Journal of Dental Sciences 19 (2024) 70–78

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Original Article

Serpin peptidase inhibitor, clade E, member 2 is associated with malignant progression and clinical prognosis in oral squamous cell carcinoma

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Received 2 February 2023; Final revision received 19 May 2023
Available online 12 June 2023

KEYWORDS
Oral cancer;
Protein expression;
Serine protease inhibitor;
SERPINE2

Abstract *Background/purpose:* The serpin peptidase inhibitor, clade E, member 2 (SERPINE2), is upregulated in breast cancer, prostate cancer, and urothelial carcinoma; however, limited information exists regarding its expression in oral cancer. Therefore, this study aimed to analyze the association between SERPINE2 expression and oral squamous cell carcinoma (OSCC) outcomes.
Materials and methods: SERPINE2 mRNA and protein expression in patients with head and neck squamous cell carcinoma and OSCC were investigated using online databases and tissue-array analysis. Its relationship with clinicopathological characteristics, OSCC prognosis and its biological function in OSCC cells were explored.
Results: Analysis using online databases revealed higher *SERPINE2* expression in tumor tissues and its role as a prognostic factor. High SERPINE2 protein levels were significantly correlated with adverse pathological parameters, including advanced clinical stage and tumor status

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https://doi.org/10.1016/j.jds.2023.05.024

($P < 0.001$), lymph nodes ($P = 0.014$), and distant metastases ($P = 0.013$). High SERPINE2 expression was associated with worse overall survival ($P < 0.001$) and was identified as an independent prognostic factor for OSCC. *In vitro* studies revealed that SERPINE2 knockdown significantly reduced cell proliferation, migration, and invasion in OSCC cell lines.
Conclusion: This study suggests that SERPINE2 may serve as a prognostic biomarker and potential therapeutic target for oral cancer.

Introduction

Oral cancer is a global public health concern associated with drinking, smoking, and betel quid chewing. Oral squamous cell carcinoma (OSCC) constitutes most oral cavity malignancies. Despite advances in diagnosis and treatment, its overall 5-year survival rate is approximately 65–70%[1]; therefore, discovering prognostic biomarkers remains essential.

Human serpin peptidase inhibitor clade E, member 2 (SERPINE2), a serine protease inhibitor superfamily member, is located on chromosome 2 q36.1, encoding a protein of 397 amino acids with a molecular weight of 43 kDa. SERPINE2 exhibits inhibitory activity against thrombin, tissue plasminogen activator, plasmin, urokinase plasminogen activator (uPA), and other serine proteinases.[2] According to its amino acid sequence, SERPINE2 belongs to the nexin protease family and is, therefore, commonly known as protease nexin-1. Various cells, including astrocytes, chondrocytes, endothelial cells, fibroblasts, macrophages, and platelets, secrete SERPINE2.[3,4] SERPINE2 is pleiotropic, and is found in various organs, with important roles in neuroprotection, thrombosis, and hemostasis.[5] *SERPINE2*-deficient mice exhibit important antithrombotic and antifibrinolytic properties.[6] Aberrant SERPINE2 expression also influences tumorigenesis of various cancers and contributes to tumor invasion and metastasis.[7–13] Furthermore, it promotes cell migration, invasion, and proliferation in esophageal squamous cell carcinoma.[14] However, information regarding its expression in OSCC remains limited.[15] Therefore, this study aimed to explore the relationship between SERPINE2 and clinicopathological parameters, OSCC prognosis, and migration and invasion *in vitro*.

Materials and methods

In silico mRNA profiles of *SERPINE2* expression and patient prognosis

TNMplot (https://www.tnmplot.com), an online analysis tool using gene array-generated data from the GEO of the NCBI and RNA-seq of TCGA, GTEx, and TARGET repositories,[16] was used to investigate and compare SERPINE2 mRNA expression in cancer and normal tissues. The Kaplan–Meier Plotter (KMplot; http://kmplot.com) was used to evaluate SERPINE2 prognostic significance in 499 head and neck squamous cell carcinoma (HNSCC) patients with 5-year overall survival rates.[17] For further analysis in the Asian population, a human OSCC mRNA expression microarray dataset [accession number GSE37991] for *SERPINE2* was selected and downloaded from the GEO (http://www.ncbi.nlm.nih.gov/geo/) with GEO2R, containing a pairwise comparison between 40 OSCC and adjacent noncancerous epithelial tissues from Taiwan.[18]

Tissue specimens

Paraffin-embedded samples were obtained from 122 OSCC patients (107 males and 15 females; mean age, 54 years; range, 26–87 years) from the Department of Pathology and Laboratory Medicine of Kaohsiung Veterans General Hospital between 1996 and 2006. The Institutional Review Board of Kaohsiung Veterans General Hospital approved the study protocol (approval code: KSVGH20-CT3-03). The 122 cases were classified according to the 2017 WHO Classification of Head and Neck Tumours,[19] staged according to the eighth edition of the American Joint Committee on Cancer Staging Manual,[20] and the hematoxylin-eosin sections were reviewed by two pathologists (HWC and TYF). None of the patients had undergone radiation or chemotherapy before surgery.

Immunohistochemistry

Samples were sliced into 4 μm sections on pre-coated slides, followed by deparaffinization in xylene, dehydration in alcohol, and antigen retrieval using a pressure cooker in 10 mM citrate buffer (pH 6.0) for 30 min. The UltraVision Quanto Detection System (Thermo Fisher Scientific, Fremont, CA, USA) was used for immunohistochemical staining. In brief, endogenous peroxidase activity and nonspecific binding were blocked by incubation with 3% hydrogen peroxide and non-immune goat serum separately. The sections were sequentially incubated at room temperature with monoclonal mouse anti-human SERPINE2 antibody (1:100; #MA5-25936; Invitrogen, Carlsbad, CA, USA) for 60 min, biotinylated secondary antibody for 10 min, and peroxidase-conjugated streptavidin for 10 min. The immunoreaction was visualized using 3,3′-diaminobenzidine and counterstained with hematoxylin, followed by clearing and mounting. Sections processed without the primary anti-SERPINE2 antibody were used as negative controls. Normal oral epithelium was used as an internal positive control. SERPINE2 expression was defined as membranous and/or cytoplasmic staining. Two

pathologists (HWC and TYF), without knowledge of the clinical information of the study participants, evaluated the slides using a semi-quantitative H-score method, as previously described[13]; the H-score ranged from 0 to 300. A consensus was reached regarding controversial cases using a multiheaded microscope.

Cell culture and lentivirus infection

Oral cancer cell lines, SAS, Ca922, and Cal27, were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA), and HSC3 cells were maintained in DMEM/F-12 (Gibco BRL, Grand Island, NY, USA), both mediums supplemented with 10% fetal bovine serum (FBS; Gibco BRL, Grand Island, NY, USA). OECM-1 cells were grown in Roswell Park Memorial Institute-1640 medium (Gibco BRL, Grand Island, NY, USA). All the cells were incubated in a humidified atmosphere of 5% CO_2 at 37 °C. Lentiviral pLKO.1 plasmid vectors containing shRNAs for human SERPINE2 [clone ID: TRCN0000290152 (shSERPINE2)] and a mammalian non-targeting control shRNA for luciferase (shLuc, clone ID: TRCN0000072246) were purchased from the National RNAi Core Facility, Academia Sinica,

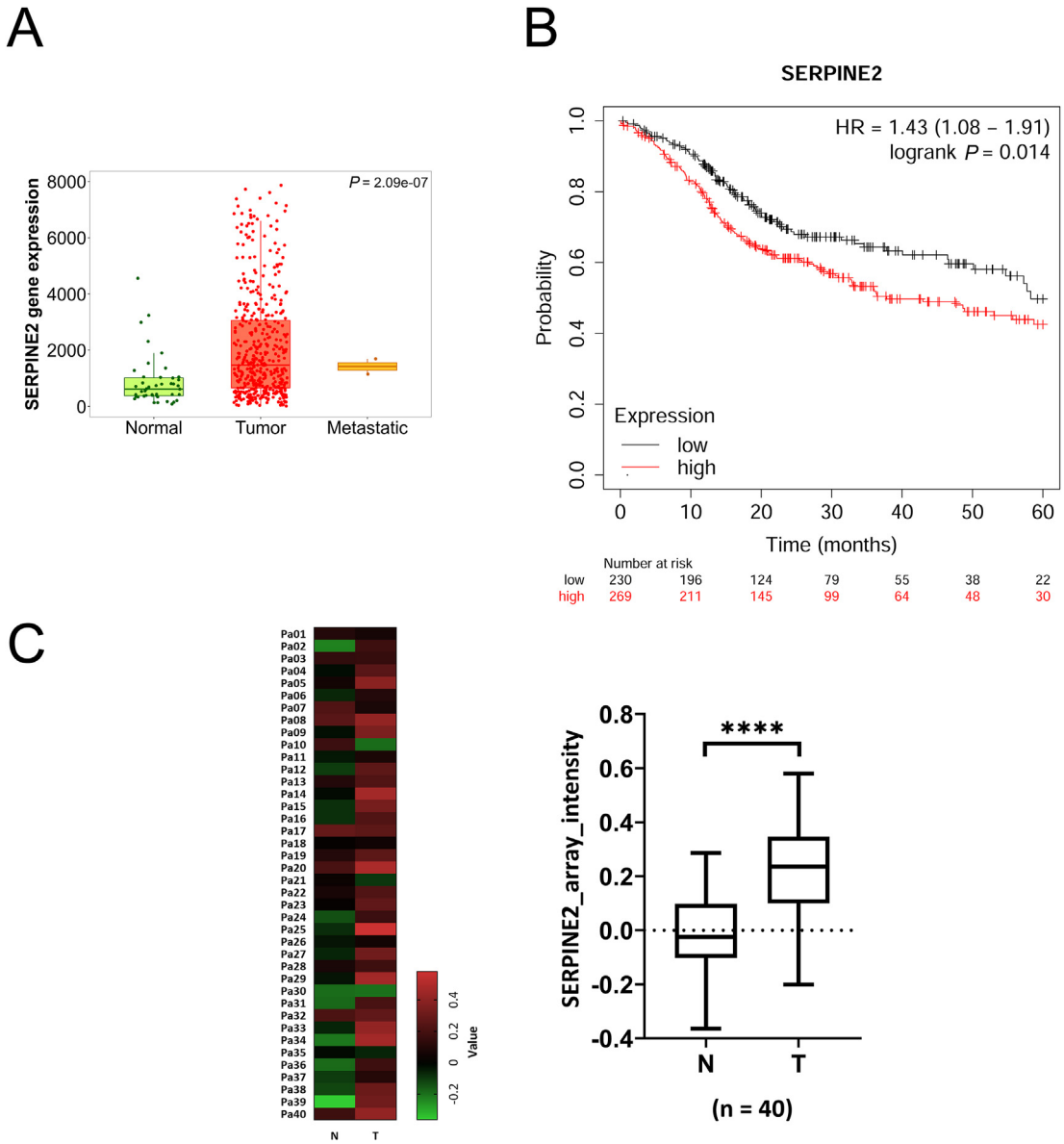

Figure 1 mRNA expression with *SERPINE2* diagnostic and prognostic values. (A) *SERPINE2* mRNA expression levels in 546 normal, tumor, and metastatic head and neck squamous cell carcinoma (HNSCC) tissues. *SERPINE2* expressions were higher in tumor tissues than in normal tissues in TNMplot ($P = 2.009e-07$). (B) High *SERPINE2* mRNA expression was significantly correlated with poor 5-year overall survival in KMPlot ($P = 0.014$). (C) Heat map and boxplot of *SERPINE2* mRNA expression in 40 OSCC tissue pairs (GSE37991). *SERPINE2* is upregulated in human OSCC. Red, upregulated; green, downregulated. Array intensity of *SERPINE2* in 40 OSCC tumors compared with their adjacent normal tissues; *SERPINE2* intensity is expressed as the log2 ratios. Data are represented as mean ± standard deviation; ****, $P < 0.0001$.

Taipei, Taiwan. Recombinant lentiviruses and transfection were conducted as described previously.[21] Stable knockdown cell lines (HSC3 and CAL27) expressing SERPINE2 shRNAs were selected for 10 days with 2.5 μg/ml puromycin after 24 h of infection.

Western blot analysis

The SERPINE2 monoclonal antibody (Invitrogen) was used at a different dilution (diluted 1:500 in 12.5 mM Tris/HCl, pH 7.6, 137 mM NaCl, 0.1% Tween 20 with 5% non-fat milk). Cultured cells were immediately washed with ice-cold phosphate-buffered saline. Ice-cold lysis buffer containing protease inhibitor cocktail tablets (Roche Diagnostics, Mannheim, Germany) and 1% NP-40 (MilliporeSigma, Burlington, MA, USA) was used to lyse cells for 15 min at 4 °C. Whole-cell lysates were obtained 15 min after centrifugation at 12,000×*g*, and the supernatant protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Western blot analysis was performed as previously described.[21]

Proliferation, migration, and invasion assay

Proliferation assay was conducted as described previously.[21] Cell migration and invasion assays were performed on a 24-well Transwell plate with an 8 μm pore size polycarbonate filter membrane (Corning, Acton, MA, USA). The wells were coated with BioCoat Matrigel (BD Biosciences,

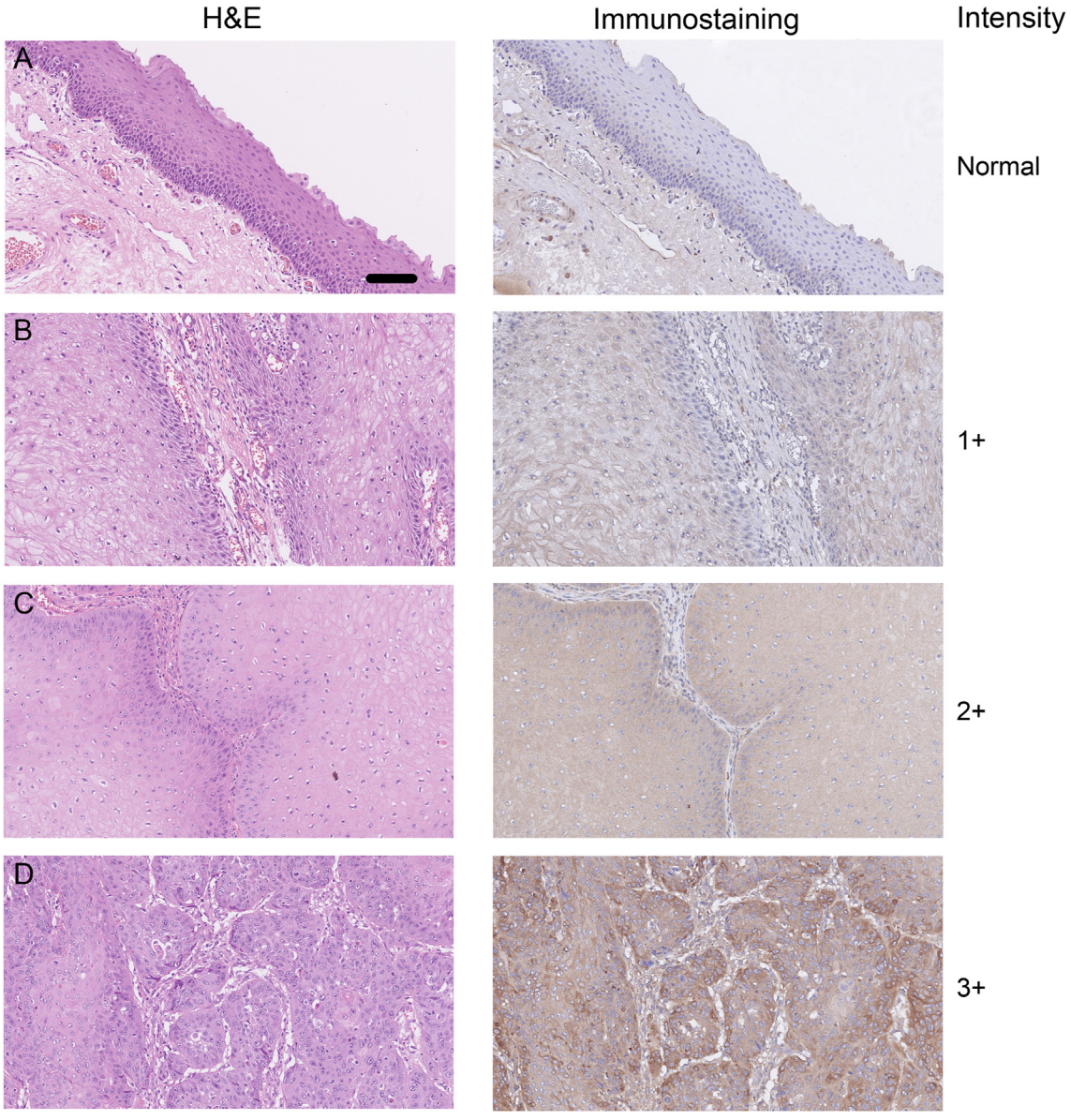

Figure 2 Representative sections of non-cancer epithelia and OSCC were stained with hematoxylin and eosin and immunostained for SERPINE2. (A) Non-cancer epithelia, (B) weak (1+), (C) moderate (2+) and (D) strong (3+). Scale bar in (A), 100 μm. The scale bar applies to all panels.

San Jose, CA, USA) for invasion assay. Cancer cell suspensions, at a cell density of 1×10^5 in 200 μl of serum-free medium, were seeded into the upper compartment for cell migration and invasion assays, and the lower chamber was filled with 10% FBS medium (Gibco BRL). After incubation for 24 h, the cells remaining in the upper chamber were removed. The filter membrane was fixed with 4% formaldehyde and stained with 5% Giemsa stain solution. The migrated and invaded cells were counted under an inverted microscope (200× magnification) in five different fields.

Statistical analysis

Statistical analyses were performed using the SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). The Mann–Whitney *U* test or Kruskal–Wallis test was used to analyze the statistical significance of the associations between SERPINE2 scores and clinicopathological parameters. Overall survival was analyzed using the Kaplan–Meier method, and the log-rank test was used for comparison based on a median cutoff immunoscore value of 160. Cox proportional hazards analyses were used to establish univariate and multivariate comparisons of survival distributions. The *in-vitro* data were expressed as mean ± standard deviation and analyzed using Student's *t*-test. All *P*-values < 0.05 were regarded as statistically significant.

Results

In silico profiles of SERPINE2 in HNSCC and OSCC patients

SERPINE2 mRNA expression in HNSCC and OSCC patients was evaluated using the TNMplot database and GSE datasets. In the TNMplot database, *SERPINE2* mRNA expression was significantly upregulated in tumor and metastatic HNSCC tissues compared to normal tissues ($P = 2.009e{-}07$) (Fig. 1A). Survival analysis using KMplot revealed that high *SERPINE2* mRNA expression conferred a significantly poor 5-year overall survival rate compared to low *SERPINE2* expression in HNSCC (Fig. 1B). In the GSE37991 dataset, using microarray data generated from 40 pairs of OSCC cohorts with a betel quid-chewing habit, SERPINE2 levels were significantly higher in tumors than in their corresponding normal samples ($P < 0.001$, Fig. 1C).

SERPINE2 protein expression is associated with poor clinical outcomes in OSCC patients

SERPINE2 was found in the cytoplasm and cell membranes of normal oral epithelial and OSCC cells, with weak positive immunoexpression in the parabasal layer of normal oral squamous epithelium (positive control; Fig. 2A). The staining distribution and intensity levels varied (Fig. 2B–D). SERPINE2 expression immunoscore was significantly associated with an advanced clinical stage ($P < 0.001$), tumor status ($P < 0.001$), lymph node ($P = 0.014$), and distant metastases ($P = 0.013$); its association with age, sex, tumor location, and histological grade was not statistically significant (Table 1). The Kaplan-Meier curve for overall survival is shown in Fig. 3. High SERPINE2 expression was significantly associated with shorter survival time ($P < 0.001$, log-rank test). Univariate analysis revealed that the decrease in overall patient survival was significantly related to advanced clinical stage, high histological grade, lymph node and distant metastasis, and high SERPINE2 expression immunoscore. Multivariate analysis indicated that high SERPINE2 expression, poorly differentiated tumors, and distant metastasis were independent prognostic factors correlated with poor overall survival in OSCC patients (Table 2).

SERPINE2 knockdown inhibits cell proliferation, migration, and invasion in OSCC cell lines

The Western blot analysis performed to evaluate SERPINE2 function in OSCC cells revealed that OECM-1, HCS3, and Cal27 cells had higher SERPINE2 expression (Fig. 4A). To examine the effect of SERPINE2 inhibition on cell proliferation and invasiveness, HSC3 and Cal27 cells were transfected with shSERPINE2 or shLuc as a control. SERPINE2 shRNA markedly reduced SERPINE2 protein expression in stable

Table 1 Relationships between the immunoscore of SERPINE2 and clinicopathological parameters in 122 patients.

Parameters	No.	Mean ± SEM	Median	*P*
Age[a]				
≤54	73	147.47 ± 7.77	160	0.132
>54	49	163.83 ± 8.86	165	
Gender[a]				
Male	107	152.59 ± 6.45	160	0.561
Female	15	164.33 ± 13.21	160	
Tumor site[b]				
Buccal	77	152.08 ± 7.29	160	0.700
Retromolar	24	148.96 ± 13.50	165	
Tongue	7	170.36 ± 25.33	170	
Others	14	165.36 ± 19.25	171	
Histological grade[b]				
Well-differentiated	45	149.00 ± 8.18	165	0.720
Moderately-differentiated	71	156.13 ± 8.35	160	
Poorly-differentiated	6	167.08 ± 30.81	183	
Stage[a]				
I–II	66	133.60 ± 7.41	153	<0.001*
III–IV	56	178.13 ± 8.34	184	
Tumor status[a]				
T1–2	78	139.20 ± 7.18	160	<0.001*
T3–4	44	180.34 ± 8.98	186	
Nodal metastasis[a]				
Absent	91	145.36 ± 6.39	160	0.011*
Present	31	179.52 ± 12.64	180	
Distant metastasis[a]				
Absent	107	148.69 ± 6.36	160	0.010*
Present	15	192.17 ± 11.12	188	

*Significance at $P < 0.05$.
[a] *P*-value by Mann–Whitney *U* test.
[b] *P*-value by Kruskal–Wallis test.

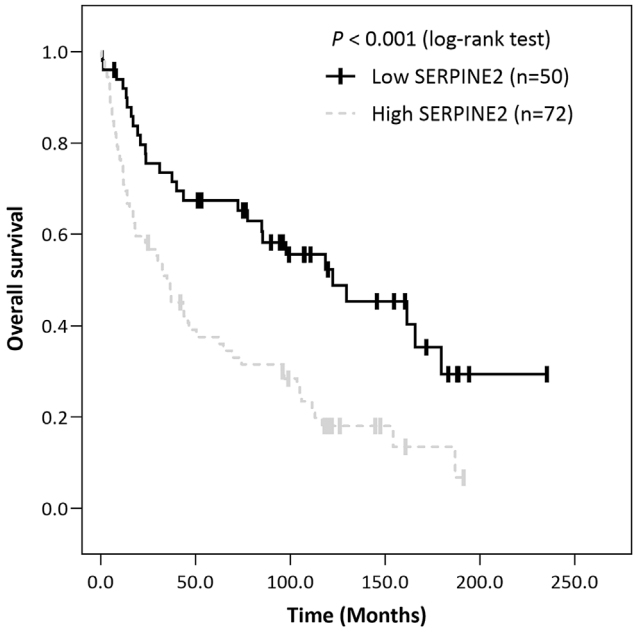

Figure 3 High SERPINE2 expression was correlated with worse overall survival rates. Kaplan–Meier survival curve in 122 OSCC patients.

clones compared to the control (Fig. 4B). SERPINE2 knockdown inhibited the growth rate, cell migration, and invasion of both HCS3 and Cal27 cells (Fig. 4C and D).

Discussion

Using the online tools TNMPlot and KMplot, SERPINE2 was demonstrated to be significantly upregulated in tumor samples, and its high expression was associated with poor HNSCC prognosis. *SERPINE2* mRNA level was significantly higher in OSCC tissues than in the corresponding normal epithelia in GSE37991, from areca nut-chewing patients. Areca nut components have been associated with oral submucosal fibrosis (OSF) through TGF-β pathway activation, and *SERPINE2* is upregulated in areca nut-treated cells.[22] OSF contributes to the epithelial-mesenchymal transition (EMT) and OSCC tumor infiltration,[23] suggesting that SERPINE2 may be correlated with oral carcinogenesis through the EMT activation pathway in OSCC and thus requires further investigation.

SERPINE2 levels were significantly increased in OSCC, highly associated with clinical stage, tumor status, lymph node metastasis, and distant metastasis, and significantly correlated with patient survival. Overall survival results were consistent with those of breast cancers,[8] gastric

Table 2 Univariate and multivariate analysis of SERPINE2 in 122 oral squamous cell carcinoma patients.

		Univariate		Multivariate	
Parameters	No.	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Age					
≤54	73	Reference			
>54	49	1.180 (0.769–1.809)	0.449		
Gender					
Male	107	Reference			
Female	15	1.508 (0.693–3.277)	0.300		
Histological grade					
Well-differentiated	45	Reference		Reference	
Moderately-differentiated	71	1.741 (1.092–2.775)	**0.020***	1.420 (0.873–2.309)	0.157
Poorly-differentiated	6	9.988 (3.930–25.387)	**<0.001***	5.711 (2.096–15.561)	**0.001***
Stage					
I–II	66	Reference		Reference	
III–IV	56	2.979 (1.926–4.608)	**<0.001***	1.370 (0.526–3.566)	0.519
Tumor status					
T1–2	78	Reference		Reference	
T3–4	44	2.782 (1.790–4.324)	**<0.001***	1.417 (0.618–3.248)	0.410
Nodal metastasis					
Absent	91	Reference		Reference	
Present	31	2.513 (1.576–4.006)	**<0.001***	1.477 (0.789–2.765)	0.223
Distant metastasis					
Absent	107	Reference		Reference	
Present	15	4.737 (2.609–8.599)	**<0.001***	2.537 (1.355–4.824)	**0.005***
SERPINE2 expression					
Low	50	Reference		Reference	
High	72	2.334 (1.467–3.715)	**<0.001***	1.737 (1.053–2.864)	**0.030***

CI: confidence interval.
*Significance at $P < 0.05$.

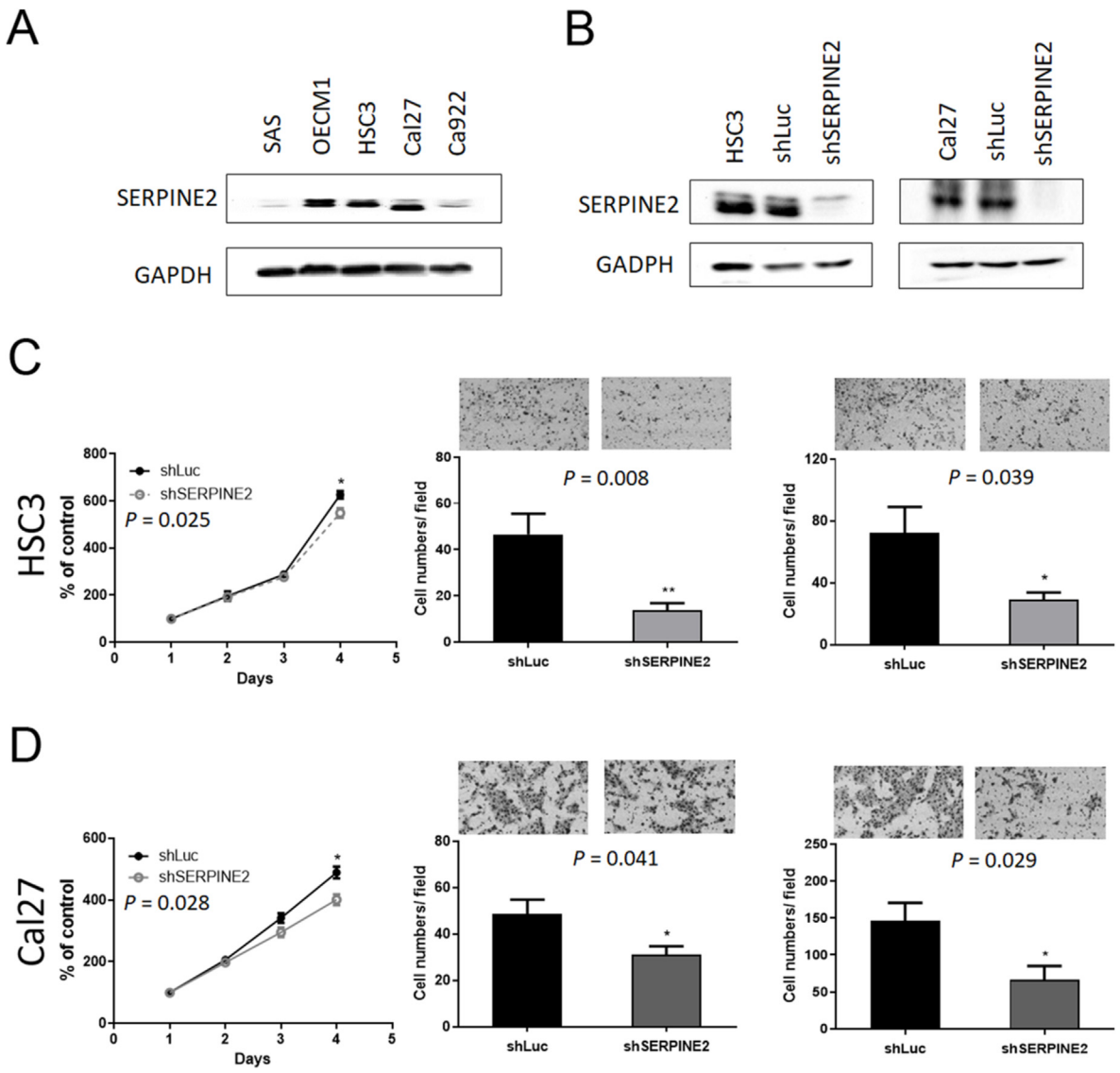

Figure 4 SERPINE2 knockdown reduced cell proliferation and invasion in HSC3 and Cal27 cells. (A) SERPINE2 expression levels in OSCC cell lines were evaluated using western blotting. (B) Immunoblotting analysis of SERPINE2 in HSC3 and Cal27 cells transduced with SERPINE2 shRNA and control shRNA (shLuc). (C) Growth curves of HSC3 cells with SERPINE2 knockdown. The migration and invasion of HSC3 cells transduced with SERPINE2 shRNA or control vector were assessed using Transwell assays. (D) Growth curves of Cal27 cells with SERPINE2 knockdown. Transwell assays were performed to analyze the migration and invasion of Cal27 cells transduced with SERPINE2 shRNA or a control vector. bar: SEM; *$P < 0.05$; **$P < 0.001$.

cancers,[2] osteosarcomas,[11] and urothelial carcinoma.[13] Furthermore, most of the commonly used patient variables were included in the regression model, and only high SERPINE2 immunoexpression, poorly differentiated tumors, and distant metastases remained significant predictors for worse overall survival rates ($P < 0.05$). The study results suggest possible roles for SERPINE2 overexpression in tumorigenesis and OSCC outcomes.

SERPINE2 affects cell proliferation, migration, and invasiveness in various cancers, including pancreatic cancer,[2] osteosarcomas,[11] ESCC,[14] and lung adenocarcinoma.[24] The association between high SERPINE2 expression and enhanced cell migration may differ from the expected function of a protease inhibitor, which should reduce ECM degradation and facilitate tumor cell invasion. Whether SERPINE2 is oncogenic or tumor-suppressing and its ability to regulate the development of a favorable microenvironment in tumors remain controversial. Xu et al.[25] showed that MMP-9 upregulates uPA and promotes tumor cell invasion via SERPINE2 cleavage. SERPINE2 in C6 glioma cells also play an anti-invasion role by regulating uPA, which induces extracellular MMP-9/2 activation.[26] A feedback model exists between SERPINE2 and matrix metalloproteinase (MMP)-9, which exerts opposite regulatory mechanisms and results in metastasis depending on their relative abundance.[2] In this study, *SERPINE2* knockdown inhibited HSC3 and Cal27 OSCC cell proliferation. A recent study on hepatocellular carcinoma suggested that BAP31 promoted cell proliferation by direct SERPINE2 regulation and MAPK pathway activation.[12] Our results also indicated

that *SERPINE2* plays an important role in cell migration and invasion in OSCC. Several studies have demonstrated that high SERPINE2 expression contributes to tumor invasion and metastasis.[7—10,14] These results suggest that SERPINE2 may have diverse biological functions in different tumor types.

Regarding the possible invasion mechanism, SERPINE2 induces cell detachment of multiple extracellular matrix proteins through a uPAR-dependent mechanism.[27] Bergeron et al.[9] confirmed that SERPINE2 expression could reduce adhesion and promote detachment in colorectal carcinoma. Furthermore, SERPINE2 can regulate breast cancer cell invasion and metastasis by stimulating extracellular signal-regulated kinase signaling and MMP-9 expressions.[7] Recently, Zhang et al.[14] showed that SERPINE2 promotes ESCC metastasis by regulating bone morphogenetic protein 4 to activate the EMT process. Additionally, Liu et al. revealed that LIM-homeodomain gene 2 promotes ESCC tumor growth and metastasis by regulating SERPINE2 expression.[28] Consistent with our findings, a study suggested that SERPINE2 may induce angiogenesis and lymphangiogenesis in OSCC.[29] SERPINE2 promoted metastasis through the glycogen synthase kinase 3β (GSK3β) signaling pathway in melanoma.[10] Fujii et al. demonstrated that transient receptor potential vanilloid 4, a Ca^{2+} channel, enhances OSCC cell proliferation via CaMKII-AKT activation.[30] The AKT/GSK3β signaling regulated EMT and metastasis in various cancers.[31] Exploring the relationship between SERPINE2 and the AKT/GSK3β signaling pathway may elucidate OSCC tumorigenesis and invasiveness. These results support that SERPINE2 could increase OSCC cell aggressiveness by promoting tumor cell growth and proliferation; however, the detailed mechanism requires further investigation.

Arroyo-Solera et al.[32] found that SERPINE1 overexpression, the phylogenetically closest relative of SERPINE2, promotes tumor aggressiveness and metastatic dissemination in HNSCC. Moreover, SERPINE1 blockade may enhance the efficacy of anti-PD-L1 therapy in melanoma patients.[33] Considering the similar function of SERPINE1, an effective therapeutic target for SERPINE2 as a promising cancer treatment strategy requires further investigation.

Overall, SERPINE2 is a valuable prognostic marker for OSCC progression, as confirmed using public databases and clinical samples. SERPINE2 may also play an active role in OSCC tumorigenesis and influence oral cancer progression by enhancing tumor cell invasiveness. Finally, the study results revealed that SERPINE2 might be a potential therapeutic target for OSCC patients.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgments

This research was supported by a grant from the Kaohsiung Veterans General Hospital (VGHKS109-074) in Taiwan, ROC. The study results are in whole or part based on data generated by TCGA Research Network (https://www.cancer.gov/tcga). Part of this study was presented at the ESMO Asia Congress 2022 in Singapore.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin* 2022;72:7—33.
2. Yang Y, Xin X, Fu X, Xu D. Expression pattern of human SERPINE2 in a variety of human tumors. *Oncol Lett* 2018;15:4523—30.
3. McKee CM, Ding Y, Zhou J, et al. Protease nexin 1 induces apoptosis of prostate tumor cells through inhibition of X-chromosome-linked inhibitor of apoptosis protein. *Oncotarget* 2015;6:3784—96.
4. Santoro A, Conde J, Scotece M, et al. SERPINE2 inhibits IL-1α-induced MMP-13 expression in human chondrocytes: involvement of ERK/NF-κB/AP-1 pathways. *PLoS One* 2015;10:e0135979.
5. Monard D. SERPINE2/protease nexin-1 in vivo multiple functions: does the puzzle make sense? *Semin Cell Dev Biol* 2017;62:160—9.
6. Francois D, Arocas V, Venisse L, et al. Hematopoietic protease nexin-1 protects against lung injury by preventing thrombin signaling in mice. *Blood Adv* 2018;2:2389—99.
7. Fayard B, Bianchi F, Dey J, et al. The serine protease inhibitor protease nexin-1 controls mammary cancer metastasis through LRP-1-mediated MMP-9 expression. *Cancer Res* 2009;69:5690—8.
8. Tang T, Zhu Q, Li X, et al. Protease nexin I is a feedback regulator of EGF/PKC/MAPK/EGR1 signaling in breast cancer cells metastasis and stemness. *Cell Death Dis* 2019;10:649.
9. Bergeron S, Lemieux E, Durand V, et al. The serine protease inhibitor serpinE2 is a novel target of ERK signaling. *Mol Cancer* 2010;9:271.
10. Wu QW. Serpine2, a potential novel target for combating melanoma metastasis. *Am J Transl Res* 2016;8:1985—97.
11. Mao M, Wang W. SerpinE2 promotes multiple cell proliferation and drug resistance in osteosarcoma. *Mol Med Rep* 2016;14:881—7.
12. Zhang X, Jiang D, Yang S, et al. BAP31 promotes tumor cell proliferation by stabilizing SERPINE2 in hepatocellular carcinoma. *Front Cell Dev Biol* 2020;8:607906.
13. Chuang HW, Hsia KT, Liao JB, Yeh CC, Kuo WT, Yang YF. SERPINE2 overexpression is associated with poor prognosis of urothelial carcinoma. *Diagnostics* 2021;11:1928.
14. Zhang J, Luo A, Huang F, Gong T, Liu Z. SERPINE2 promotes esophageal squamous cell carcinoma metastasis by activating BMP4. *Cancer Lett* 2020;469:390—8.
15. Gao S, Krogdahl A, Sorensen JA, Kousted TM, Dabelsteen E, Andreasen PA. Overexpression of protease nexin-1 mRNA and protein in oral squamous cell carcinomas. *Oral Oncol* 2008;44:309—13.
16. Á Bartha, Győrffy B. TNMplot.com: a web tool for the comparison of gene expression in normal, tumor and metastatic tissues. *Int J Mol Sci* 2021;22:2622.
17. Győrffy B. Survival analysis across the entire transcriptome identifies biomarkers with the highest prognostic power in breast cancer. *Comput Struct Biotechnol J* 2021;19:4101—9.
18. Lee CH, Wong TS, Chan JY, et al. Epigenetic regulation of the X-linked tumour suppressors BEX1 and LDOC1 in oral squamous cell carcinoma. *J Pathol* 2013;230:298—309.
19. El-Naggar A, Chan J, Grandis J, Takata T, Slootweg P. *WHO Classification of Head and Neck Tumours*, fourth ed. Lyon: IARC, 2017.
20. Ridge JA, Lydiatt WM, Patel SG, et al. Lip and oral cavity. In: Amin MB, Edge SB, Greene FL, et al., eds. *AJCC Cancer Staging Manual*, eighth ed. New York: Springer, 2017:79—94.
21. Cheng MF, Huang MS, Lin CS, et al. Expression of matriptase correlates with tumour progression and clinical prognosis in oral squamous cell carcinoma. *Histopathology* 2014;65:24—34.

22. Khan I, Kumar N, Pant I, Narra S, Kondaiah P. Activation of TGF-β pathway by areca nut constituents: a possible cause of oral submucous fibrosis. *PLoS One* 2012;7:e51806.
23. Chen PN, Lin CW, Yang SF, Chang YC. Oral submucous fibrosis stimulates invasion and epithelial-mesenchymal transition in oral squamous cell carcinoma by activating MMP-2 and IGF-IR. *J Cell Mol Med* 2021;25:9814—25.
24. Dokuni R, Nagano T, Jimbo N, et al. High expression level of serpin peptidase inhibitor clade E member 2 is associated with poor prognosis in lung adenocarcinoma. *Respir Res* 2020;21:331.
25. Xu D, McKee CM, Cao Y, Ding Y, Kessler BM, Muschel RJ. Matrix metalloproteinase-9 regulates tumor cell invasion through cleavage of protease nexin-1. *Cancer Res* 2010;70:6988—98.
26. Pagliara V, Adornetto A, Mammi M, et al. Protease nexin-1 affects the migration and invasion of C6 glioma cells through the regulation of urokinase plasminogen activator and matrix metalloproteinase-9/2. *Biochim Biophys Acta* 2014;1843:2631—44.
27. Czekay R-P, Loskutoff DJ. Plasminogen activator inhibitors regulate cell adhesion through a uPAR-dependent mechanism. *J Cell Physiol* 2009;220:655—63.
28. Li X, Wu X, Chen H, Liu Z, He H, Wang L. LHX2 enhances the malignant phenotype of esophageal squamous cell carcinoma by upregulating the expression of SERPINE2. *Genes* 2022;13:1457.
29. Sasahira T, Kurihara-Shimomura M, Shimomura H, Kirita T. SERPINE2 is an oral cancer-promoting factor that induces angiogenesis and lymphangiogenesis. *Int J Clin Oncol* 2021;26:1831—9.
30. Fujii S, Tajiri Y, Hasegawa K, et al. The TRPV4-AKT axis promotes oral squamous cell carcinoma cell proliferation via CaMKII activation. *Lab Invest* 2020;100:311—23.
31. Liu JJ, Li LZ, Xu P. Upregulation of TRPM8 can promote the colon cancer liver metastasis through mediating Akt/GSK-3 signal pathway. *Biotechnol Appl Biochem* 2022;69:230—9.
32. Arroyo-Solera I, Pavon MA, Leon X, et al. Effect of serpinE1 overexpression on the primary tumor and lymph node, and lung metastases in head and neck squamous cell carcinoma. *Head Neck* 2019;41:429—39.
33. Del Rosso M, Fibbi G, Laurenzana A, Margheri F, Chilla A. A possible role for PAI-1 blockade in melanoma immunotherapy. *J Invest Dermatol* 2021;141:2566—8.