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# Proteasome Subunit Alpha Type-7 Expression Suppresses Cutaneous Squamous Cell Carcinoma Progression by Inhibiting Cancer-associated Cytokines

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**Abstract.** *Background/Aim: Cutaneous squamous cell carcinoma (cSCC) is a common non-melanoma skin cancer, and its incidence is increasing. Proteasome subunit alpha type-7 (PSMA7) has been found to be aberrantly expressed in several cancers. However, whether it functions as a tumor suppressor or oncogene in the pathogenesis of cancers, particularly cSCC, remains controversial. Here, we aimed to investigate the functions of PSMA7 in cSCC pathogenesis. Patients and Methods: Clinicopathological characteristics were evaluated in 131 patients with cSCC using tissue sections. The expression of PSMA7, nucleotide-binding oligomerization domain-containing protein 1 (NOD1), and mitochondrial antiviral signaling protein (MAVS) was determined in cSCC tissue sections using immunohistochemical staining. The effect of PSMA7 expression on the biological behavior of cSCC cells was investigated in vitro. Results: High immunoreactivity of PSMA7 (high-PSMA7) was detected in 53 (40.5%) patients with cSCC and was significantly associated with histologic grade (p=0.008) and favorable recurrence-free survival (p=0.018). The expression of PSMA7 and NOD1 (p=0.026) and MAVS (p=0.032) was negatively correlated in cSCC tissues. Contrary to the results of the cohort study, cell viability and invasiveness significantly decreased after PSMA7 down-regulation in cSCC cells in vitro. mRNA expression of tumor necrosis factor-alpha, interleukin-1 alpha (IL-1α), IL-6, and IL-8 were significantly increased after PSMA7 down-regulation in cSCC cells (all p=0.002). Conclusion: PSMA7-mediated degradation of NOD1 and MAVS as well as the subsequent reduction of the cancer-associated cytokine network may be a crucial mechanism of the antitumoral function of PSMA7 in patients with cSCC.*

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*Key Words:* Cancer associated cytokine network, cSCC, pathogenesis, MAVS, NOD1, PSMA7.

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Cutaneous squamous cell carcinoma (cSCC) is the second most common non-melanoma skin cancer in humans. cSCC accounts for 20% of skin cancers, and its incidence has increased dramatically (1, 2). Although cSCC often shows benign clinical features, it may also demonstrate metastasis, recurrence, and even lead to death (3).

Caucasian background, older age, UV-light radiation exposure, and immune suppression are common risk factors for cSCC occurrence (4). In our previous study, we found that a history of organ transplantation, diabetes, and other malignancies and histologic differentiation status were

significantly related to the recurrence of cSCC; it is treated with Mohs micrographic surgery, which usually demonstrates a higher cure rate than conventional wide excision treatment (5). The pathogenesis of cSCC may be complicated, resulting in misdiagnosis and adverse outcomes. Identification of reliable biomarkers and investigation of the related molecular mechanisms can provide a basis for pathogenesis research and can be subsequently used for early diagnosis and development of effective therapeutic strategies.

The proteasome is a pivotal component of the ubiquitin–proteasome system (UPS), which is involved in protein homeostasis (6). It can recognize and subsequently ubiquitinate proteins and further mediate their proteolysis and degradation, thereby influencing various biological behaviors of the cells, such as cell growth, differentiation, apoptosis, cell cycle progression, gene transcription, and signal transduction (7).

Previous studies have demonstrated that the UPS is associated with the pathogenesis of many neurodegenerative and myodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (8-10). Additionally, accumulating evidence has shown that the UPS can participate in malignant transformation in various cancers by controlling some key transcription factors, including p53, hypoxia inducible factor 1α (HIF1α), c-jun, methionine adenosyltransferase 2A (MATα2), c-Fos, androgen receptors, nuclear factor-κB (NF-κB), signal transducer and activator of transcription 3, and c-Myc (11).

The 26S proteasome, a key player of the UPS, is composed of two large complexes: the 19S regulatory complex and the 20S catalytic core (12). As an alphatype subunit of the 20S proteasome core complex, proteasome subunit alpha type-7 (PSMA7) participates in the regulation of proteasomal activity.

Aberrant expression of PSMA7 has been detected in various types of cancers (13, 14); PSMA7 may affect cancer pathogenesis through the degradation of proteins, which play pivotal roles in cancer progression. Previous studies have suggested that nucleotide-binding oligomerization domain-containing protein 1 (NOD1) and mitochondrial antiviral signaling protein (MAVS), well-known contributors to innate immunity, are possible degradation targets of PSMA7 (15, 16). Beyond its regulatory roles in the innate immune system, major functions of NOD1 and MAVS expression in cancer cells as well as in the tumor microenvironment (TME) have been found in various types of cancers (17-19). As a possible regulator of both NOD1 and MAVS, PSMA7 is thought to have multifaceted functions in cancer progression, which may affect not only the tumor cells themselves, but also the tumor microenvironment.

In this study, we investigated the clinicopathological significance of PSMA7 expression in patients with cSCC and further evaluated the effect of PSMA7 expression on the biological behavior of cSCC cells *in vitro*. This study explores new molecular mechanisms as well as diagnostic and therapeutic targets for cSCC.

Table I. *Clinicopathological characteristics of 131 patients with cutaneous squamous cell carcinoma.*

| Clinicopathological variable | Values, n (%) |
|---|---|
| Total no. of cases | 131 |
| Age | |
| Mean age (range) | 72.95 (30-90) |
| Sex | |
| Male | 62 (47.3) |
| Female | 69 (52.7) |
| Site | |
| Head and neck | 111 (84.7) |
| Acral | 17 (13.0) |
| Trunk | 2 (1.5) |
| Extremity | 1 (0.8) |
| Size, cm | |
| Median size (range) | 1.7 (0.3-5.0) |
| Differentiation | |
| WD | 62 (47.3) |
| MD | 59 (45.0) |
| PD | 10 (7.6) |
| Invasion of subcutaneous fat | |
| No | 114 (87.0) |
| Yes | 17 (13.0) |
| Recurrence | |
| No | 113 (86.3) |
| Yes | 18 (13.7) |

WD: Well-differentiated; MD: moderately differentiated; PD: poorly differentiated.

## Patients and Methods

*Patients in the cSCC cohort*. cSCC specimens were obtained from 131 patients who underwent Mohs micrographic surgery at the Department of Dermatology, Severance Hospital, Seoul, South Korea, from 2000 to 2017. This study was approved by the Institutional Review Board for Bioethics of the Yonsei University Health System, Severance Hospital (4-2018-0331). Clinicopathological characteristics of the patients are shown in Table I. Written informed consent has been obtained from the patient(s) to publish this paper.

*Immunohistochemistry*. The surgical specimens were cut into 4 μm tissue sections and deparaffinized with xylene and graded ethanol series. Antigen retrieval and blocking of endogenous peroxidase activity were performed sequentially as previously described (20). PSMA7 (rabbit monoclonal antibody, working dilution 1:250; Abcam, Cambridge, MA, USA, Cat. No. ab133502), NOD1 (rabbit polyclonal antibody, working dilution 1:200; Abcam, ab217798), and MAVS (rabbit polyclonal antibody, working dilution 1:200; Abcam, ab264147) antibodies were used in this study. As a negative control, the primary antibody was replaced by phosphate buffered

Table II. *Primers used in this study.*

| Gene | Primer | Primer sequences (5' to 3') |
|---|---|---|
| TNF-α | Forward | CAGAGGGCCTGTACCTCATC |
| | Reverse | GGAAGACCCCTCCCAGATAG |
| IL-1α | Forward | CCCAAAACCATCACAGGTAG |
| | Reverse | GCACACCCAGTAGTCTTGCT |
| IL-6 | Forward | TGACCTGAGCACCTTCTTTC |
| | Reverse | GGTGGAGAGCTTTCAGTTCA |
| IL-8 | Forward | GTTTTGCCAAGGAGTGCTAA |
| | Reverse | CCAGACAGAGCTCTCTTCCA |
| GAPDH | Forward | GTTTTGCCAAGGAGTGCTAA |
| | Reverse | CCAGACAGAGCTCTCTTCCA |

TNF-α: Tumor necrosis factor-alpha; IL: interleukin; GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

saline. Secondary antibody incubation and visualization were performed using REAL EnVision HRP Rabbit/Mouse Detection System (Dako, Carpinteria, CA, USA).

The weighted histoscore method was used to score PSMA7, NOD1, and MAVS protein expression in the cSCC tissues. As described previously, the total histoscore was calculated based on the staining intensity and percentage of positive cells (21). For further analysis, the patients were divided into two groups: low (total histoscore <100) and high (total his-toscore ≥100) levels of protein expression groups based on the total histoscore of each protein in patients with cSCC.

*Cell culture and establishment of PSMA7-knockdown cSCC cells*. The human cSCC cell lines A431 and HSC-1 were purchased from the Korean Cell Line Bank and the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan), respectively, and used in the *in vitro* study. Cell lines were cultured in Roswell Park Memorial Institute 1640 medium (Gibco Biosciences, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Invitrogen, Waltham, MA, USA). Scrambled (Scr)-small interfering (si) RNA or PSMA7-siRNA (Bioneer, Seoul, Republic of Korea) was transfected using Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions. The knockdown efficiency of PSMA7 in each cell line was evaluated using western blotting.

*Western blotting*. Total protein was extracted from each group of cSCC cells using cell lysis buffer (Cell Signaling Technology, Danvers, MA, USA). Protein samples obtained from each group of cells were resolved using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). PSMA7 (Rabbit monoclonal antibody, working dilution 1:1,000; Abcam, Cat. No. ab133502), NOD1 (rabbit polyclonal antibody, working dilution 1:1,000; Abcam, ab217798), and MAVS (rabbit polyclonal antibody, working dilution 1:1,000; Abcam, ab264147) antibodies were used as primary antibodies in this study. Anti-rabbit IgG (working dilution 1:5,000, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was used as the secondary antibody, and visualization was performed using an enhanced chemiluminescence detection system (Pierce Biotechnology Inc., Rockford, IL, USA).

*Quantitative real-time polymerase chain reaction*. Total RNA was isolated from A431 and HSC-1 cells following transfection with Scr-siRNA or PSMA7-siRNA using TRIzol reagent (Invitrogen). Complementary DNA synthesis was performed using oligo (dT) primers and GoScriptTM Reverse Transcriptase (Promega, Madison, WI, UA), according to the manufacturer's instructions. Tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1α, IL-6, and IL-8 mRNA expression in each group of cells was detected using the Fast SYBRTM Green Master Mix (Thermo Fisher Scientific) on an Applied Biosystems (Foster City, CA, USA) instrument. Primers used in this study are listed in Table II.

*Effect of PSMA7 knockdown on the biological behavior of cSCC cells*. The invasion ability of the cells was also comparatively investigated in each group of cSCC cells using Transwell invasion assay, as previously described (22). For analysis, $6\times10^4$ cells were seeded in each upper chamber and the invading cells through the Matrigel were counted after 36 h. To investigate the effect of PSMA7 on the proliferation or viability of cSCC cells, the number of viable cells was compared in each group of cells. For analysis, each group of cells was seeded in a 6 well plate ($6\times10^3$ cells/well) and counted at the indicated time points after trypan blue (Sigma-Aldrich, St. Louis, MO, USA) staining.

*Statistical analysis*. The effect of PSMA7 down-regulation on the biological behavior of cSCC cells was investigated using the Mann-Whitney *U*-test. The association between protein expression and clinicopathological variables was investigated using the chi-square test and Fisher's exact test. The prognostic significance of the expression of each protein was investigated using Kaplan-Meier analysis and log-rank test. SPSS version 26 (IBM Corp, Armonk, NY, USA) was used for all the statistical analyses. Statistical significance was set at $p<0.05$.

## Results

*Clinicopathological characteristics of patients with cSCC*. As shown in Table I, 131 patients with cSCC were included in the cohort. The mean age at diagnosis was 72.95, and the male-to-female ratio was 0.90. The most common cancer sites were the head and neck (n=111, 84.7%), followed by the acral (n=17, 13.0%), trunk (n=2, 1.5%), and extremities (n=1, 0.8%). In our cohort, 10 (7.6%) patients showed poorly differentiated histologic grades, and 17 (13.0%) showed cancer cell invasion into subcutaneous fat. The median follow-up period was 12 months, and 18 (13.7%) patients experienced recurrence during follow-up.

*Clinicopathological significance of PSMA7 expression in the cSCC cohort*. PSMA7 expression was detected in the nuclei and cytoplasm of tumor cells in cSCC tissue samples. A representative expression pattern of PSMA7 is shown in Figure 1A (i and ii). Of 131 patients with cSCC, 53 (40.5%) showed high immunoreactivity for PSMA7 expression (high-PSMA7), which was significantly lower in patients with a poorly differentiated grade (0, 0%) than in patients with a well-differentiated (30, 48.4%) or moderately differentiated

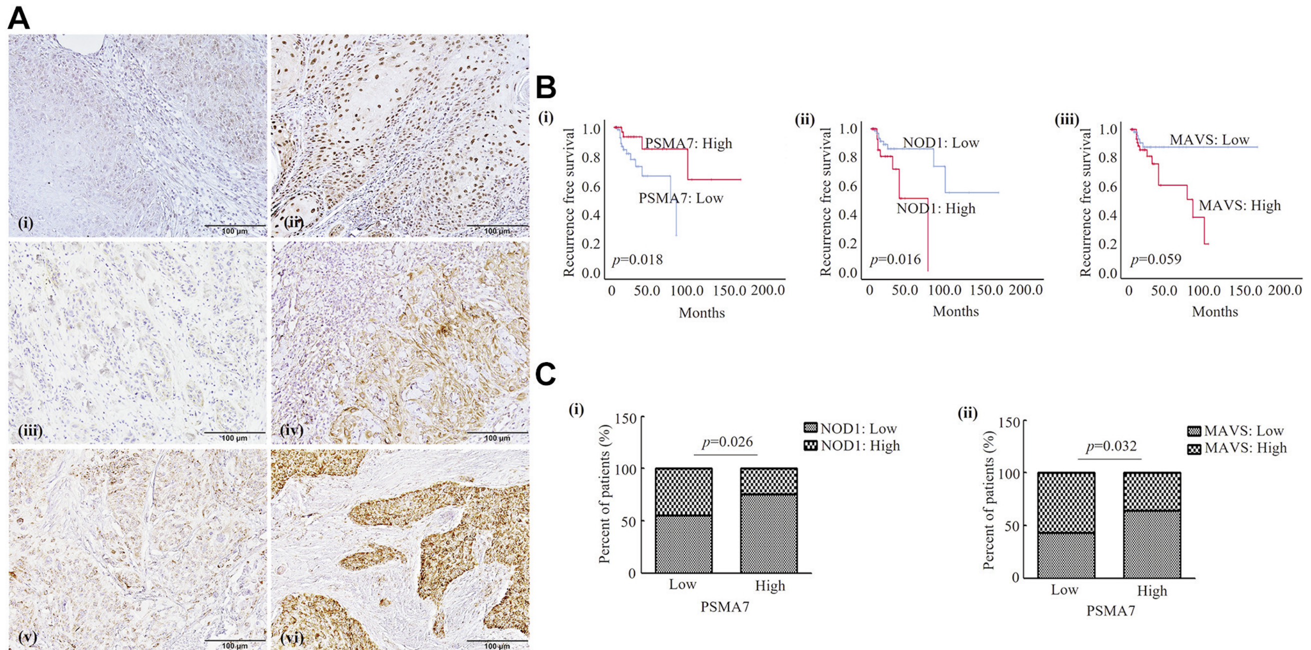


Figure 1. *Proteasome subunit alpha type-7 (PSMA7), nucleotide-binding oligomerization domain containing protein 1 (NOD1), and mitochondrial antiviral signaling protein (MAVS) expression in patients with cutaneous squamous cell carcinoma (cSCC). (A) Representative patterns of low and high expression of PSMA7 (i-ii), NOD1 (iii-iv), and MAVS (v-vi) (Original magnification: 200×; Scale bar: 100 μm). (B) Prognostic significance of PSMA7 (i), NOD1 (ii), and MAVS (iii) in patients with cSCC. (C) Association of PSMA7 expression with NOD1 (i) and MAVS (ii).*

(23, 39.0%) histologic grade ($p$=0.008) (Table III). Patients with high-PSMA7 showed a more favorable recurrence-free survival (RFS) than those with low immunoreactivity for PSMA7 (low-PSMA7) ($p$=0.018, mean survival duration 25.64 months for high-PSMA7 *vs*. 17.37 months for low-PSMA7) (Figure 1B i). There was no significant association between PSMA7 expression and other clinicopathological variables such as age, sex, lesion site, tumor size, and depth of invasion (Table III).

We also measured the expression of NOD1 and MAVS proteins, possible degradation targets of PSMA7. Representative expression patterns of NOD1 and MAVS are shown in Figure 1A (iii-vi). NOD1 and MAVS were both detected in the cytoplasm of tumor cells in the cSCC tissues. High immunoreactivity for NOD1 (high-NOD1) was found in 48 (36.6%) patients with cSCC. Patients with high-NOD1 had a lower RFS than those with low NOD1 immunoreactivity (low-NOD1) ($p$=0.016, mean survival duration 14.333 months for high-NOD1 *vs*. 24.410 months for low-NOD1) (Figure 1B ii). No significant association was found between NOD1 expression and other clinicopathological variables, such as age, sex, lesion site, histologic grade, tumor size, and depth of invasion (Table III). High immunoreactivity of MAVS (high MAVS) was found in 63 (48.1%) patients with cSCC. No significant association was found between MAVS expression and other clinicopathological variables, such as age, sex, lesion site, histologic grade, tumor size, depth of invasion, and RFS (Table III) (Figure 1B iii).

A significant association was found between the expression of PSMA7 and that of NOD1 and MAVS. High-NOD1 was detected more often in patients with low-PSMA7 (35, 44.9%) than in those with high-PSMA7 (13, 24.5%) ($p$=0.026). Similarly, high MAVS was also detected more often in patients with low-PSMA7 (44, 56.4%) than in those with high-PSMA7 (19, 35.8%) ($p$=0.032) (Figure 1C).

*Effect of PSMA7 down-regulation on cSCC cells*. PSMA7 down-regulation and increased expression of NOD1 and MAVS were observed in the PSMA7-siRNA group compared to the scr-siRNA group of HSC-1 cells. Moreover, NOD1 expression was also increased after PSMA7 down-regulation in A431 cells. Because the expression of MAVS in A431 cells is very weak, we did not detect the effect of PSMA7 on the expression of MAVS in A431 cells (Figure 2A).

The number of invading cells in the Matrigel-coated Transwell assay was compared in the scr- and PSMA7-siRNA groups of A431 and HSC-1 cells. The number of invading cells was 0.72- and 0.52-fold lower in the PSMA7-siRNA group than in the scr-siRNA group in both A431 and HSC-1 cells (both $p$<0.001) (Figure 2B).

Table III. *Clinicopathological significance of PSMA7, NOD1, and MAVS expression in patients with cutaneous squamous cell carcinoma.*

| Variables | Total no. of cases | PSMA7 | | *p*-Value | NOD1 | | *p*-Value | MAVS | | *p*-Value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Low | High | | Low | High | | Low | High | |
| Age | | | | | | | | | | |
| <73 | 52 (39.7) | 26 (50.0) | 26 (50.0) | 0.071 | 38 (73.1) | 14 (26.9) | 0.061 | 29 (55.8) | 23 (44.2) | 0.473 |
| ≥73 | 79 (60.3) | 52 (65.8) | 27 (34.2) | | 45 (57.0) | 34 (43.0) | | 39 (49.4) | 40 (50.6) | |
| Sex, n (%) | | | | | | | | | | |
| Male | 62 (47.3) | 37 (59.7) | 25 (40.3) | 0.976 | 44 (71.0) | 18 (29.0) | 0.087 | 34 (54.8) | 28 (45.2) | 0.525 |
| Female | 69 (52.7) | 41 (59.4) | 28 (40.6) | | 39 (56.5) | 30 (43.5) | | 34 (49.3) | 35 (50.7) | |
| Site, n (%) | | | | | | | | | | |
| Head and neck | 111 (84.7) | 68 (61.3) | 43 (38.7) | 0.274 | 70 (63.1) | 41 (36.9) | 1 | 56 (50.5) | 55 (49.5) | 0.803 |
| Acral | 17 (13.0) | 8 (47.1) | 9 (52.9) | | 11 (64.7) | 6 (35.3) | | 10 (58.8) | 7 (41.2) | |
| Trunk | 2 (1.5) | 2 (100) | 0 (0) | | 1 (50.0) | 1 (50.0) | | 1 (50.0) | 1 (50.0) | |
| Extremity | 1 (0.8) | 0 (0) | 1 (100) | | 1 (100) | 0 (0) | | 1 (100) | 0 (0) | |
| Size, cm | | | | | | | | | | |
| ≤2 | 85 (64.9) | 48 (56.5) | 37 (43.5) | 0.357 | 53 (62.4) | 32 (37.6) | 0.85 | 40 (47.1) | 45 (52.9) | 0.146 |
| >2 | 46 (35.1) | 30 (65.2) | 16 (34.8) | | 30 (65.2) | 16 (34.8) | | 28 (60.9) | 18 (39.1) | |
| Differentiation | | | | | | | | | | |
| WD | 62 (47.3) | 32 (51.6) | 30 (48.4) | 0.008 | 41 (66.1) | 21 (33.9) | 0.285 | 30 (48.4) | 32 (51.6) | 0.457 |
| MD | 59 (45.0) | 36 (61.0) | 23 (39.0) | | 38 (64.4) | 21 (35.6) | | 34 (57.6) | 25 (42.4) | |
| PD | 10 (7.6) | 10 (100) | 0 (0) | | 4 (40.0) | 6 (60.0) | | 4 (40.0) | 6 (60.0) | |
| Invasion of subcutaneous fat | | | | | | | | | | |
| No | 114 (87.0) | 68 (59.6) | 46 (40.4) | | 72 (63.2) | 42 (36.8) | | 59 (51.8) | 55 (48.2) | |
| Yes | 17 (13.0) | 10 (58.8) | 7 (41.2) | 1 | 11 (64.7) | 6 (35.3) | 1 | 9 (52.9) | 8 (47.1) | 1 |

PSMA7: Proteasome subunit alpha type-7; NOD1: nucleotide-binding oligomerization domain-containing protein 1; MAVS: mitochondrial antiviral signaling; WD: well-differentiated; MD: moderately differentiated; PD: poorly differentiated.

The number of viable cells was compared in the scr- and PSMA7-siRNA groups of A431 and HSC-1 cells. The number of viable A431cells was 0.86- and 0.67-fold lower in the PSMA7-siRNA group than in the scr-siRNA group at 36 and 72 h of culture ($p$=0.041 and $p$=0.002, respectively). Similarly, the number of viable HSC-1 cells was 0.68- and 0.43-fold lower in the PSMA7-siRNA group than in the scr-siRNA group at 36 and 72 h of culture ($p$=0.004 and $p$=0.002, respectively) (Figure 2C).

The expression of cytokines related to NF-κB signaling was also compared in the scr- and PSMA7-siRNA groups of A431 and HSC-1 cells. TNF-α mRNA expression was 4.64- and 3.48-fold higher in the PSMA7-siRNA group than in the scr-siRNA group of both A431 and HSC-1 cells (both $p$=0.002). Similarly, significantly increased IL-6 (1.57- and 6.69-fold, respectively) and IL-8 (5.78- and 3.61-fold, respectively) mRNA expression was found in the PSMA7-siRNA group than in the scr-siRNA group of both A431 and HSC-1 cells (all $p$=0.002). Moreover, IL-1α expression was 4.71-fold higher in the PSMA7-siRNA group than in the scr-siRNA group of HSC-1 cells ($p$=0.002). There was no significant difference in IL-1α expression in A431 cell after PSMA7 down-regulation (Figure 2D).

## Discussion

The role of PSMA7 in tumor progression remains controversial. Both oncogenic and tumor-suppressive functions of PSMA7 have been suggested in various types of cancers. Some researchers have found that high PSMA7 expression is positively correlated with liver metastasis in patients with colorectal cancer and has an independent impact on poor prognosis of patients with gastric cancer (13, 14). Moreover, depletion of PSMA7 can attenuate the tumorigenic activity, motility, and invasion ability of colorectal cancer cells *in vitro* (23). In contrast, some studies have found that PSMA7 over-expression can attenuate proliferation, invasion, and tumorigenic abilities of lung adenocarcinoma cell lines (24). In this study, we found oncogenic activities of PSMA7 in cSCC cells *in vitro*. We observed that the number of cells and the invasion ability were significantly decreased after the down-regulation of PSMA7 in cSCC cell lines. Contrary to what was expected from the findings of the *in vitro* study, high PSMA7 expression was associated with improved disease outcomes in our cohort. These conflicting results may be attributed to the regulatory function of PSMA7 in the TME.

In addition to the biological characteristics of tumor cells, the TME also plays a critical role in patient prognosis. As a

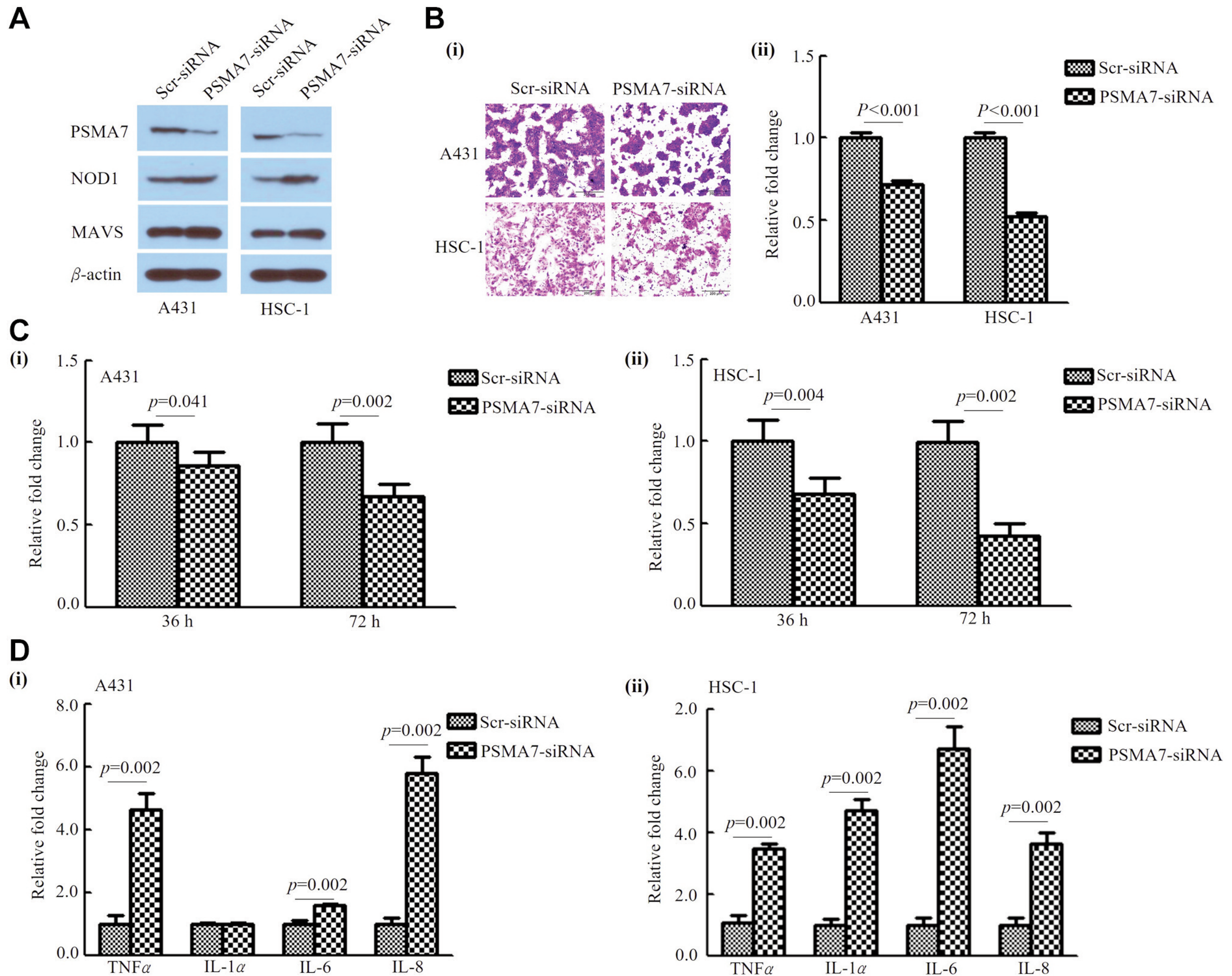


Figure 2. *The effect of proteasome subunit alpha type-7 (PSMA7) expression on the biological behavior of cutaneous squamous cell carcinoma (cSCC) cells. (A) PSMA7 down-regulation as well as both nucleotide binding oligomerization domain containing protein 1 (NOD1) and mitochondrial antiviral signaling (MAVS) expression was determined using western blot analysis. (B) Representative pattern of invading cells in each group (i). Invasion ability of the cells was significantly decreased after PSMA7 down-regulation (ii). (C) Number of viable cells was significantly decreased after PSMA7 down-regulation at the indicated time point. (D) Tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1α, IL-6, and IL-8 expression was significantly increased after PSMA7 down-regulation in cutaneous squamous cell carcinoma cells (i-ii).*

dynamic coordinator of tumor progression, the TME can largely influence tumor cell survival, growth, invasion, and metastasis (25, 26). Increased evidence suggests that the inflammatory TME suppresses or promotes tumor progression in various types of cancers. The suppressive function of the inflammatory TME in tumor progression largely depends on antitumor immune reactions mediated by both the innate and adaptive immune systems. Antitumor immune reactions are initiated by cytotoxic immune cells, such as natural killer cells and CD8 positive T cells, which are activated during the recognition of genetic alterations in tumor cells. However, the host cannot eliminate all tumor cells by antitumor immune reactions, and some tumor cells can escape tumor-immunosurveillance through a complex mechanism (27). In addition, various cytokines and chemokines secreted by leukocytes that are rich in the inflammatory TME can promote cancer proliferation, invasion, and metastasis (28, 29). The inflammatory TME may promote tumor progression by regulating the cancer-associated cytokine network rather than by promoting antitumor immunity.

NOD1 and MAVS, possible degradation targets of PSMA7, are crucial mediators of the inflammatory TME. NOD1 is a member of the NOD-like receptor family and is involved in innate immune responses, the first line of defense against invading pathogens. Under ligand sensing,

NOD1 can trigger the proinflammatory signaling pathway *via* the activation of NF-kB and mitogen-activated protein (MAP) kinase signaling pathways (30-32). As a well-known mediator of innate immunity, MAVS can also modulate inflammatory responses by activating the NF-κB pathway (33). As a crucial functional molecular link between NOD1 and MAVS, NF-κB is an inducible transcription factor that can promote the expression of many genes that play an important role in immune and inflammatory responses, such as various cytokines, including TNF-α, IL-1, IL-6, and IL-8 (34). In this study, PSMA7 expression was negatively associated with both NOD1 and MAVS expression in cSCC tissues. Furthermore, down-regulation of PSMA7 resulted in an increase in both TNF-α, IL-1 α, IL-6, and IL-8 expression in cSCC cells *in vitro*.

Growing evidence suggests that inflammation promotes cancer progression (35, 36). As major drivers of chronic inflammation, the involvement of TNF-α, IL-1 α, IL-6, and IL-8 in cancer progression have also been suggested in various types of malignancies, including cSCC. Studies have shown that TNF-α deficiency attenuates chemically induced skin carcinogenesis in mice (37). Moreover, TNF-α has been shown to promote cancer growth, invasion, metastasis, and chemoresistance in various types of cancers (38-42). Similar pro-tumoral functions of IL-1 α, IL-6, and IL-8 have also been suggested in various types of cancers, including cSCC (43-46). Some studies have shown that the antitumoral activity of TNF-α is also observed after acute local administration of TNF-α, while chronic and persistent TNF-α stimuli can promote the progression of cancers (47-49). In this study, PSMA7 down-regulation attenuated the oncogenic activities of cSCC cells. This may be due to the production of TNF-α mediated by PSMA7 down-regulation *in vitro*, which may not be sufficient to promote cancer progression in terms of quantity and duration. In contrast, PSMA7 down-regulation induced TNF-α production in patients with cSCC during cancer progression and may have tumor-promoting roles. Moreover, unlike the *in vitro* experimental conditions of cancer cells, cSCC tissues are composed of various key cellular components that contribute to cancer progression, and the cancer-associated cytokines can also largely affect the cells in the TME, thus further triggering new events related to the tumor-supporting roles of the TME. For example, IL-6 and IL-8 can contribute to cancer cell immune evasion by controlling the biological behaviors of cells in the TME, such as tumor-associated macrophages, dendritic cells, tumor-associated neutrophils, and myeloid derived suppressor cells (46, 50-54).

Cellular heterogeneity is a common feature of tumor tissues and represents the genotypic and phenotypic differences between subpopulations of cells. Even if the proliferative and invasive abilities were decreased in some subpopulations of cancer cells by PSMA7 down-regulation, it is not surprising that other subpopulations of cancer cells would evade immune surveillance and complete their invasion and metastasis under the effect of a cancer-associated cytokine network. Thus, PSMA7 may function as a favorable prognostic indicator in patients with cSCC *via* the control of cancer-associated cytokines.

In conclusion, our results suggest that PSMA7 mediated degradation of NOD1 and MAVS as well as the subsequent reduction of cancer-associated cytokine networks may be a crucial mechanism of the antitumoral function of PSMA7 in patients with cSCC. The details of the functions and related mechanisms of PSMA7 in cSCC pathogenesis require further investigation.

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## Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

## Authors' Contributions

Methodology, X.X.S. and M.P.; Software, M.P and K.Y.K.; Validation, X.H.R. and S.G.L.; Formal analysis, M.P. and K.Y.K.; Investigation: X.X.S. and M.P.; Writing-original draft preparation: M.P. and X.X.S.; Writing-review and editing, K.Y.C., M.R.R., and Z.H.J.; Supervision, M.R.R. and Z.H.J. All Authors have read and agreed to the published version of the manuscript.

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