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Original Article

Proteasome activator subunit 3 promotes pancreatic cancer growth via c-Myc-glycolysis signaling axis

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ABSTRACT

Pancreatic cancer has the worst prognosis among all cancers and novel markers and therapeutic targets are desperately needed for this terribly deadly disease. Proteasome activator subunit 3 (PSME3) is highly involved in the initiation and progression of many human cancers. However, the potential effect of PSME3 on pancreatic cancer remains largely unknown. In the present study, we first found that PSME3 was significantly upregulated in pancreatic cancer cells and tissues at both mRNA and protein levels using qRT-PCR, western blot analysis, Oncomine data mining and immunohistochemical analysis. High PSME3 expression was positively correlated with tumor size and pM stage, and was significantly correlated with poor prognosis in pancreatic cancer patients revealed by Kaplan–Meier analysis. Gene set enrichment analysis demonstrated that the gene sets related to cell proliferation and metastasis were positively correlated with elevated PSME3 expression. Consistently, silencing of PSME3 suppressed cell proliferation and invasive capacity of pancreatic cancer. Mechanistically, PSME3 inhibited the degradation of c-Myc and thus enhanced glycolysis, which ultimately led to the oncogenic effects of PSME3 on pancreatic cancer. Collectively, our data suggest that PSME3 plays oncogenic roles in pancreatic cancer by inhibiting c-Myc degradation to promote glycolysis, and could serve as a novel therapeutic target for pancreatic cancer treatment.

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Introduction

Pancreatic cancer is one of the most aggressive human malignancies that has not yet been successfully controlled [1]. Despite many decades of intensive research and many novel therapeutic strategies, the prognosis of patients with pancreatic cancer still remains bleak. Therefore, it is urgently needed to improve our understanding of the molecular mechanisms that drive the initiation and progression of pancreatic cancer, and to develop more efficient therapeutic options.

Proteasome activator subunit 3 (PSME3), one of the subunits of 11S proteasome regulator, also known as REG gamma (REGγ) and PA28γ, has been shown to regulate the degradation of many important regulatory proteins, including the oncogenic steroid receptor coactivator-3 (SRC-3), the cell cycle inhibitors (p16, p21 and p53) and ERα [2–9]. Several studies have identified PSME3 as an important regulator of cell proliferation and body growth in mice. For example, PSME3-deficient mice displayed growth retardation, immune defects and cell specific mitotic defects, and PSME3-knockdown cells exhibited decreased cell proliferation and increased cell apoptosis [10,11]. Recently, the importance of PSME3 in carcinogenesis has been highlighted by numerous studies that have shown that PSME3 is overexpressed in multiple human cancers, including liver, breast, thyroid, colorectal, lung and endometrial cancer [12–16]. Specially, PSME3 promotes the malignant potential of breast cancer by facilitating cell proliferation, suppressing cell apoptosis, and inhibiting immune activity, indicating its functional diversity in tumor progression [17]. However, the expression pattern and possible cellular functions of PSME3 in pancreatic cancer remain largely unknown.

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In the present study, we first found that PSME3 was highly expressed in pancreatic cancer cells and tissues at both mRNA and protein levels. High PSME3 expression was positively correlated with tumor size and pM stage. Then we showed that high PSME3 was significantly correlated with poor prognosis in pancreatic cancer patients using Kaplan–Meier analysis. We further confirmed the oncogenic roles of PSME3 in cell proliferation and invasion of pancreatic cancer by loss-of-function study. Finally, we demonstrated that PSME3 enhanced glycolysis through inhibiting c-Myc degradation, leading to its oncogenic implications in pancreatic cancer. Our findings provide novel insights into the functions of PSME3 in pancreatic cancer and suggest that PSME3 may serve as a promising therapeutic target for pancreatic cancer.

Materials and methods

Cell culture and reagent

The human pancreatic cancer cell lines, BxPC3, MiaPaCa-2, PANC-1, SW1990 and HPAC and the nonmalignant Human pancreatic ductal epithelial (HPDE) cells were purchased from the American Type Culture Collection (ATCC, USA). All cells were maintained in DMEM medium containing 10% fetal bovine serum and incubated at 37 °C with 5% CO_2. Cycloheximide and galactose were purchased from Sigma-Aldrich (Shanghai, China).

Quantitative RT-PCR analysis (qRT-PCR)

The analysis of qRT-PCR was performed using SYBR Premix Ex TaqTM (TaKaRa, Japan) on a Lightcycler system (Roche Diagnostics). The primers for PSME3 gene were as follows: Forward 5′-ATGTCAGAGTGGATCTGAG-3′ and Reverse 3′-TACAGTCTCACCTAGACTC-5′. Relative gene expression analysis was performed using the $2^{(-\triangle\triangle Ct)}$ method, with β-actin used as a housekeeping control. Each experiment was conducted in duplicate.

Western blotting analysis

Protein expression was detected using a western blot assay, as previously described [18]. Briefly, total cell lysate was extracted by RIPA lysis buffer (Beyotime Biotechnology). Approximately 30 μg of protein was separated on a 10% SDS-PAGE and transferred into a PVDF membrane. After blocking with 5% skimmed milk, the membranes were incubated overnight with primary antibodies against PSME3 (Proteintech, 14907-1-AP), c-Myc (Abcam, ab32072) and β-actin (Abcam, ab8227), followed by incubation with secondary antibodies. Visualization was done using the ECL methods (Invitrogen) according to the manufacturer's recommendations.

Oncomine analysis

The mRNA expression level of PSME3 in pancreatic cancer tissues was analyzed using Oncomine (https://www.oncomine.org/). The log-transformed and normalized expression values of PSME3 were extracted, analyzed, and read on Oncomine. A *P* value less than 0.05 was selected as a threshold to lower the false discovery rate.

Immunohistochemical analysis

The tissue microarray containing 81 pancreatic cancerous tissues and matched adjacent noncancerous tissues from 44 cases was purchased from Shanghai Outdo Biotech Inc. Immunostaining was performed following the standard procedures. Primary anti-PSME3 antibody (Proteintech, 14907-1-AP), horseradish peroxidase-labeled anti-rabbit secondary antibody and DAB were used to visualize the immunoreactivity. The degree of immunoreactivity was evaluated and scored by two independent investigators blinded to the clinical data. A semiquantitative scoring method based on both staining intensity and the percentage of positively stained cells was used to calculate the staining index (SI) as previously described [19]. Cutoff values for high and low expression of PSME3 were chosen based on a measurement of heterogeneity using the log-rank test with respect to overall survival. The optimal cutoff was identified as an SI score of greater than or equal to 4, which was considered to be high expression, and less than or equal to 3, which was considered to be low expression.

Gene set enrichment analysis (GSEA)

To gain insight into PSME3-mediated molecular pathways in pancreatic cancer, GSEA was performed using the Broad Institute GSEA version 4.0 software. The dataset GSE15471 consisted of 39 pancreatic cancer tissues and matched normal pancreatic tissue samples was downloaded from the NCBIs Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). The gene sets used for the enrichment analysis were downloaded from the Molecular Signatures Database (MsigDB, http://software.broadinstitute.org/gsea/index.jsp). The gene sets with a false discovery rate (FDR) less than 0.25 were considered as significantly enriched.

Transfection

All transfections were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Cells were transfected with PSME3 siRNAs or negative control (GenePharma Inc., Shanghai, China) at a concentration of 50 nM.

Cell proliferation assay

MiaPaCa2 and SW1990 cells were seeded into 96-well plates in triplicate at a density of 4000 cells/well and then transfected with PSME3 siRNAs or negative control. Cell viability was measured at day 1, 3 and 5 post-transfection, using the Cell Counting Kit (CCK8, Dojindo, Japan) according to the manufacturer's instructions. The absorbance at 450 nm was measured using a multifunctional microplate reader (Bio-Rad Laboratories, Hercules, CA).

Cell invasion assay

MiaPaCa2 and SW1990 cells were seeded into matrigel coated transwell chambers (BD Biosciences) in triplicate at a density of 2×10^4 cells/well in 200 μl serum-free DMEM medium. Then 700 μl DMEM containing 10% FBS was added into the lower compartment of the transwell chamber. After incubation for 48 h, invaded cells were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet, and then counted under a light microscope at a magnification of 200.

Glucose consumption and lactate production assay

MiaPaCa2 and SW1990 cells were seeded into 6-well plates at a density of 5×10^5 cells/well and then transfected with PSME3-specific siRNAs. After 48 h, culture medium were collected for analysis. Glucose and lactate levels in the medium were measured using the glucose colorimetric assay kit (BioVision) and Lactate Assay Kit (Sigma) respectively, according to the manufacturer's protocol. All values were normalized to cellular protein concentration.

Statistical analysis

Analysis of PSME3 differential expression between pancreatic cancer tissues and nontumorous pancreatic tissues was performed on Oncomine with one-tailed unpaired *t* test. Other statistical data were analyzed using GraphPad Prism 5.0 software. Data were represented as mean ± standard deviation (SD). The two-tailed Student t-test was used to reveal statistical significance. Overall survival was evaluated by the Kaplan–Meier analysis and the log-rank test. A *P* value less than 0.05 was considered to be statistically significant.

Results

PSME3 is commonly upregulated in pancreatic cancer cells and tissues

To explore the functional role of PSME3 in pancreatic cancer, we first determine the expression level of PSME3 in five pancreatic cancer cell lines by qRT-PCR. As shown in Fig. 1A, BxPC3, MiaPaCa2, SW1990, PANC-1 and HPAC cells all exhibited a significantly higher level of PSME3 gene compared with nonmalignant HPDE cells, especially MiaPaCa2 and SW1990 cells ($P < 0.001$). In line with this result, western blot analysis also demonstrated that PSME3 protein levels were remarkably elevated in all five pancreatic cancer cell lines compared with HPDE cells (Fig. 1B).

To further determine whether PSME3 is also overexpressed in clinical samples of human pancreatic cancer, We performed data mining in publicly available pancreatic cancer datasets using the Oncomine platform. Data from five independent datasets consistently showed that the mRNA level of PSME3 in pancreatic cancer tissues was significantly higher than that in their normal counterparts (Fig. 1C). To confirm this observation at protein level, we performed immunohistochemical analysis in a pancreatic cancer tissue microarray which was constructed from pancreatic cancer patients with clinical follow-ups. As demonstrated in Fig. 1D, both the aberrantly strong cytoplasmic and nuclear immunoreactivity of PSME3 was observed in malignant ductal epithelium, while no or weak positive signal was found in normal pancreatic ductal cells, implying the existence of an enrichment of PSME3 in human pancreatic cancer tissues. We further calculated the staining index (SI) scores by multiplying the percentage of positive tumor cells by

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