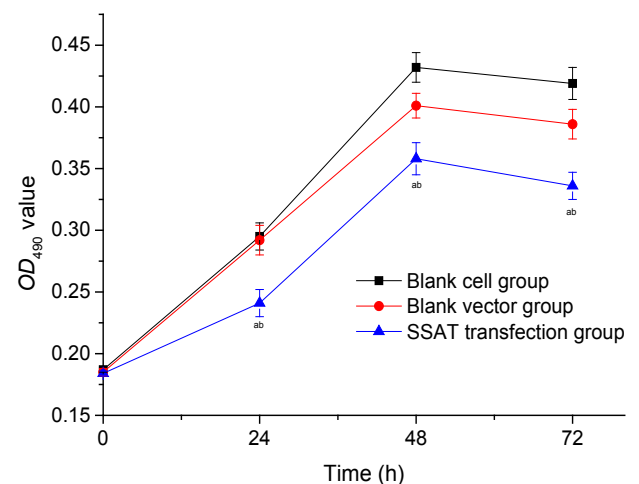


Fig. 2. Effect of SSAT transfection on the proliferation of prostate cancer cells by MTT assay (n = 3). ^a $P < 0.05$, compared to the blank cell group; ^b $P < 0.05$, compared to the blank vector group

Table I. Comparison of apoptosis rate and mortality rate in each group.

Parameter	Blank cell group (n=3)	Blank vector group (n=3)	SSAT transfection group (n=3)	P value
Mortality rate of prostate cancer cells (%)	2.27±0.46	6.60±0.53 ^a	18.30±1.23 ^{ab}	<0.05
Apoptosis rate of prostate cancer cells (%)	4.97±1.61	5.72±1.57 ^a	7.39±2.71 ^{ab}	<0.05

^aP <0.05, compared to the blank cell group; ^bP <0.05, compared to the blank vector group.

The cells in each group were cultured under hypoxia for 48 h. The results of flow cytometry showed that compared to the blank cell group and the blank vector group, the mortality rate and apoptosis rate of prostate cancer cells in the SSAT transfection group were elevated (P <0.05, Table I). Tian *et al.* (2012) demonstrated that overexpression of SSAT by DENSPM treatment induces cell detachment and apoptosis in glioblastoma.

In summary, transfection of SSAT under hypoxic conditions can inhibit the proliferation and promote apoptosis of prostate cancer cells. It suggests that transfection of SSAT may regulate the proliferation and apoptosis of prostate cancer cells via the Akt/GSK-3β/β-catenin signaling pathway. However, whether SSAT has a cross-linking effect with other signaling pathways in exerting this regulatory function still remains unclear, and further research is needed in the future.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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