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ORIGINAL RESEARCH

Identification of potential biomarkers and analysis of prognostic values in head and neck squamous cell carcinoma by bioinformatics analysis

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Abstract: The purpose of this study was to find disease-associated genes and potential mechanisms in head and neck squamous cell carcinoma (HNSCC) with deoxyribonucleic acid microarrays. The gene expression profiles of GSE6791 were downloaded from the Gene Expression Omnibus database. Differentially expressed genes (DEGs) were obtained with packages in R language and STRING constructed protein–protein interaction (PPI) network of the DEGs with combined score >0.8. Subsequently, module analysis of the PPI network was performed by Molecular Complex Detection plugin and functions and pathways of the hub gene in subnetwork were studied. Finally, overall survival analysis of hub genes was verified in TCGA HNSCC cohort. A total of 811 DEGs were obtained, which were mainly enriched in the terms related to extracellular matrix (ECM)–receptor interaction, ECM structural constituent, and ECM organization. A PPI network was constructed, consisting of 401 nodes and 1,254 edges and 15 hub genes with high degrees in the network. High expression of 4 genes of the 15 genes was associated with poor OS of patients in HNSCC, including *PSMA7*, *ITGA6*, *ITGB4*, and *APP*. Two significant modules were detected from the PPI network, and the enriched functions and pathways included proteasome, ECM organization, and ECM–receptor interaction. In conclusion, we propose that *PSMA7*, *ITGA6*, *ITGB4*, and *APP* may be further explored as potential biomarkers to aid HNSCC diagnosis and treatment.

Keywords: head and neck squamous cell carcinoma, interaction network, prognostic biomarkers, function and pathway analysis

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, with ~650,000 new cases and nearly 350,000 patient deaths from HNSCC annually.[1] Prognosis remains poor, and the 5-year survival rates for HNSCC patients continue to be <50%. Local tumor recurrence, distant metastasis, and therapeutic resistance appear to be the major contributing factors for this low survival rate.[2]

Previously identified biomarkers can help in predicting the prognosis of HNSCC. However, their clinical application is limited. Currently, there is no evidence-based recommendation for altering the treatment of patients with HNSCC by the expression of individual biomarkers.[3] Therefore, it is crucial to investigate the molecular mechanisms involved in proliferation, apoptosis, and invasion of HNSCC and discover more effective biomarkers of HNSCC to improve diagnosis and prevention of the disease.

Currently, genetic and genomics research is developing rapidly, which helps us to understand the potential mechanisms of some diseases.[4,5] For example, microarray

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analysis is widely used in the field of cancer genetics research, which may measure gene expression on a genome-wide scale simultaneously.[6]

In the present study, the biological informatics approach was used to analyze the gene expression profiles in HNSCC, and functional analysis was performed to identify differentially expressed genes (DEGs) between HNSCC and normal control. Subsequently, network analysis was applied for the DEGs and a protein–protein interaction (PPI) network was constructed; then, we investigated whether the hub gene of the subnetwork could reduce the overall survival (OS) in TCGA database. Through analyzing their biological functions, pathways, and OS, we may bring to light the underlying mechanisms of HNSCC development and identify the potential candidate biomarkers for diagnosis, prognosis, and drug targets.

Materials and methods

Microarray data

Microarray expression profiles of GSE6791[7] were downloaded from Gene Expression Omnibus database for identifying DEGs of HNSCC. GSE6791, which was already deposited in GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array, Santa Clara, CA, USA), consisted of 42 HNSCC samples and 14 normal epithelial samples.

Data preprocessing and identification of DEGs

The raw array data were subjected to background correction and quartile data normalization. Then, the DEGs between HNSCC samples and normal controls were identified using the empirical Bayes approach in linear models for the microarray data (limma) package.[8] $|\log FC| > 1$ and $P < 0.05$ were selected as the cutoff criterion.

Functional and pathway enrichment analysis of DEGs

The Database for Annotation, Visualization, and Integrated Discovery (DAVID),[9] which is a comprehensive set of functional annotation tools, has been used for systematic and integrative analysis of large gene lists. In this work, the significant gene ontology (GO) biological process terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of the identified DEGs were performed using DAVID database with the thresholds of $P < 0.05$.

Modules from the PPI network

To evaluate the interactive relationships among DEGs, the DEGs were mapped to the Search Tool for the Retrieval of Interacting Genes(STRING) database.[10] Then, the interaction relationships of DEGs were selected to construct the PPI network (combined score >0.8) and visualized using Cytoscape.[11] The Molecular Complex Detection (MCODE) plugin[12] in Cytoscape was used to screen the modules of PPI network, using cutoff values as follows: MCODE scores >15 and number of nodes >15. Moreover, the function and pathway enrichment analysis of DEGs in each module was performed using DAVID.

Survival analysis of the hub gene

OS analysis was performed using HNSCC samples from the TCGA dataset and mRNA *Z*-score data files were downloaded from the cBioPortal.[13] Patients were classified into high or low expression based on whether *Z*-score expression was $>$ median (high) or $<$ median (low). Based on these categories, log-rank analysis and Kaplan–Meier plots were produced using Prism Software (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Identification of DEGs

The total number of samples analyzed was 42 HNSCC samples, along with 14 normal epithelial samples. After data preprocessing, DEG analysis was performed using the limma software package. A total of 811 genes were identified after the analyses of GSE6791, including 550 upregulated and 261 downregulated genes.

GO and KEGG pathway enrichment analyses

We uploaded all 811 DEGs to the online software DAVID to identify overrepresented GO categories and KEGG pathways. GO analysis results showed that the most overrepresented GO terms in biological processes were enriched in extracellular matrix (ECM) organization, antigen processing and presentation of exogenous peptide antigen via major histocompatibility class I, transporter associated with antigen processing-dependent, and collagen catabolic process. In addition, the most enriched GO terms in molecular function and cellular component were threonine-type endopeptidase activity and extracellular exosome, respectively. On the other hand, the most enriched KEGG pathway terms were as follows: ECM–receptor interaction, amebiasis, proteasome, focal adhesion, and small cell lung cancer (Table 1).

Table 1 Functional and pathway enrichment analysis of upregulated and downregulated DEGs in HNSCC

ID	Go term	Count	*P*-value
GO_function			
GO_BP:0030198	ECM organization	43	2.07E–18
GO_BP:0002479	Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent	23	5.77E–15
GO_BP:0030574	Collagen catabolic process	21	1.10E–12
GO_BP:0060337	Type I interferon signaling pathway	20	1.13E–11
GO_BP:0031145	Anaphase-promoting complex-dependent catabolic process	21	8.10E–11
GO_CC:0070062	Extracellular exosome	213	2.62E–20
GO_CC:0005576	Extracellular region	129	1.88E–13
GO_CC:0005615	Extracellular space	113	4.42E–13
GO_CC:0005578	Proteinaceous ECM	41	1.51E–12
GO_CC:0031012	ECM	43	2.31E–12
GO_MF:0004298	Threonine-type endopeptidase activity	11	4.09E–09
GO_MF:0005201	ECM structural constituent	17	1.38E–08
GO_MF:0005518	Collagen binding	14	1.05E–06
GO_MF:0004252	Serine-type endopeptidase activity	28	1.18E–05
GO_MF:0005515	Protein binding	429	1.27E–05
KEGG_PATHWAY			
Hsa:04512	ECM–receptor interaction	23	1.23E–10
Hsa:05146	Amebiasis	25	2.11E–10
Hsa:03050	Proteasome	15	1.25E–08
Hsa:04510	Focal adhesion	30	2.76E–07
Hsa:05222	Small cell lung cancer	16	1.52E–05

Note: Top five terms were selected according to *P*-value.
Abbreviations: DEGs, differentially expressed genes; HNSCC, head and neck squamous cell carcinoma; GO, gene ontology; BP, biological process; ECM, extracellular matrix; MHC, major histocompatibility; TAP, transporter associated with antigen processing; CC, cellular component; MF, molecular function.

Coexpression network analysis of DEGs

To interpret the biological meaning of the identified DEGs, we constructed a coexpression network for the DEGs with a combined score >0.8 and with significant interaction relation composed of 401 nodes and 1,254 edges by STRING database analysis (Figure 1). From the coexpression network of the selected DEGs, the top 15 hub genes were determined according to the number of the interacting edges: *CDK1*, *PTK2*, *ITGAV*, *APP*, *COL1A1*, *MMP9*, *AURKA*, *BMP2*, *ITGB4*, *CDC20*, *SDC4*, *COL1A2*, *ITGA6*, *PSMA7*, and *STAT1* (Table 2). The distinct modules of 401 DEGs and their interacting genes were further identified by the MCODE using Cytoscape software. Among the modules, two subnetworks with >15 nodes were selected (Figure 2), and enrichment analysis showed that the genes in the subnetworks were mainly associated with proteasome, ECM–receptor interaction, protein digestion and absorption, and focal adhesion (Table 3).

Hub genes were validated as an independent predictor for OS in the TCGA cohort

We subsequently sought to assess the significance of expression of 15 hub genes in HNSCC. Therefore, the relation between expression of 15 hub genes and OS in the TCGA HNSCC cohort (461 patients) was verified, and the patients were divided into low or high expression groups according to the median expression. Our results showed that poor OS was associated only in those patients with high expression of *PSMA7* (HR: 1.60 [1.20–2.10], *P*=0.0009) in the TCGA HNSCC cohort, as well as *ITGA6* (HR: 1.32 [1.00–1.75], *P*=0.0472), *ITGB4* (HR: 1.38 [1.05–1.83], *P*=0.0113), and *APP* (HR: 1.40 [1.04–1.87], *P*=0.0113; Figure 3).

Conclusion

Despite advances in surgical, chemotherapy, and medical therapy, the overall mortality of HNSCC has remained virtually unchanged over the past decades. The lethality of HNSCC is mainly due to difficulties in detecting it at an early stage and the lack of effective treatments for patients in advanced stages. Interestingly, bioinformatics plays a major role in the analysis and interpretation of genomic and proteomic data.[14] For example, some researchers focus on bioinformatics, nanogenomics, and nanoproteomics aspects of contemporary nanodentistry and summarize some proteomics and proteogenomics approaches for oral diseases.[15,16] Therefore, in the present study, we attempted to utilize comprehensive bioinformatics methods to explore the potential molecular mechanism of HNSCC to improve survival rate and prevention.

In this study, a total of 811 DEGs were screened, consisting of 550 upregulated genes and 261 downregulated genes. Moreover, we selected two significant modules with several key DEGs (like *PSMA7*, *ITGA6*, and *ITGB4*) in HNSCC regulatory network, and functional enrichment analyses showed that these key DEGs were mainly enriched in ECM–receptor interaction, which is closely related to cancer. Finally, survival analysis of these hub genes revealed that four overexpressed genes were significantly correlated with poor OS of patients in the TCGA HNSCC cohort, and these included *PSMA7*, *ITGA6*, *ITGB4*, and *APP*.

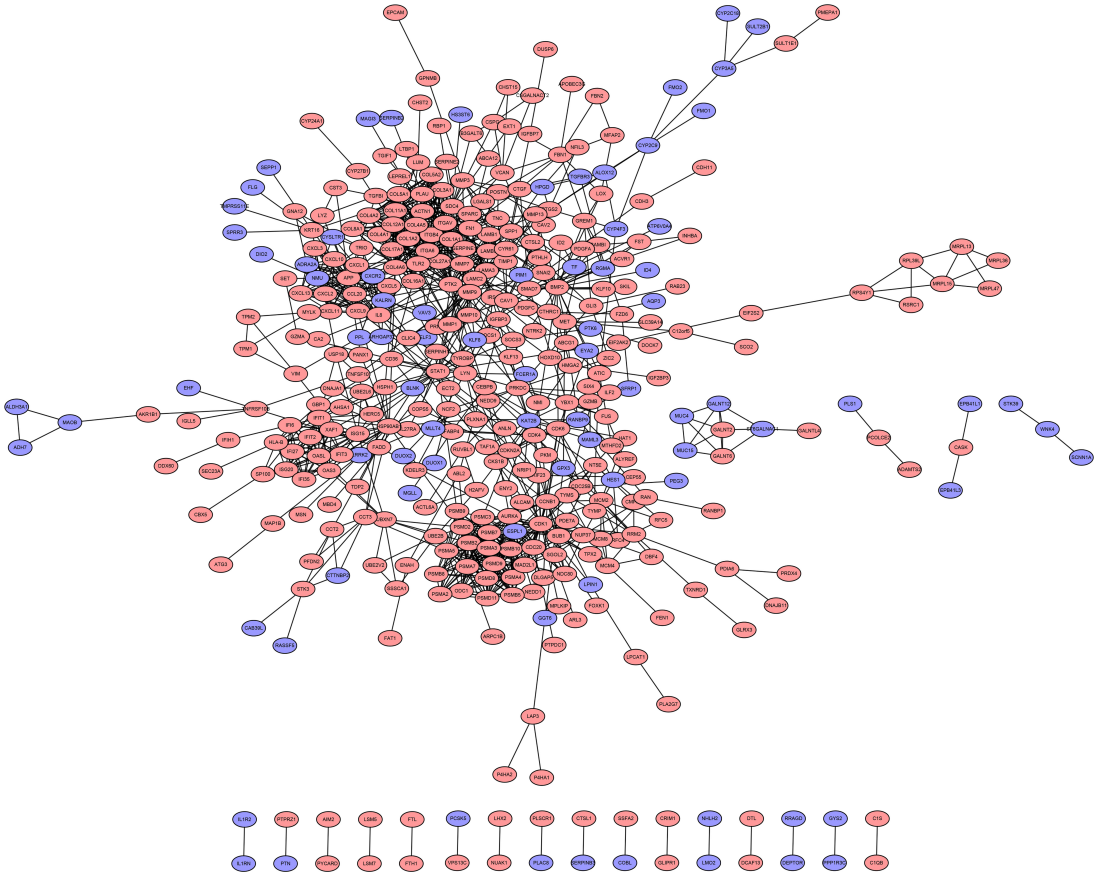

Figure 1 PPI network of differentially expressed genes.
Notes: Blue represents downregulated DEGs; red represents upregulated DEGs.
Abbreviations: PPI, protein–protein interaction; DEGs, differentially expressed genes.

The data showed that *PSMA7* is involved in "module 1" of the gene coexpression network, which is enriched in the proteasome pathway. Many studies have suggested that proteasome promotes the degradation of oxidatively damaged proteins that play a role in the cell cycle and transcription, which are essential for cancer improvement. Previously, it was reported that *PSMA7* inhibits the proliferation, tumorigenicity, and invasion of human lung adenocarcinoma cells.[17] Similar results also showed that high expression of *PSMA7* is associated with liver metastasis in colorectal cancer.[18] Besides, Hu et al also found depletion of *PSMA7* inhibited cell growth, invasion, and migration in RKO cells and strongly suppressed the tumorigenic ability of RKO cells in vivo.[19] Taken together, we speculate that the overexpression of *PSMA7* may contribute to HNSCC progression and correlate with a poor prognosis.

Table 2 The hub genes that had a degree >22 in PPI network

Gene	Regulation	Degree
CDK1	Up	36
PTK2	Up	34
ITGAV	Up	29
APP	Up	28
COL1A1	Up	27
MMP9	Up	27
AURKA	Up	26
BMP2	Up	26
ITGB4	Up	26
CDC20	Up	25
SDC4	Up	25
COL1A2	Up	23
ITGA6	Up	23
PSMA7	Up	23
STAT1	Up	23

Abbreviation: PPI, protein–protein interaction.

On the other hand, *ITGA6* and *ITGB4*, which are found in "module 2" in PPI network, were associated with the ECM–receptor interaction pathway, and belong to the integrin family, which participates in cell adhesion as well as cell surface-mediated signaling. Interactions between cells and the ECM could lead to the direct or indirect control of cellular processes of adhesion, migration, differentiation, proliferation, and apoptosis.[20] As previously reported, silencing of

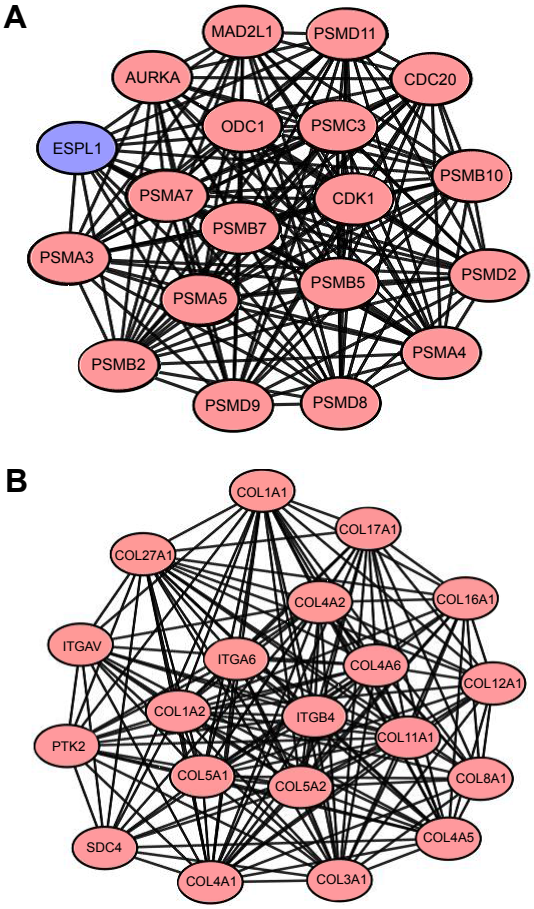

Figure 2 Functional modules in the PPI network.
Notes: From PPI networks of DEGs with combined score >0.8, we clustered two functional modules, using MCODE: module 1 (**A**) and module 2 (**B**). Blue represents downregulated DEGs; red represents upregulated DEGs.
Abbreviations: PPI, protein–protein interaction; DEGs, differentially expressed genes; MCODE, Molecular Complex Detection.

ITGA6 genes significantly inhibited cell migration and invasion in head and neck cancer cells and hepatocellular carcinoma cells.[21,22] Similarly high *ITGA6* expression was shown to enhance invasion in models of metastatic breast cancer.[23] Moreover, Kwon et al[24] found *ITGA6* is a possible target for antibody-related diagnostic and therapeutic modalities in esophageal squamous cell carcinoma. Meanwhile, *ITGB4* regulates migration and invasion in models of metastatic prostate cancer.[25] Moreover, Masugi et al[26] found that knockdown of *ITGB4* reduced the migration and invasion and that upregulation of *ITGB4* promoted cell scattering and motility in pancreatic ductal adenocarcinoma cells. Besides, our study shows that *ITGB4* was associated with poor prognosis in HNSCC; similar results have also been shown in pancreatic ductal adenocarcinoma patients.[27] Together, we speculate that *ITGA6* and *ITGB4* in ECM–receptor interaction signaling pathway may play a significant role in HNSCC.

Table 3 Functional and pathway enrichment analysis of the DEGs in modules

ID	Description	Count	*P*-value
Module 1			
GO_BP:0031145	Anaphase-promoting complex-dependent catabolic process	17	1.73E–36
GO_BP:0051436	Negative regulation of ubiquitin protein ligase activity involved in mitotic cell cycle	16	4.09E–34
GO_BP:0051437	Positive regulation of ubiquitin protein ligase activity involved in regulation of mitotic cell cycle transition	16	1.27E–33
GO_BP:0006521	Regulation of cellular amino acid metabolic process	14	2.99E–30
GO_BP:0043161	Proteasome-mediated ubiquitin-dependent protein catabolic process	16	8.04E–27
GO_CC:0000502	Proteasome complex	12	5.82E–24
GO_CC:0005839	Proteasome core complex	8	2.77E–17
GO_CC:0005829	Cytosol	19	4.58E–14
GO_CC:0005654	Nucleoplasm	16	2.87E–10
GO_CC:0005634	Nucleus	18	1.40E–08
GO_MF:0004298	Threonine-type endopeptidase activity	8	2.90E–17
GO_MF:0005515	Protein binding	18	1.49E–05
Hsa:03050	Proteasome	12	2.16E–21
Module 2			
GO_BP:0030198	ECM organization	17	5.98E–29
GO_BP:0030574	Collagen catabolic process	12	7.33E–23
GO_BP:0030199	Collagen fibril organization	7	2.78E–12
GO_BP:0071230	Cellular response to amino acid stimulus	7	9.11E–12
GO_BP:0007155	Cell adhesion	9	1.70E–08
GO_CC:0005788	Endoplasmic reticulum lumen	15	1.42E–24
GO_CC:0005581	Collagen trimer	11	5.76E–19
GO_CC:0005576	Extracellular region	15	1.27E–11
GO_CC:0031012	ECM	9	2.85E–10
GO_CC:0005578	Proteinaceous ECM	8	5.96E–09
GO_MF:0005201	ECM structural constituent	11	4.30E–20
GO_MF:0048407	Platelet-derived growth factor binding	5	3.76E–10
GO_MF:0038132	Neuregulin binding	3	1.20E–05
Hsa:04512	ECM–receptor interaction	15	5.56E–25
Hsa:04974	Protein digestion and absorption	13	4.91E–20
Hsa:04510	Focal adhesion	15	1.76E–19
Hsa:05146	Amebiasis	12	7.43E–17
Hsa:04151	PI3K-Akt signaling pathway	15	2.72E–16
Hsa:05222	Small cell lung cancer	7	3.22E–08

Abbreviations: DEGs, differentially expressed genes; GO, gene ontology; BP, biological process; MHC, major histocompatibility; TAP, transporter associated with antigen processing; CC, cellular component; ECM, extracellular matrix; MF, molecular function.

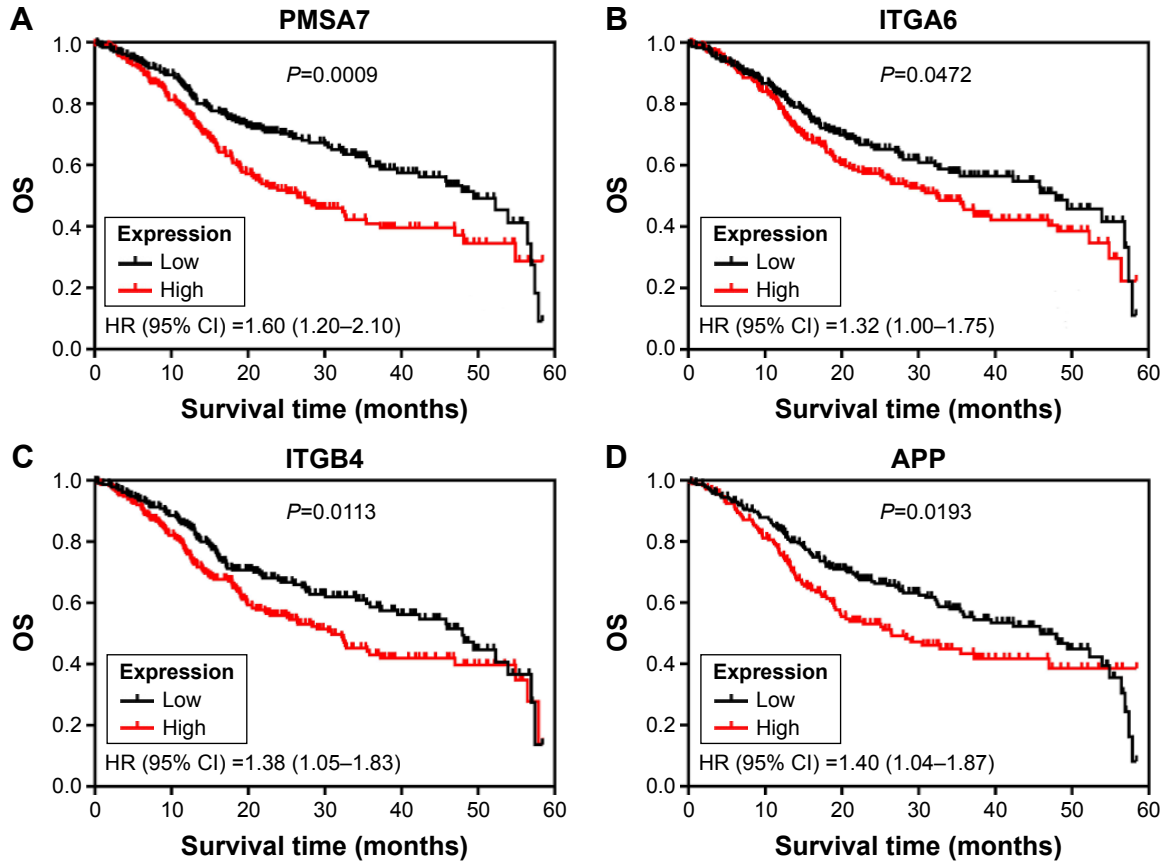

Figure 3 Kaplan–Meier curves depicting OS in the TCGA HNSCC cohort with high and low expression of PMSA7 (**A**), ITGA6 (**B**), ITGB4 (**C**) and APP (**D**), respectively.
Abbreviations: OS, overall survival; HNSCC, head and neck squamous cell carcinoma; HR, hazard ratio; CI, confidence interval.

Amyloid-β precursor protein (*APP*) is the highly conservative single transmembrane protein with a receptor-like structure that has been shown to be involved in Alzheimer disease,[28] but its function in normal physiological is unclear. Interestingly, *APP* is increased in many different cancers, such as colon cancer, pancreatic cancer, and thyroid cancer.[29–31] Lim et al[32] found that overexpression of *APP* is found both in malignant breast cancer cell lines and in human breast cancer tissues, and *APP* could regulate cell growth, apoptosis, and motility of breast cancer, possibly via engagement of AKT-mediated signaling pathways. Similarly, *APP* could promote cell growth in pancreatic cancer cells.[31] In addition, Ko et al[33] found a significant increase of *APP* in an oral squamous cell carcinoma (OSCC) tissue and also that OSCC patients with high mRNA levels of *APP* had poor prognoses. The abovementioned studies show that *APP* may be involved in the pathogenesis of malignant tumors by affecting cell growth or apoptosis, thereby supporting our findings.

In summary, the current study was intended to identify DEGs with comprehensive bioinformatics analysis to find the potential biomarkers and predict progression of diseases. We found that hub genes of complex networks, such as *PSMA7*, *ITGA6*, *ITGB4*, and *APP*, may be exploited as a prognostic tool for HNSCC. Finally, our results suggested that proteasome and ECM–receptor interaction may be important in the development of HNSCC. However, further experimental studies are still required to prove our findings and determine the potential clinical value of these as biomarkers.

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Disclosure

The authors report no conflicts of interest in this work.

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