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RESEARCH PAPER

PSMC2 knockdown exerts an anti-tumor role in nasopharyngeal carcinoma through regulating AKT signaling pathway

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ABSTRACT

Nasopharyngeal carcinoma is a major public health problem in several countries, particularly in Southeast Asia and North Africa. However, the mechanism underlying the malignant biological behaviors of nasopharyngeal carcinoma is not fully clear. Our study intended to investigate the functional importance and molecular mechanism of proteasome 26 S subunit ATPase 2 (PSMC2) in the progression of nasopharyngeal carcinoma. We examined the expression of PSMC2 in both nasopharyngeal carcinoma tissues and normal healthy tissues using immunohistochemistry (IHC). Additionally, we conducted a series of cell experiments to verify the functional roles of PSMC2 and to explore the underlying pathway involved. The results revealed that PSMC2 was significantly upregulated in nasopharyngeal carcinoma tissues compared to normal tissues. Moreover, high PSMC2 was shown to closely correlate with the pathological stage and tumor infiltrate in nasopharyngeal carcinoma patients. Functionally, we observed a suppression of nasopharyngeal carcinoma progression upon knocking down PSMC2. This was evidenced by inhibited cell proliferation and migration *in vitro*, as well as impaired cell growth *in vivo*, along with increased apoptosis. Mechanistically, the inhibitory effects of PSMC2 silence on nasopharyngeal carcinoma could be reversed by the addition of AKT activator. Overall, our study sheds light on a novel mechanism underlying the development and progression of nasopharyngeal carcinoma, with PSMC2 exerting a positive regulatory role through the modulation of the AKT signaling pathway. A deeper understanding of PSMC2 may contribute to the development of improved treatment strategies for nasopharyngeal carcinoma.

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Introduction

Nasopharyngeal carcinoma is a tumor type that originates in the epithelial cells of the nasopharynx [1]. Nasopharyngeal carcinoma is characterized by distinct geographical distribution and is particularly frequent in East and Southeast Asia [2–4]. Over the past decade, there has been a gradual decline in the incidence of nasopharyngeal carcinoma and a significant decrease in mortality [5]. This can be attributed to advancements in radiotherapy and chemotherapy, which have led to excellent local control of the disease. However, the challenge lies in the treatment of metastatic nasopharyngeal carcinoma, as there is still a lack of effective therapies for distant metastases. Therefore, better understanding of the pathogenesis and metastatic mechanisms is essential for the development of novel therapeutics for nasopharyngeal carcinoma.

Proteasome 26S subunit ATPase 2 (PSMC2) is an important component of the 26S proteasome 19S regulatory subunit, which is located on the genome at 7q22.1-q22.3. PSMC2 is involved in various cellular processes, including cell differentiation and proliferation, apoptosis, DNA damage repair, energy metabolism and signal transduction [6]. Emerging evidence suggested that PSMC2, as a functional protein, promotes tumor progression in a variety of human tumors, such as ovarian cancer [7], colorectal cancer [8], osteosarcoma [9], pancreatic cancer [10], and hepatocellular carcinoma [11]. For instance, studies have shown that depleting PSMC2 expression suppressed ovarian cancer cell proliferation [7]. In pancreatic cancer, PSMC2 has been associated with cell proliferation and apoptosis [10]. Additionally, in osteosarcoma patients, high levels of PSMC2 expression have

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been observed, and knocking down PSMC2 diminished cell proliferation, migration, and ameliorated apoptosis, highlighting its oncogenic role in osteosarcoma [9]. However, whether PSMC2 participates in the development and progression of nasopharyngeal carcinoma has not been widely discussed.

In this study, we first proved the abnormal expression of PSMC2 in nasopharyngeal carcinoma tissue using a tissue microarray, then explored the biological significance and clinical relevance of PSMC2 in nasopharyngeal carcinoma patients. Additionally, we conducted an analysis to unveil the underlying molecular mechanism through which PSMC2 operates within the context of nasopharyngeal carcinoma.

Materials and methods

Cell lines, tissues and animals

In this study, we utilized human immortalized nasopharyngeal epithelial cells NP69 and four different human nasopharyngeal carcinoma cell lines, namely 5-8F, C666–1, CNE-2Z, and HONE-1. They were provided by American type culture collection (ATCC). For cell culture, 5-8F and CNE-2N cells were kept in 1640 medium supplemented with 10% fetal bovine serum (FBS). NP69, C666–1, and HONE-1 cells were grown in H-DMEM +10%FBS medium.

The experiments involving human tissue samples received support from the Scientific Research and Clinical Trial Ethics Office, The First Affiliated Hospital of Zhengzhou University. A tissue microarray, containing 74 cases of nasopharyngeal carcinoma tissues and 26 normal (chronic pharyngitis) tissues from healthy individuals, was provided by Shanghai Bioscienceres Co., Ltd. (Shanghai, China).

Regarding the animal experiments, all procedures were approved by the Scientific Research and Clinical Trial Ethics Office, The First Affiliated Hospital of Zhengzhou University. We procured female BALB-C nude mice, aged 4 weeks and weighing approximately 21 g, from Beijing Weitong Lihua Experimental Animal Technical Co., Ltd. (Beijing, China). The mice were housed in cages with a maximum of 5 mice per cage under controlled environmental conditions, including a temperature range of 22–25°C, humidity of 50–60%, and a 12-h light/dark cycle. They had access to an ample supply of water and food throughout the study.

Immunohistochemistry (IHC)

The melanoma and non-tumor sections were deparaffinized and subjected to antigen retrieval using 1× EDTA solution (Beyotime Biotechnology Co., Ltd, Shanghai, China). Following that, the sections were blocked with 3% H_2O_2 for 5 min to inhibit endogenous peroxidase activity. Then, the sections were incubated overnight at 4°C with primary antibodies, including PSMC2 (1:50, biorbyt, #orb30409) and Ki-67 (1:100, Abcam, #ab16667). After the primary antibody incubation, the sections were treated with a secondary antibody (goat anti-rabbit IgG H&L (HRP):1:400, Abcam). To visualize the antibody-antigen complexes, the sections were subjected to DAB staining together with hematoxylin counterstaining (Baso DiagnosticsInc., Zhuhai, China). Finally, the slides were sealed with neutral resin (China National Pharmaceutical Group Co., Ltd, Beijing, China), and then the images were captured and analyzed under an optical microscope. The staining results were assessed using an IHC scoring standard, which categorized the staining results as negative (0), positive (1–4), ++ positive (5–8), or +++ positive (9–12). This scoring system takes into account the staining intensity (ranging from weak to strong) and the extent of staining, which is graded as 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%), or 4 (76–100%).

Establishment of stably infected cells

The lentivirus expressing human PSMC2 short hairpin RNA (shPSMC2) was prepared using the following procedure: The RNA interference target sequence was designed based on the PSMC2 gene template by Shanghai Bioscienceres Co., Ltd. (Shanghai, China). After that, a single-stranded DNA oligo containing interference sequence was chemically synthesized and annealed to form a double-stranded DNA. This double-stranded DNA was then ligated into the BR-V-108 vector

and transferred into prepared TOP 10 E. coli competent cells. Positive recombinants were identified by PCR analysis and further confirmed by sequencing. Finally, the qualified plasmids were used for lentivirus packaging.

For lentiviral infection, C666–1 and CNE-2Z cells (2×10^5) were infected with lentiviral particles containing shPSMC2 or a control shRNA (shCtrl) at a concentration of 1×10^8 TU/mL under ENI.S +Polybrene conditions. Next, the cells were cultured in their respective growth medium for 72 h. The efficiency of PSMC2 knockdown was then evaluated.

RNA extraction, cDNA synthesis and qRT-PCR

After lentivirus infection, total RNA of C666–1 and CNE-2Z cells was isolated using TRIzol reagent (Sigma, St. Louis, MO, USA) for cDNA synthesis and quantitative real-time PCR (qRT-PCR). 2.0 μg RNA was reverse transcribed into cDNA using Promega M-MLV Kit (Promega, Heidelberg, Germany). qRT-PCR was performed using the SYBR Green Master Mix Kit (Vazyme, Nanjing, Jiangsu, China) and the Applied Biosystems 7500 Sequence Detection system. GAPDH was used as an internal normalization control. The relative expression of mRNA was evaluated based on the $2^{-\Delta\Delta Ct}$ method. The primers sequences (5′-3′) were listed as follows: the forward primer of PSMC2 is CAGC ACTCTGGGATTTGGCT, the reverse primer is TTT CTATCCACGCCCACTCTC; the forward primer of GAPDH is TGACTTCAACAGCGACACCCA, the reverse primer is CACCCTGTTGCTGTAGCCAAA.

Western blot assay

After lentivirus infection, C666–1 and CNE-2Z cells were collected to extract total protein. The proteins were separated using a 10% SDS-PAGE gel and subsequently subjected to western blot analysis. Firstly, the PVDF membranes were blocked with TBST solution containing 5% skim milk for 1 h at room temperature. Then, the membranes were incubated with primary antibodies, including PSMC2 (1:2000, Proteintech, #14905–1-AP), Bax (1:2000, Abcam, #ab18273), Bcl-w (1:1000, CST, #2724), AKT (1:3000, CST, #4691S), p-AKT (1:1000, Proteintech, #66444–1-Ig), p-STAT1 (1:2000, Abcam, ab109461), p-STAT3 (1:3000, Abcam, ab76315), β-Catenin (1:2000, Proteintech 66,379–1-Ig) and GAPDH (1:3000, Proteintech, #60004–1-lg). Following this, the membranes were incubated with secondary antibodies, such as Goat Anti-Rabbit (1:3000, Beyotime, #A0208) and Goat Anti-Mouse (1:3000, Beyotime, #A0216), for 2 h at room temperature. After that, the membranes were washed three times with TBST solution (10 min/time). Finally, the color rendering was conducted using the ECL +plusTM Western Chemiluminescent HRP Substrate kit.

Cell proliferation detection using the MTT assay

C666–1 and CNE-2Z cells, after being infected with shPSMC2 and shCtrl, were seeded into 96-well plates at a density of 5×10^3 cells/well. To evaluate cell proliferation, 10 μl of 5 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution, from Beijing Dingguo Biotechnology Co., Ltd., Beijing, China, was added to each well. At the conclusion of the experiments, the MTT solution allowed for the quantification of cell viability and proliferation.

Cell proliferation detection using the CCK-8 assay

C666–1 cells with shPSMC2 were plated in a 96-well plate with a volume of 100 μl/well. The cells were then exposed to a 20 μM AKT activator (SC79) for 24 h. Then, 10 μl CCK-8 regent was added to each well. 4 h later, the 96-well plate was placed on a shaker for 2–5 min to ensure proper mixing. The optical density (OD) value was detected at 450 nm using a microplate reader, allowing for the assessment of cell viability and proliferation.

Colony formation experiment

C666–1 and CNE-2Z cells were infected with shPSMC2 and shCtrl lentiviruses and followed by seeding into a 6-well plate (2 mL/well). The cells were allowed to grow for 5 days to form visible colonies. The presence of colonies was documented using a fluorescence microscope (Olympus, Tokyo, Japan). Finally, the cells were washed with PBS, fixed with 1 mL 4% paraformaldehyde, and stained with 500 μL

Giemsa (Dingguo, Shanghai, China). This staining allowed for the identification and counting of the individual colonies.

Detection of cell apoptosis by fluorescence activated cells sorting (FACS)

After lentivirus infection, C666–1 and CNE-2Z cells were cultured in a 6-well plate with a volume of 2 mL/well. When the cell confluence reached 85%, the cell suspension was centrifuged at 1,300 rpm, and the supernatant was discarded. Then, the cells were washed with D-Hanks (4°C, pH = 7.2 ~ 7.4). To assess apoptosis, the cells were stained in the dark by adding 10 μL Annexin V-APC (eBioscience, San Diego, CA, USA). The cell apoptosis level was evaluated using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

Transwell assay

Cell migration was detected using Transwell assays. The indicated cells (5×10^4) were seeded in 100 μl of FBS-free medium onto a fibronectin-coated polycarbonate membrane inserted in a Transwell kit (Corning, Kennebunk, ME, USA). In the lower chamber, 500 μl of medium containing 10% FBS was added as a chemoattractant. Following an appropriate incubation time, the cells adhering to the lower surface were fixed and stained with 1% crystal violet solution for 1 min. Subsequently, the migrated cells were counted under a microscope in three random fields.

Human phospho-kinase array-membrane

To investigate the impact of PSMC2 knockdown on phospho-kinases, we employed a Human Phospho-Kinase Array-Membrane assay in C666–1 cells. The cells were lysed using 2 × Cell Lysis Buffer, and the resulting lysates were used for further analysis. The Handling Array membranes were then blocked using 2 mL of 1×Wash Buffer II and incubated overnight at 4°C with the cell lysates and 1×Biotin-conjugated Anti-Cytokines. Finally, the membranes were visualized and analyzed using a chemiluminescence imaging system, allowing for the detection and quantification of the signals.

Mouse tumor cell xenograft assay

BALB/c nude mice were subcutaneously injected in the right flank with 1×10^7 C666–1 cells infected with shCtrl or shPSMC2, followed by their division into two groups ($n = 10$/group). The dimensions of the tumors, including their length and width, were regularly measured to calculate the tumor volume according to the formula (tumor volume=π/6×L×W×W) throughout the experimental period. After 29 days of injection, the mice were sacrificed, and the tumors were resected, weighed and examined for Ki-67 expression.

Statistical analysis

Data were presented as the mean ± standard deviation (SD), and statistical analysis was conducted using analysis of variance (ANOVA) or the unpaired t-test for two-group comparisons. All experiments were repeated at least three times. $P < 0.05$ was considered to be statistically significant.

Results

PSMC2 is frequently upregulated in nasopharyngeal carcinoma

In order to elucidate the roles of PSMC2 in the pathogenesis of nasopharyngeal carcinoma, we first set out to examine its protein expression in nasopharyngeal carcinoma tissues in comparison to normal tissues from individuals with chronic pharyngitis. We found that PSMC2 was strongly expressed in nasopharyngeal carcinoma tissues relative to normal tissues (Figure 1a). Specifically, we observed an increase in PSMC2 expression in 43.2% of the evaluated tumor samples ($n = 74$), whereas the expression was found to be low in 26 cases of normal tissues (Table 1). Furthermore, our subsequent analyses, including the Mann-Whitney U test, demonstrated a correlation between PSMC2 levels and both pathological stage and tumor infiltrate (Table 2). These findings were further validated through Spearman's rank correlation analysis (Table 3). In addition, the investigation by qRT-PCR and western blot analysis indicated that in comparison to NP69 cells, the mRNA and protein levels of PSMC2 were significantly higher in the 5-8F, C666–1, CNE-2Z, and HONE-1 cell lines. Notably, the expression levels were particularly

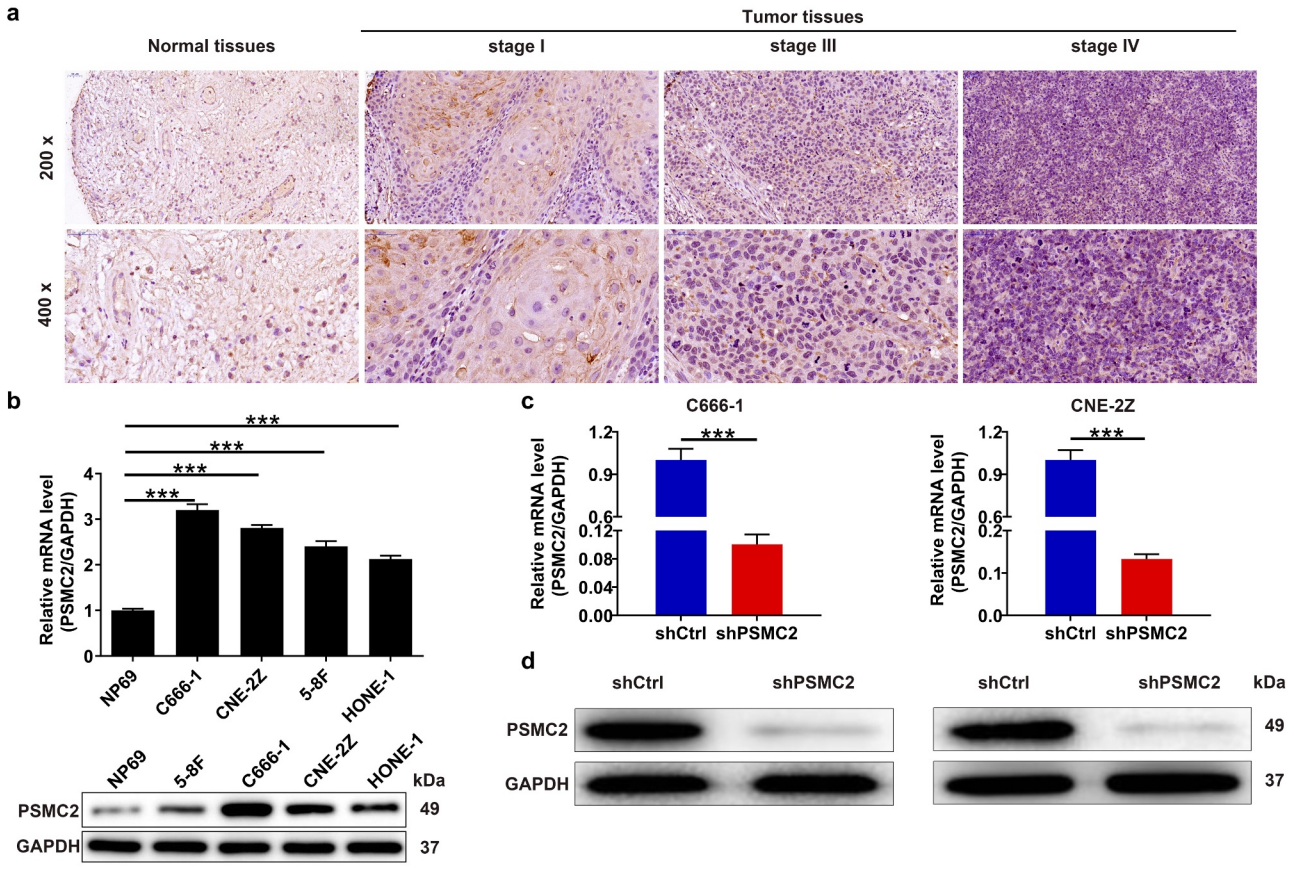

Figure 1. PSMC2 was upregulated in nasopharyngeal carcinoma and PSMC2 knockdown cell models were constructed. (a) the protein expression levels of PSMC2 in nasopharyngeal carcinoma tumor tissues and normal (chronic pharyngitis) tissues from healthy were determined by immunohistochemical staining. (b) the PSMC2 mRNA and protein expression in NP69 and nasopharyngeal carcinoma cell lines was detected by qRT-PCR and western blot analysis, respectively. (c, d) the knockdown efficiencies of PSMC2 were detected by qRT-PCR (c) and western blot (d). Results were presented as mean ± SD. ***$P < 0.001$.

Table 1. Expression patterns of PSMC2 in nasopharyngeal carcinoma tissues and para-carcinoma tissues revealed in immunohistochemistry analysis.

	Tumor tissue		Normal tissue		
PSMC2 expression	Cases	Percentage	Cases	Percentage	*P* value
Low	42	56.8%	26	100%	<0.001
High	32	43.2%	0	-	

elevated in C666–1 and CNE-2Z cells (Figure 1b), which were employed in subsequent loss-function experiments. Collectively, PSMC2 acts as a tumor promotor in nasopharyngeal carcinoma development.

Deletion of PSMC2 results in enhanced cell vitality and motility *in vitro*

In order to investigate the consequences of PSMC2 depletion, we utilized siRNA targeting PSMC2 to knock down its expression in C666–1 and CNE-2Z cell lines, both of which exhibited relatively higher levels of endogenous PSMC2. As expected, this resulted in the inhibition of PSMC2 expression at both mRNA and protein levels, as depicted in (Figure 1c,d) respectively. Subsequently, we evaluated the impacts of PSMC2 inhibition on cellular behaviors. Our findings demonstrated a notable decrease in the proliferation of C666–1 and CNE-2Z cells following PSMC2 knockdown (Figure 2a). Additionally, the formation of colonies generated from PSMC2-deficient cells was significantly reduced compared to cells infected with shCtrl (Figure 2b), aligning with the results obtained from the proliferation assays.

Table 2. Relationship between PSMC2 expression and tumor characteristics in patients with nasopharyngeal carcinoma.

Features	No. of patients	PSMC2 expression		*P* value
		low	high	
All patients	74	42	32	
Age (years)				0.791
≤51	38	21	17	
<51	36	21	15	
Gender				0.905
Male	62	35	27	
Female	12	7	5	
Stage				0.001
1	18	14	4	
2	22	8	14	
3	2	0	2	
4	2	0	2	
Tumor infiltrate				0.001
1	18	14	4	
2	22	8	14	
3	2	0	2	
4	2	0	2	
Grade				0.117
1	4	3	1	
2	10	6	4	
3	14	5	9	

Table 3. Relationship between PSMC2 expression with tumor infiltrate and pathological stage in patients with nasopharyngeal carcinoma.

		PSMC2
Tumor infiltrate	Spearman correlation	0.502
	Signification (double-tailed)	0.001
	N	44
Stage	Spearman correlation	0.502
	Signification (double-tailed)	0.001
	N	44

Moreover, we investigated the migration abilities of the cells and observed a decreased migration capability in both C666–1 and CNE-2Z cells when PSMC2 expression was downregulated (Figure 2c). Interestingly, the PSMC2-deficient cells exhibited higher rates of apoptosis. Flow cytometry analysis revealed a more than threefold increase in apoptotic capability compared to the shCtrl group (Figure 2d). Taken together, these findings suggested that PSMC2 plays a crucial role in regulating cell proliferation, migration and apoptosis during the development of nasopharyngeal carcinoma.

Deletion of PSMC2 results in arrested tumor outgrowth *in vivo*

To investigate the effects of PSMC2 depletion on tumor cell growth *in vivo*, we conducted an experiment involving the subcutaneous implantation of PSMC2-deficient C666–1 cells into BALB/c nude mice. The subsequent monitoring of tumor growth over a period of 29 days revealed an interesting observation: the tumors derived from PSMC2-deficient C666–1 cells exhibited substantially smaller size compared to those derived from cells infected with shCtrl (Figure 3a). This reduction in tumor size was evident not only in terms of tumor weight (Figure 3b) but also in tumor volume (Figure 3c). Furthermore, our analysis of Ki67 patterns, a marker for proliferation, in xenografts generated from C666–1 cells depleted of PSMC2 supported our *in vitro* proliferation assays (Figure 3d). The Ki67 patterns were notably decreased in the PSMC2-depleted xenografts, further highlighting the crucial role of PSMC2 in promoting the outgrowth of tumor cells *in vivo*. These findings collectively demonstrated that PSMC2 depletion has a significant impact on the growth of tumor cells.

PSMC2 mediates nasopharyngeal carcinoma via AKT signaling pathway

In this section, we aimed to unveil the molecular mechanism through which PSMC2 regulates the proliferation and apoptosis of nasopharyngeal carcinoma cells. Thus, we employed the Human Phospho-Kinase Array-Membrane to assess alterations in the phosphorylation status of various kinases in C666–1 cells following PSMC2 downregulation. We observed a decrease in the levels of several elements in response to PSMC2 depletion. These elements included Akt1/2/3 (S473), CREB (S133), EGFR (Y1086), eNOS (S1177), Chk-2 (T68), c-jun (S63), Fgr (Y412), Hsp27 (S78/S82), p53 (S15), p53 (S46), p53 (S392), JNK1/2/3 (T183/Y185, T221/Y223), Lck (Y394), Lyn (Y397), Msk1/2 (S376/S360), p70 S6K (T389), p70 S6K (T421/S424), PRAS40 (T246), p38α (T180/Y182), PDGF Rβ (Y751), Src (Y419), PYK2 (Y402), RSK1/2 (S221/S227), RSK1/2/3 (S380/S386/S377), STAT2 (Y689), STAT5a/b (Y694/Y699), WNK1 (T60), Yes (Y426), STAT1 (Y701), STAT3 (Y705), STAT3 (S727), β-Catenin and Hsp60 (Figure 4a). To further validate these findings, we performed western blot analysis, and the results indicated that the protein level of p-AKT exhibited the most significant reduction in PSMC2-silenced C666–1

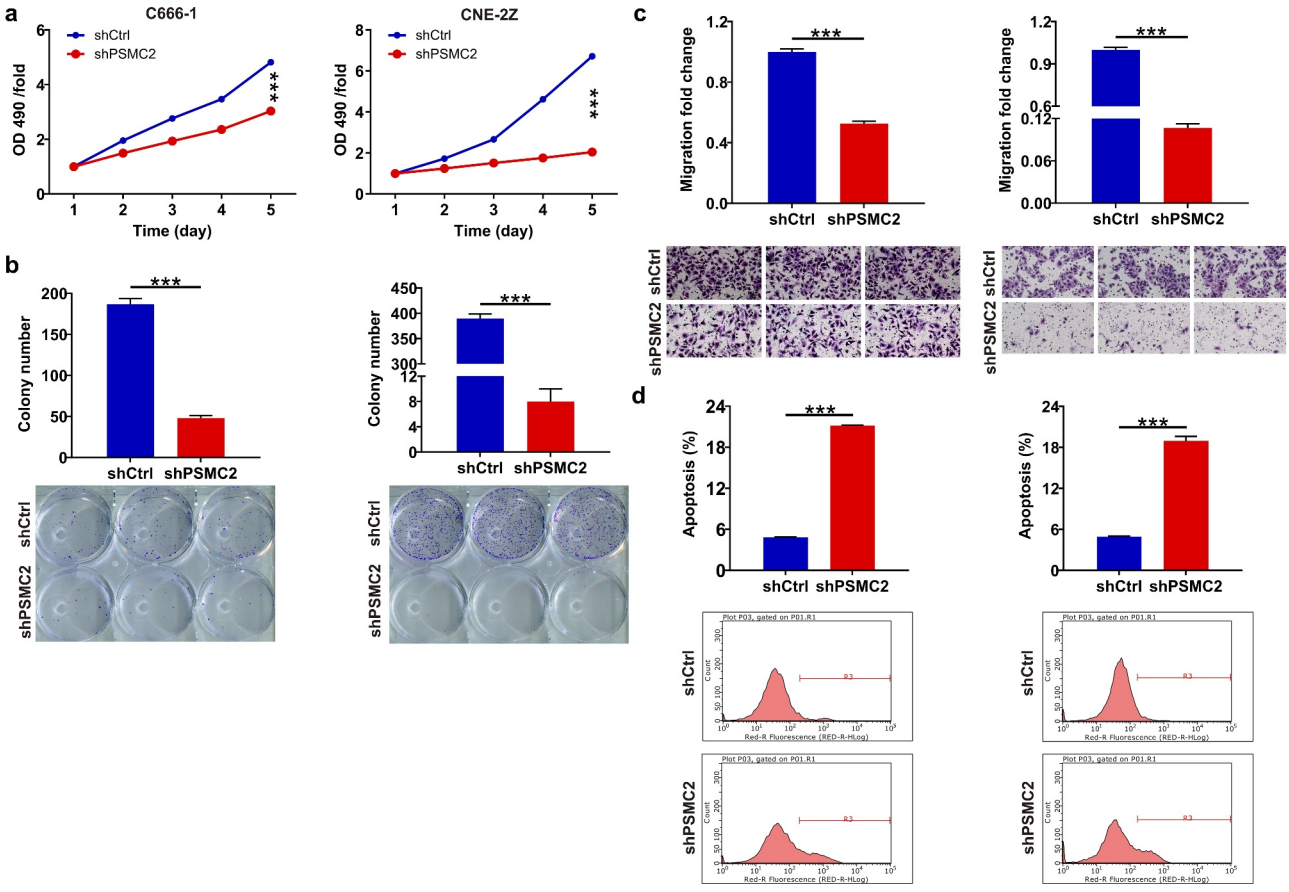

Figure 2. PSMC2 knockdown inhibited nasopharyngeal carcinoma cell proliferation and migration as well as enhanced cell apoptosis. (a, b) After lentiviral infection, C666–1 and CNE-2Z cell proliferation was evaluated by MTT assay (a) and colony forming assay (b). (c) After lentiviral infection, the changes of C666–1 and CNE-2Z cell migration were detected by transwell assay. (d) the effects of PSMC2 knockdown on C666–1 and CNE-2Z cell apoptosis were examined by fluorescence activated cells sorting (FACS). Results were presented as mean ± SD. ***$P < 0.001$.

cells (Figure 4b). It is well-known that the AKT pathway plays a crucial role in cell proliferation and apoptosis, which has been implicated in the progression of various malignant tumor, including nasopharyngeal carcinoma [12–14]. This implied that the AKT pathway could play a role in PSMC2-moderated nasopharyngeal carcinoma cell growth and apoptosis. To investigate this further, we treated PSMC2-deficient C666–1 and CNE-2Z cells with an AKT pathway activator and assessed various cellular behaviors and responses. As presented in (Figure 4c,d) in comparison to the shCtrl group, the shPSMC2 group exhibited suppressed cell proliferation and enhanced apoptosis. However, upon treatment with the AKT activator, the reversal of these changes was observed. At the molecular level, compared to the shCtrl group, the shPSMC2 group showed reduced levels of PSMC2, P-AKT, and Bcl-w proteins, along with increased expression of Bax. Following AKT activator treatment, the alterations in these factors were reversed (Figure 4e). In summary, we concluded that PSMC2 regulates nasopharyngeal carcinoma via the AKT pathway.

Discussion

In this study, by conducting IHC analysis on a tissue microarray, we found that PSMC2 was overexpressed in nasopharyngeal carcinoma. Besides, abundant PSMC2 was linked to patients' pathological stage and tumor infiltrate. Interestingly, we demonstrated that PSMC2 is essential for various malignant processes, including cell viability, migration, apoptosis, and regulation of tumor growth. Herein, we proposed that PSMC2 serves as a cancer-promoting factor in nasopharyngeal carcinoma.

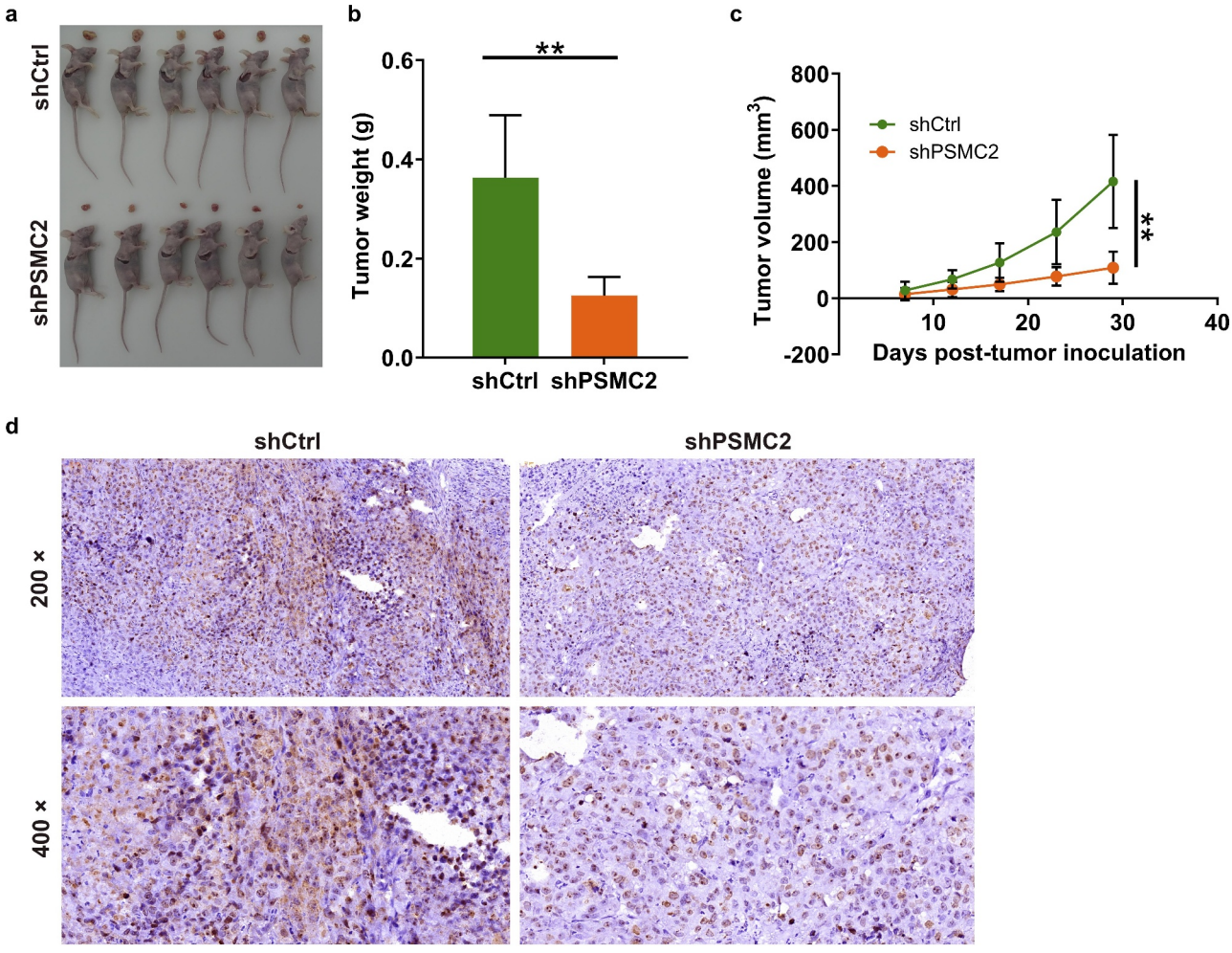

Figure 3. PSMC2 knockdown suppressed nasopharyngeal carcinoma tumor growth *in vivo*. (a) a nude mice model of PSMC2 knockdown was constructed. The photos of tumor derived from C666–1 cells infected with shCtrl and shPSMC2. (b) the weight of tumors was measured after sacrificing mice. (c) the volume of tumors was tested from feeding to sacrifice. (d) the value of Ki-67 was detected by IHC in tumor sections. Results were presented as mean ± SD. $^{**}P < 0.01$.

Previous studies have demonstrated PSMC2 overexpression to be associated with poor prognosis and aggressive tumor behaviors. For instance, in colorectal cancer, it was demonstrated that high expression of PSMC2 was associated with a lower overall survival rate compared to those with low PSMC2 expression [8]. Similarly, in patients with pancreatic cancer, high PSMC2 expression was correlated with a poor prognosis [10]. Besides, abundant PSMC2 was closely related to the pathological stage of skin cutaneous melanoma [15]. Duan *et al.* discovered a significant link between PSMC2 expression and both tumor infiltrate and tumor stage in hepatocellular carcinoma [16]. Likewise, in our research focused on nasopharyngeal carcinoma, we observed a similar pattern. PSMC2 expression was found to be closely associated with the pathological stage and tumor infiltrate in patients with this type of cancer. Moreover, PSMC2 was ubiquitously expressed in nasopharyngeal carcinoma samples compared to normal tissues. These findings strengthen the growing body of evidence highlighting the significance of PSMC2 in various cancer types, including nasopharyngeal carcinoma.

On the other hand, we suggested that PSMC2 knockdown remarkably inhibited cell proliferation and migration *in vitro*, as well as tumor growth *in vivo*, while facilitating apoptosis. Impaired apoptosis not only plays a crucial role in cancer development [17], but it also poses a major hurdle to effective treatment strategies [18]. In our study, cell apoptosis induced by PSMC2 knockdown is complex and involved in multi-apoptotic proteins. The Bcl-2 family, consisting of pro-apoptotic and anti-apoptotic subfamilies [19], serves as critical regulators

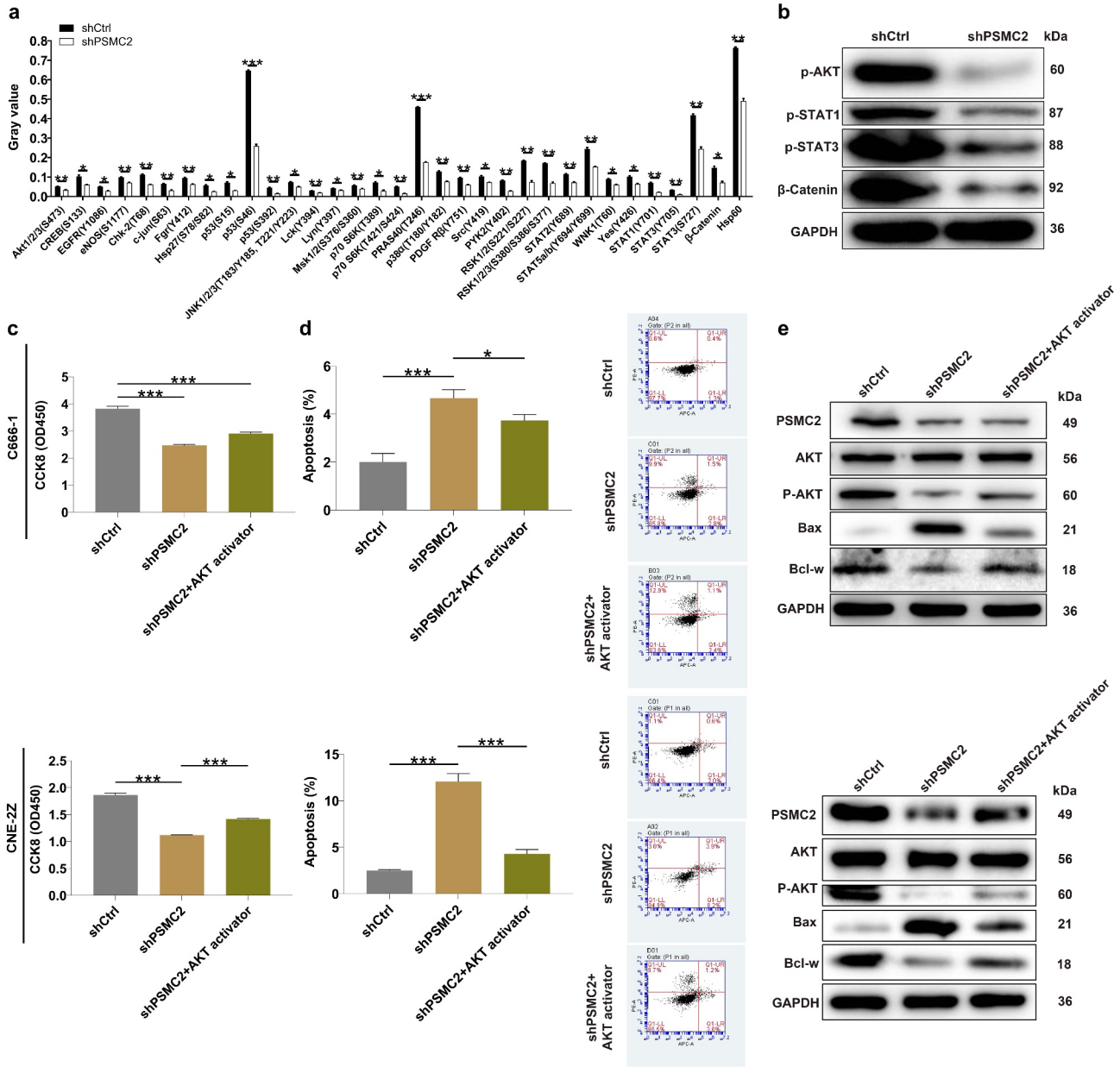

Figure 4. The exploration of molecular mechanism behind PSMC2 regulating nasopharyngeal carcinoma. (a) the phosphorylated protein levels in C666–1 cells with PSMC2 depletion were measured using human Phospho-Kinase Array-Membrane. (b) the levels of p-AKT, p-STAT1, p-STAT3 and β-Catenin in PSMC2-depleted C666–1 cells were assessed by western blot. (c, d) After SC79 treatment, the alterations of proliferation and apoptosis of C666–1 and CNE-2Z cells with PSMC2 depletion were detected by CCK8 assay (c) and flow cytometry experiment (d), respectively. (e) After SC79 treatment, the protein changes of PSMC2, AKT, p-AKT, Bax and Bcl-w in C666–1 and CNE-2Z cells with PSMC2 depletion were assessed by western blot. Results were presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

of cellular life and death signals. Among these family members, Bax and Bcl-w, which are frequently dysregulated in cancer [20,21], act as pro-apoptotic and anti-apoptotic factors, respectively. Our findings demonstrated that knocking down PSMC2 might regulate nasopharyngeal carcinoma cell apoptosis by increasing Bax and decreasing Bcl-w. Based on these findings, we postulated that PSMC2 mediated cell apoptosis by regulating Bax/Bcl-w expression. These findings unveiled a novel mechanism associated with PSMC2 and provide insights toward the development of a promising therapeutic target for nasopharyngeal carcinoma.

Furthermore, our study delved into the underlying mechanism through which PSMC2 operates. We observed that depletion of PSMC2 led to suppression

of p-AKT expression, which was subsequently reversed upon treatment with SC79. Many published documents have highlighted the significance of the AKT signaling pathway in the regulation of nasopharyngeal carcinoma [22–24]. Upregulation of the AKT pathway in patients with nasopharyngeal carcinoma is associated with a poorer prognosis [25]. Abnormalities in AKT pathway components have been implicated in multiple growth-related pathologies and several types of cancers, including nasopharyngeal carcinoma. For example, microRNA-613 exerts anti-angiogenic effect on nasopharyngeal carcinoma cells by downregulating FN1 and inactivating AKT signaling pathway [12]. Besides, Fan *et al.* suggested that YBX3 mediates the metastasis of nasopharyngeal carcinoma via AKT signaling pathway [13]. NOTCH2 negatively regulates metastasis and epithelial-Mesenchymal transition via TRAF6/AKT in nasopharyngeal carcinoma [26]. Consistent with these findings, our study reaffirmed the pivotal role of the AKT pathway in the progression of nasopharyngeal carcinoma.

We also discussed the potential underlying mechanisms or pathways through which PSMC2 may contribute to the malignant progression of nasopharyngeal carcinoma. PSMC2 is a component of the 26S proteasome, which plays a crucial role in protein degradation [6]. Overexpression of PSMC2 may lead to enhanced proteasome activity, resulting in the accelerated degradation of tumor suppressors and regulatory proteins, thereby promoting nasopharyngeal carcinoma cell proliferation, survival, and metastasis. Previous studies have indicated that PSMC2 may contribute to the dysregulation of cell cycle progression and apoptosis in osteosarcoma [9]. Changes in PSMC2 expression levels may disrupt the balance between cell proliferation and programmed cell death, leading to uncontrolled cell growth and resistance to apoptosis in nasopharyngeal carcinoma. Furthermore, PSMC2 has been associated with the regulation of DNA repair mechanisms [27]. Increased PSMC2 expression may contribute to DNA repair capacity in nasopharyngeal carcinoma cells, enabling them to overcome DNA damage and maintain genomic stability, thus facilitating tumor progression and resistance to therapy.

In summary, our study demonstrated that depletion of PSMC2 in human nasopharyngeal carcinoma cells resulted in suppressed proliferation and migration, along with enhanced apoptosis. Targeting PSMC2 could offer opportunities for the development of novel therapeutic interventions specifically tailored for nasopharyngeal carcinoma treatment. However, further investigation is required to translate these findings into clinical applications. For example, assessing PSMC2 expression in patient tissue samples or utilizing preclinical animal models will be essential to validate PSMC2 as a prognostic biomarker or a therapeutic target for nasopharyngeal carcinoma patients. Additionally, exploring the effectiveness and safety of PSMC2-targeted therapies, such as small molecule inhibitors or gene therapies, in preclinical and clinical settings will be crucial. We acknowledge the challenges associated with clinical translation, and it is worth considering the potential limitations and obstacles that may arise. For instance, off-target effects, resistance mechanisms, and potential side effects need to be carefully evaluated when developing PSMC2-targeted therapies.

Disclosure statement

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Ethics statement

This animal experiment was approved by Scientific Research and Clinical Trial Ethics Office, The First Affiliated Hospital of Zhengzhou University.

Author contributions

Jin Su and Kun Feng designed this research. Jin Su, ShouSen Hu and ShiPing Ding operated the cell and animal experiments. Jin Su, ShouSen Hu and Kun Feng conducted the data processing and analysis. Jin Su and ShiPing Ding completed the manuscript which was reviewed by ShouSen Hu, ShiPing Ding, Jin Su and Kun Feng. All the authors have confirmed the submission of this manuscript.

Data availability statement

The data used and analyzed during the current study are available from the corresponding author on reasonable request.

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