Scientific Research Report

Poor Prognosis of Oral Squamous Cell Carcinoma Correlates With *ITGA6*

Churen Zhang *, *Qiaoling Cai*, *Jianguo Ke* **

Department of Stomatology, The First Affiliated Hospital of Xiamen University, School of Medicine, Xiamen University, China

ARTICLE INFO

Article history:
Received 19 March 2022
Received in revised form
27 May 2022
Accepted 28 May 2022
Available online 9 July 2022

Key words:
ITGA6
Oral squamous cell carcinoma
Autophagy

ABSTRACT

Objectives: Oral cancer is the ninth most common cancer worldwide and a leading cause of cancer-related death. Oral squamous cell carcinoma (OSCC) accounts for 90% of all oral cancers. Autophagy is a conserved essential catabolic process related to OSCC. The aim of this study was to elucidate diagnostic and prognostic autophagy-related biomarkers in OSCC.
Methods: The OSCC gene expression data set was obtained from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) between the OSCC samples and adjacent healthy tissues were identified by R software. The Human Autophagy Database was screened, which revealed 222 autophagy-related genes. The autophagy-related DEGs were identified. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were applied. Protein–protein interaction network analysis was performed in the STRING database. cytoHubba in the Cytoscape software was applied to determine the top 10 hub genes. The data set of patients with OSCC from The Cancer Genome Atlas (TCGA) was used to evaluate the prognostic value of the 10 hub genes. The association between prognosis-related hub genes and immune infiltrates was explored.
Results: Twenty-seven autophagy-related DEGs were identified. The top 10 hub genes were *CCL2, CDKN2A, CTSB, CTSD, CXCR4, ITGA6, MAP1LC3A, MAPK3, PARP1*, and *RAB11A*. *ITGA6* was identified as the most efficient biomarker. Receiver operating characteristic curve analysis indicated that *ITGA6* had the highest diagnostic accuracy for OSCC (area under the curve = 0.925). *ITGA6* expression was significantly related to immune infiltrates.
Conclusions: The autophagy-related gene *ITGA6* might be an efficient diagnostic and prognostic biomarker in OSCC.

© 2022 The Authors. Published by Elsevier Inc. on behalf of FDI World Dental Federation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Introduction

Oral cancer is the ninth most frequently occurring cancer worldwide and a leading cause of cancer-related death.[1] About 350,000 new cases of oral cancer were diagnosed in 2018, with approximately 170,000 associated deaths.[2] Oral cancer is highly prevalent in India and Southeast Asia, representing approximately 40% of all malignancies, compared to about 4% in Western countries.[3] Oral cancer generally refers to malignant lesions that originate in the oral cavity, including buccal mucosa, tongue, vermilion border of the lip, floor of the mouth, palate, and gingiva.[4] Oral squamous cell carcinoma (OSCC), which is an aggressive malignancy associated with high morbidity and mortality, accounts for 90% of all oral tumours.[5] Main risk behaviours for OSCC include tobacco smoking, alcohol consumption, and betel nut chewing. Moreover, environmental risk factors and genetic factors increase the incidence of OSCC.[6] Betel nuts caused up to 8,222 cases of OSCC in Changsha, the capital city of Hunan province in China, resulting in about ¥5 billion financial loss in 2016.[7] For OSCC, the prognosis is closely linked to the stage of disease at diagnosis; the later the diagnosis, the worse the prognosis.[8] According to the data from the Surveillance, Epidemiology, and End Results (SEER) programme from the United States, if OSCC has not metastasised, the 5-year survival rate is approximately 85.1%. Moreover, the 5-year survival rate of patients with lymph node metastases and

* *Corresponding author*. Department of Stomatology, The First Affiliated Hospital of Xiamen University, School of Medicine, Xiamen University, 55 Zhenhai Avenue, Siming District, Xiamen, 361003, China.
** Jianguo ke is the co-corresponding author.
E-mail addresses: 18311321821@163.com (C. Zhang), caiqiaoling12003@163.com (Q. Cai), 13666009910@163.com (J. Ke).
https://doi.org/10.1016/j.identj.2022.05.010
0020-6539/© 2022 The Authors. Published by Elsevier Inc. on behalf of FDI World Dental Federation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

distant metastases significantly decreased to 66.8% and 40.1%, respectively.[9] Despite advances in cancer treatment, the 5-year survival rate of advanced-stage OSCC remains poor.[10] The pathogenesis of OSCC is still not clear. The accumulation of genetic defects and epigenetic abnormalities play a significant role in the initiation and development of OSCC.[11] The mutant antioncogene p53 accelerates the development of OSCC, possibly through the acquisition of ability to invade surrounding tissues.[12] Clinical examination in combination with histopathologic evaluation are the major tools for diagnosis of OSCC.[13] Clinically relevant indexes, including histological features with TNM staging help in assessment of prognosis. However, these indexes remain inadequate in accurately predicting survival in these patients.[14] Therefore, there is an urgent unmet need for the development of reliable diagnostic and prognostic biomarkers.

Autophagy is a conserved essential catabolic process, by which damaged cellular components are transferred to the lysosome for degradation, thereby participating in the metabolic cycle.[15] Autophagy plays crucial roles in a wide variety of biological processes. Defects in autophagy have been linked to several human diseases, including cancer.[16] However, autophagy is a double-edged sword in tumourigenesis. Autophagy selectively eliminates superfluous or damaged organelles and aggregated proteins to maintain cellular homeostasis and decrease cell damage.[17] This is the protective aspect of autophagy that inhibits the development of cancer in healthy cells. On the contrary, autophagy also promotes the survival and metabolic fitness of established tumours.[17] Previous studies have explored the association between autophagy and OSCC. On the basis of The Cancer Genome Atlas (TCGA) database, some autophagy-related genes (ARGs) related to OSCC have been identified.[18, 19] However, some of the identified genes have not been adequately validated. Consequently, there is an unmet need to explore prognostic target biomarkers in OSCC.

We hypothesised that some kinds of ARGs were efficient prognostic target biomarkers in OSCC. This research was conducted to obtain a deeper understanding of the unrealised clinical efficacy of ARGs as diagnostic and prognostic biomarkers in patients with OSCC. RNA-sequencing data of OSCC tissues and adjacent healthy tissues were obtained from Gene Expression Omnibus (GEO) data sets (GSE74530).[20] Autophagy-related differentially expressed genes were identified. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted to identify the functions and involved pathways. Protein–protein interactions (PPI) were constructed, and potential hub genes were identified. Data of OSCC patients from TCGA were used as the validation set to establish the diagnostic and prognostic value of the hub genes.

Material and methods

Sources of data

The OSCC microarray data set (GSE74530) was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74530). GSE74530 was in the GPL570 platform ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array) and included 6 oral tumours and 6 adjacent non-involved oral tissues from 6 participants who had signed informed consent forms in the original research.[20] The original study was performed according to the Declaration of Helsinki (version 2002) and approved by the Internal Review Board of the Ohio State University.[20] The Human Autophagy Database (http://www.autophagy.lu/index.html) was screened, and we found 222 autophagy-related genes. The UCSC XENA database (https://xenabrowser.net/datapages/) included TCGA and GTEx RNAseq data in transcripts per million reads format (TPM), which were uniformly processed through the Toil process. After log2 conversion, RNAseq data in TPM format was applied for analysis and comparison of different cancer types. The data sets for OSCC used in this study included RNAseq data in level 3 HTSeq-FPKM format from the TCGA (https://portal.gdc.cancer.gov/) Head and Neck Squamous Cell Carcinoma (HNSC) project. The samples belonging to oral cancer sites (alveolar ridge, base of tongue, buccal mucosa, floor of mouth, hard palate, oral cavity, oral tongue) were retained. Samples that were from non-oral cancer sites (hypopharynx, larynx, lip, oropharynx, tonsil) were excluded. Fragments per kilobase per million (FPKM) RNAseq data were converted to TPM format and log2 transformed. Finally, 328 OSCC samples and 32 healthy samples were included.

Differentially expressed gene (DEG) analysis

Then, the box diagram was conducted through ggplot2 to estimate the standardisation of the samples, and the clustering analysis between the OSCC group and the healthy group was done by principal component analysis (PCA). Linear models were fitted by limma package of R software (4.1.0) to further determine the differential expressed genes between the OSCC group and the healthy group.[21] DEGs were defined as genes with adjusted P value <.05 and $|\log2(FC)|>1.0$. Subsequently, a Venn diagram was constructed to find the overlapping parts of the DEGs and the ARGs, which revealed the critical autophagy-related DEGs. Correlation between the autophagy-related DEGs was calculated using Spearman correlation coefficient (a correlation coefficient with $P < .05$ was considered statistically significant).

Functional enrichment analysis of autophagy-related DEGs

GO and KEGG pathways were analysed using the package "GO plot" for the DEGs. A P value <.05 was required for the enriched GO/KEGG terms. GO was composed of cellular component (CC), biological process (BP), and molecular function (MF).

PPI network analysis

STRING (https://string-db.org/) was used for construction of protein interactions. Cytoscape software was used to visualise the PPI. In addition, cytoHubba, a plug-in of Cytoscape, was applied to confirm the top 10 hub genes through the maximal clique centrality algorithm.[22]

Autophagy-related gene-based diagnostic and prognostic signature

The data set of patients with OSCC from TCGA-OSCC were separated into high-expression and low-expression groups by the median of the expression data of the identified autophagy-related genes. Next, the overall survival (OS) in the TCGA-OSCC cohort was calculated and visualised by survival package and survminer package. The accuracy of the autophagy-related genes differentiating tumour tissue and healthy tissue was reflected by the area under the curve (AUC) calculated by pROC package. The autophagy-related genes with significant influence on OS and AUC values >0.9 were defined as valuable biomarkers of OSCC. Furthermore, the mRNA expression of valuable biomarkers in different cancer types was analysed. The immunohistochemistry analysis results of proteins of the valuable biomarkers expressed in OSCC and healthy tissues were obtained from the Human Protein Atlas database (www.proteinatlas.org).

Correlation between ITGA6 *and immune infiltrates in OSCC*

We analysed the correlation of *ITGA6* expression with the infiltration of 24 different kinds of immune cells, including activated dendritic cells (aDC); B cells; $CD8^+$ T cells; cytotoxic cells; dendritic cells (DCs); eosinophils; immature DCs (iDCs); macrophages; mast cells; neutrophils; Natural Killer (NK) CD56bright cells; NK CD56dim cells; NK cells; plasmacytoid DC (pDCs); T cells; T helper cells; T central memory (Tcm) cells; T effector memory (Tem) cells; T follicular helper (Tfh) cells; T gamma delta (Tgd) cells; Th1 cells; Th17 cells; Th2 cells; and Treg cells. The markers for the 24 immune cells and the classification and description of the specific cells can be found in an article published in *Immunity*. The immune infiltration algorithm used was ssGSEA in the GSVA package. The correlation between *ITGA6* expression and immune infiltrates was evaluated by Spearman correlation coefficients.

Results

The autophagy-related DEGs of OSCC

The median of each sample from GSE74530 was oriented on a horizontal line; this indicated that the degree of normalisation between samples was good (Figure 1A). The samples of each group were obviously separated in the PCA plot, and the ratio of PC1 and PC2 was high. This indicated that there was a significant difference between the groups (Figure 1B). Based on the criterion of DEGs, 1573 up-regulated genes and 613 down-regulated genes were identified (Figure 1C). The heatmap shows the expression of the top 20 up-regulated genes and down-regulated genes (Figure 1D). The overlapping parts of the DEGs and autophagy-related genes included 27 genes, shown in the Venn diagram (Figure 1E). Spearman correlation analysis of the 27 autophagy-related DEGs was conducted (Figure 1F).

Functional enrichment analysis of autophagy-related DEGs

GO/KEGG pathways of the 27 autophagy-related DEGs were analysed. The top 5 enriched BPs of the autophagy-related DEGs included processes utilising autophagic mechanism, autophagy, leukocyte migration, regulation of neuron projection development, and positive regulation of neurogenesis. The top 5 enriched MFs of the autophagy-related DEGs were composed of cell-substrate junction, cell-substrate adherens junction, collagen-containing extracellular matrix, focal adhesion, and late endosome. The enriched CCs of the autophagy-related DEGs were composed of laminin binding, insulin-like growth factor binding, and insulin-like growth factor I binding (Figure 2A). The top 5 enriched KEGG pathways were human papillomavirus infection, apoptosis, influenza A, regulation of actin cytoskeleton, and human cytomegalovirus infection (Figure 2B).

PPI network construction and identification of hub genes

PPI analysis was conducted to identify the interaction between the autophagy-related DEGs, which are shown in the interacted circular plot (Figure 3A). The number of interactions of each gene with other genes is also shown, and the gene *CTSB* was found to have the most interactions with other genes (Figure 3B). In addition, the top 10 hub genes were identified through cytoHubba and were as follows: *CCL2, CDKN2A, CTSB, CTSD, CXCR4, ITGA6, MAP1LC3A, MAPK3, PARP1*, and *RAB11A* (Figure 3C). Furthermore, based on the expression data from TCGA-OSCC, *CDKN2A, CTSB, CTSD, CXCR4, ITGA6*, and *PARP1* were found highly expressed in OSCC, and *CCL2, MAP1LC3A, MAPK3*, and *RAB11A* were identified as less expressed in OSCC (Figure 3D).

Autophagy-related gene-based diagnostic and prognostic signature

The 328 OSCC samples from TCGA-OSCC were separated into high-expression and low-expression groups by taking the median of the expression data of *CCL2, CDKN2A, CTSB, CTSD, CXCR4, ITGA6, MAP1LC3A, MAPK3, PARP1*, and *RAB11A*, respectively. Cox regression analysis results indicated that there was a statistically significant difference in the OS time distribution of the *ITGA6* group ($P = .043$; Figure 4A), but not the other groups (Figure 4B–J). In addition, receiver operating characteristics (ROC) curve analysis indicated that *ITGA6* had the highest diagnostic accuracy value for OSCC (AUC = 0.925; Figure 5A); the AUC value of the other 9 hub genes ranged between 0.579 and 0.813 (Figure 5B–J).

Protein expression of ITGA6 *in OSCC and mRNA expression of* ITGA6 *in different cancer types*

Immunohistochemistry showed that the ITGA6 protein had low expression in healthy oral mucosa and high expression in OSCC. The mRNA expression of *ITGA6* showed statistically significant differences between different cancer types and their corresponding healthy tissues (Supplementary Figure 1).

Correlation between ITGA6 *and immune infiltrates in OSCC*

The *ITGA6* expression was significantly related to B cells ($r = -0.139$, $P = .012$), $CD8^+$ T cells ($r = -0.194$, $P < .001$),

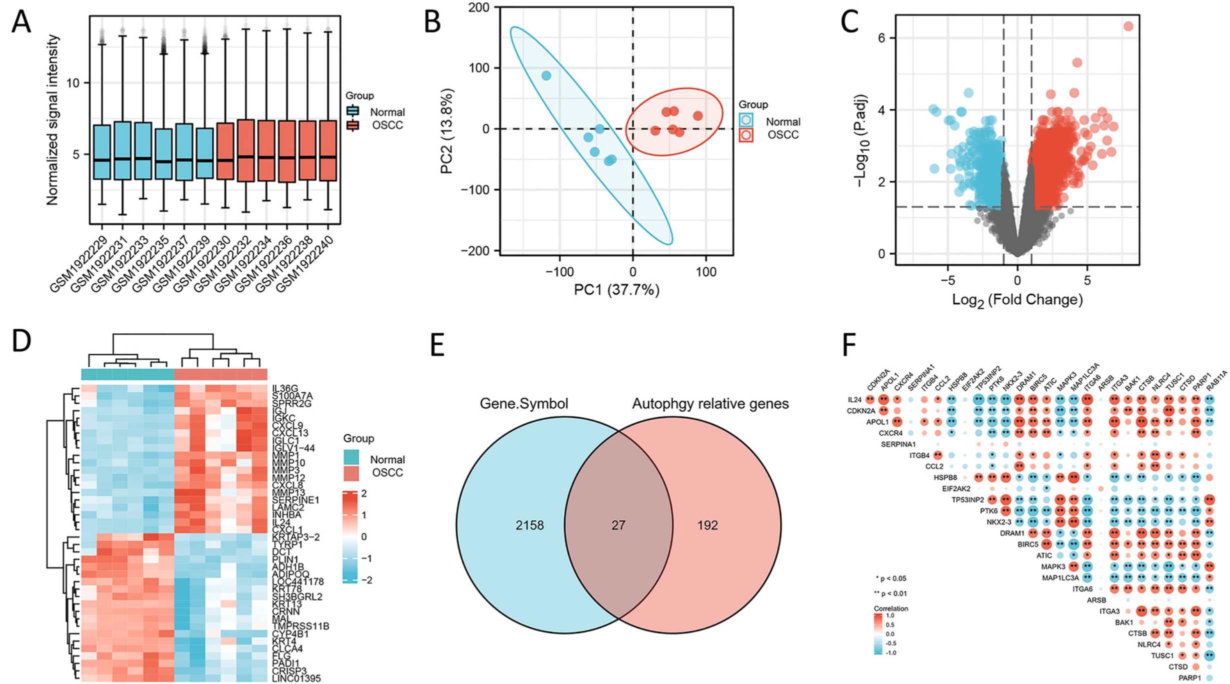

Fig. 1 – Identification of autophagy-related differentially expressed genes (DEGs) in oral squamous cell carcinoma (OSCC). A, mRNA expression level of each sample in the GSE74530. The degree of normalisation between samples was satisfactory. B, Principal component analysis plot indicates that there were significant differences between the groups. C, Volcano plot shows the DEG expression between OSCC and adjacent healthy tissues. Red points represent the up-regulated genes; blue points represent the down-regulated genes. A total of 1573 up-regulated genes and 613 down-regulated genes were identified. D, The heatmap shows the expression of the top 20 up-regulated genes and down-regulated genes. E, The Venn diagram shows the intersection of the DEGs and autophagy-related genes, including 27 genes, which are the autophagy-related DEGs. F, Spearman correlation analysis of the 27 autophagy-related DEGs.

cytotoxic cells ($r = -0.222$, $P < .001$), eosinophils ($r = 0.255$, $P < .001$), neutrophils ($r = 0.186$, $P < .001$), NK CD56bright cells ($r = -0.223$, $P < .001$), pDC ($r = -0.260$, $P < .001$), T cells ($r = -0.116$, $P = .036$), T helper cells ($r = 0.214$, $P < .001$), Tcm ($r = 0.291$, $P < .001$), Tgd ($r = 0.405$, $P < .001$), Th1 cells ($r = 0.202$, $P < .001$), and Th2 cells ($r = 0.332$, $P < .001$). The P value is shown on the x-axis, and the immune cells are represented on the y-axis (Supplementary Figure 2).

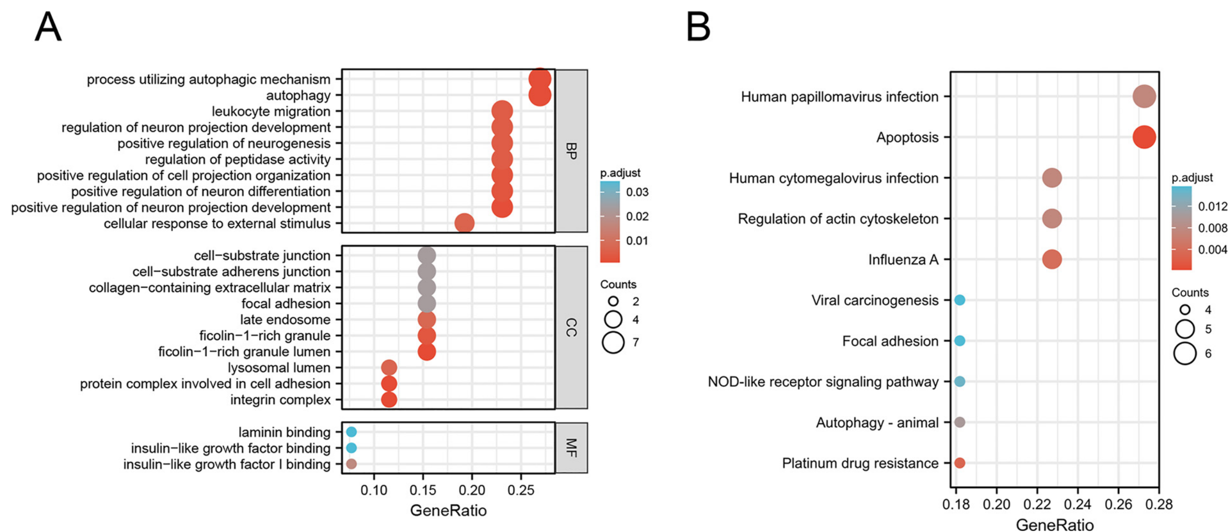

Fig. 2 – Functional enrichment analysis of autophagy-related differentially expressed genes (DEGs). A, Gene Ontology enrichment analysis of the 27 autophagy-related DEGs, including biological process, molecular function, and cellular component. B, The enriched Kyoto Encyclopedia of Genes and Genomes pathways of the 27 autophagy-related DEGs.

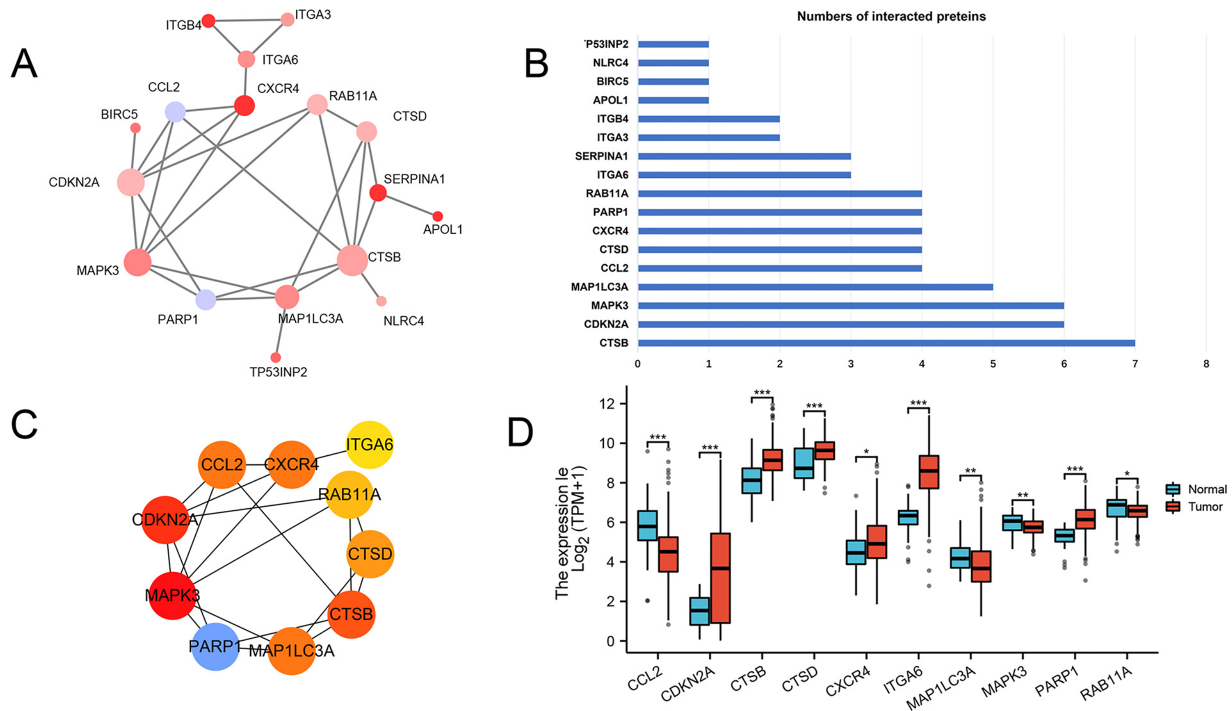

Fig. 3 – Protein–protein interaction (PPI) network construction and identification of hub genes. A, STRING database was used for PPI analysis to identify the interaction between the autophagy-related differentially expressed genes (DEGs). B, The number of interactions of each gene with other genes is shown. C, The top 10 hub genes were identified through cytoHubba; they are *CCL2, CDKN2A, CTSB, CTSD, CXCR4, ITGA6, MAP1LC3A, MAPK3, PARP1*, and *RAB11A*. D, The expression level of the 10 hub genes in oral squamous cell carcinoma (OSCC) samples and healthy tissues, according to the data from The Cancer Genome Atlas–OSCC.

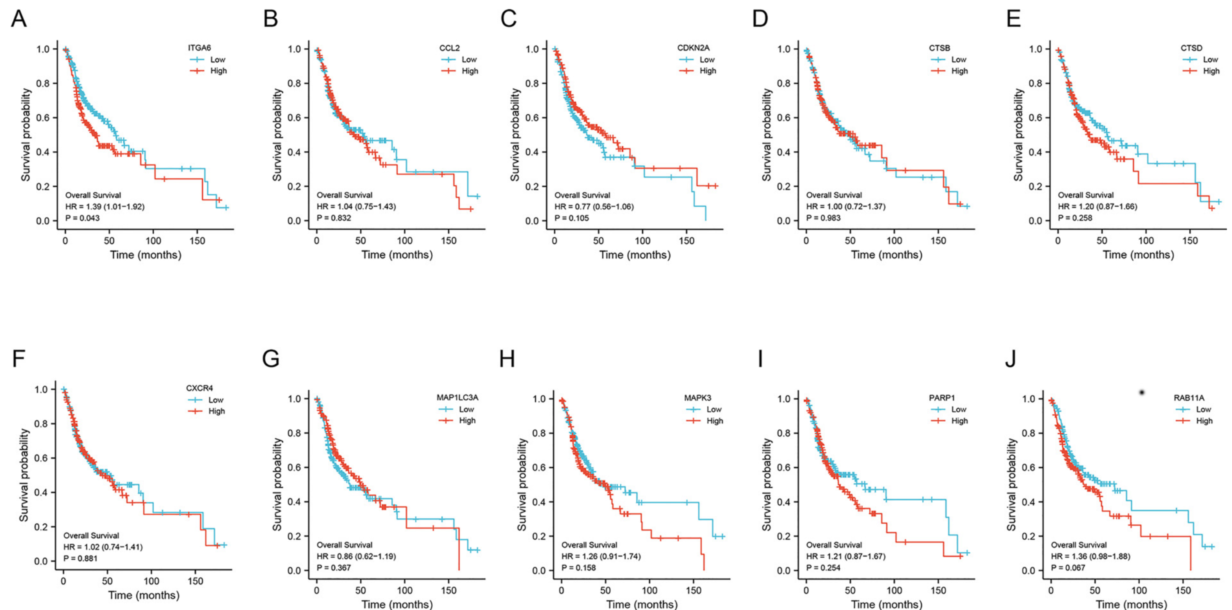

Fig. 4 – Kaplan–Meier curves of overall survival of patients with oral squamous cell carcinoma (OSCC); patients were divided into a high-expression group and a low-expression group by the median of the expression data of the 10 hub genes from The Cancer Genome Atlas–OSCC. A, High expression of *ITGA6* was related to lower overall survival of patients with OSCC, P = .043. B–J, The expression of *CCL2* (P = .832), *CDKN2A* (P = .105), *CTSB* (P = .983), *CTSD* (P = .258), *CXCR4* (P = .881), *MAP1LC3A* (P = .367), *MAPK3* (P = .158), *PARP1* (P = .254), and *RAB11A* (P = .067) did not significantly influence the overall survival of patients with OSCC.

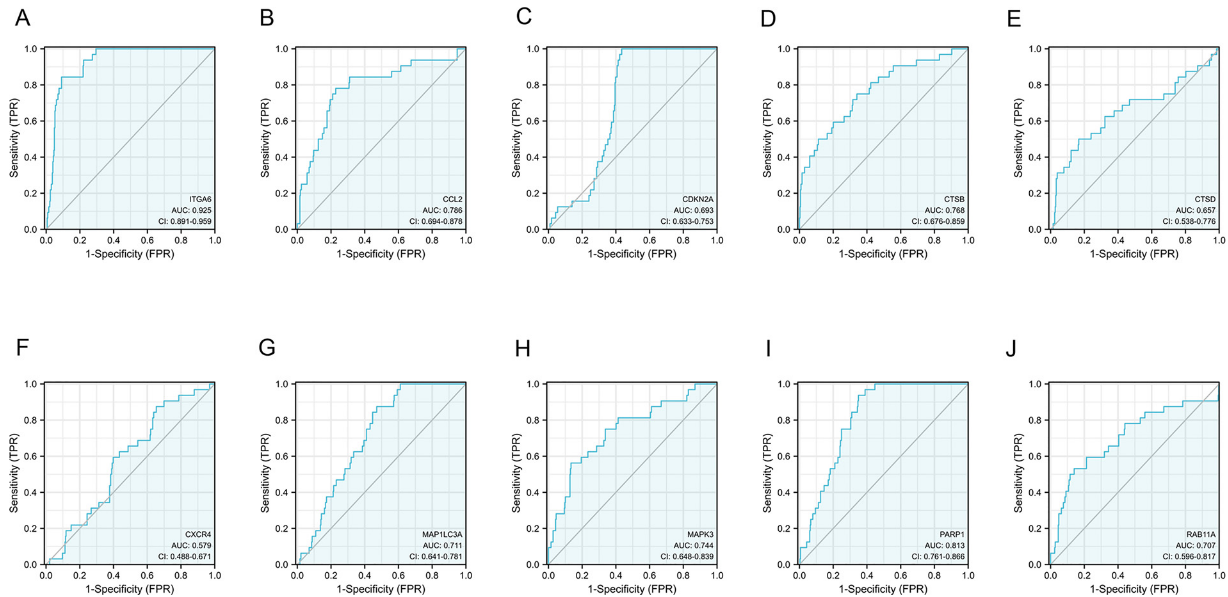

Fig. 5 – Receiver operating characteristic analysis indicated the diagnostic accuracy value of the 10 hub genes. A, The diagnostic accuracy value of *ITGA6* was highest, with area under the curve (AUC) = 0.925. B–J, The diagnostic accuracy values of the other 9 hub genes were as follows: *CCL2* (AUC = 0.786), *CDKN2A* (AUC = 0.693), *CTSB* (AUC = 0.768), *CTSD* (AUC = 0.657), *CXCR4* (AUC = 0.579), *MAP1LC3A* (AUC = 0.711), *MAPK3* (AUC = 0.744), *PARP1* (AUC = 0.813), and *RAB11A* (AUC = 0.707).

Discussion

OSCC remains one of the most life-threatening diseases worldwide. In spite of refinements in treatment of OSCC, the 5-year survival rates are still disappointingly low.[10] Therefore, there is a desperate need for identification of new prognostic biomarkers to aid in clinical management and development of novel therapies that can improve outcomes.[2] Accumulating evidence indicates that autophagy contributes to OSCC development, accelerating metabolic activity and thereby promoting cancer cell survival.[23] However, currently, objective analysis of the role of autophagy in the pathogenesis and prognosis of OSCC remains limited and inconclusive. Previous studies have found that various autophagy-related genes, including *ATG9A*, *ATG16L1*, and *LC3*, were correlated with the pathologic characteristics or prognosis of OSCC.[24] Similar prognostic models of oral cancers or head and neck squamous cell carcinoma based on TCGA data have been established. Fei *et al*.[25] found that the prognostic model based on the autophagy-related genes (*WDR45*, *MAPK9*, *VEGFA*, and *ATIC*) proved to be effective. They established a prognostic model based on multiple genes, which had better prognostic ability in OSCC. Liu et al[26] illustrated that *FADD* and *NKX2-3* were prognosis-related genes in HNSC, which they further verified with cellular experiments. Fang et al[27] used different algorithms (univariate Cox regression analyses and LASSO regression method) to identify the major autophagy-related genes in HNSC, which further proved to be related to immune cell infiltration. Huang et al[28] identified *ATG12* and *BID* as potential independent prognostic biomarkers of OSCC. Li et al[29] built a prognosis autophagy signature of HNSC; the high-risk group had a lower survival rate than the low-risk group. In the present study, gene expression profiles of OSCC were utilised to identify and validate functional autophagy-related genes, which were related to the pathogenesis and prognosis of OSCC. Finally, *ITGA6*, which has not been studied before, was identified. *ITGA6* could accurately distinguish between OSCC and healthy tissues, with AUC = 0.925. High-level expression of *ITGA6* was associated with poor prognosis. *ITGA6* could potentially be applied to prognostic stratification of OSCC, which would contribute to a personalised treatment regimens and provide new avenues for targeted autophagy therapy.

Through GO/KEGG functional analyses, it was found that differentially expressed autophagy genes were enriched in autophagy, cell matrix interactions, human papillomavirus (HPV) infection, and so on. Studies focusing on the role of autophagy have found that autophagy promotes membrane transport and activates intracellular signaling pathways.[30,31] It has been reported that autophagy regulates tumour progression through the nuclear factor κB (NF-κB) pathway. NF-κB can silence apoptosis signals to promote survival and cell migration of tumour cells.[32] The enrichment of HPV infection indicated the interaction amongst autophagy, immune response, and tumour microenvironment. Previous studies have found that there is higher immune cell infiltration in the tumour microenvironment of HPV-positive patients with head and neck tumours.[33,34]

The gene integrin subunit alpha 6 (*ITGA6*) encodes alpha 6 subunit, which is a member of the integrin alpha chain family of proteins. Alpha 6 subunit interacts with a beta 1 (*ITGB1*) or beta 4 (*ITGB4*) subunit to form an integrin. *ITGA6:ITGB4* integrin accelerates the progression of tumourigenesis, whilst alpha 6 beta 1 integrin can inhibit the erb-b2 receptor tyrosine kinase 2 (*ERBB2*) signal.[35] *ITGA6:ITGB4* binds to *IGF1*, and this binding is essential for insulin like growth factor 1 (*IGF1*) signaling. *ITGA6:ITGB4* binds to *IGF2* and this binding is essential for *IGF2* signaling.[36] The *ERBB2* signal is constitutively activated in various tumours, leading to imbalanced cellular

proliferation and metastasis.[37] *ITGA3* and *ITGA6* were found to be highly expressed in HNSC and correlated with poor prognosis of HNSC.[38] *NLRC4, PARP1, PTK6, CDKN2A*, and *BIRC5* were reported to be associated with *ITGA6* in lung adenocarcinoma.[39] High expression of *ITGA6* was associated with low survival rates in lung adenocarcinoma.[40] Similar results were found in the present study. In laryngeal squamous cell carcinoma (LSCC), miR-144-3p targeting *ITGA6* participated in tumourigenesis and progression of LSCC.[41] Circular RNA FAT1(e2) has been reported to interact with miR-30e-5p to further regulate *ITGA6* and finally enhance proliferation and migration of colorectal cancer cells.[42] The present study also found a significantly high expression of *ITGA6* in colon adenocarcinoma, head and neck squamous cell carcinoma, and many different cancers, indicating the important role of *ITGA6* in tumourigenesis.

ITGA6 was found to promote the phosphorylation of protein kinase B to activate phosphatidylinositol-4,5-bisphosphate3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway, contributing to the invasion seen in pancreatic cancer.[43] Under healthy nutritional conditions, mTOR inhibited autophagy; under starvation conditions, mTOR promoted autophagy.[44] It has been observed that activation of mTOR suppresses autophagy, resulting in progression of OSCC.[45]

Our results suggest that the *ITGA6* expression level was associated with the infiltration levels of a range of immune cells, including B cells, $CD8^+$ T cells, cytotoxic cells, eosinophils, neutrophils, NK CD56bright cells, pDCs, T cells, T helper cells, Tcm cells, Tgd cells, Th1 cells, and Th2 cells. The *ITGA6* expression level negatively correlated with B and $CD8^+$ T cell infiltration. B cells were key players in inflammatory and immune reactions and were related to the prolonged survival of patients with cancer.[46] B cells, as important indicators, portend a good prognosis and showed an intensely positive effect on clinical outcomes in human colorectal tumours.[47] $CD8^+$ T cells are considered major drivers of antitumour immunity. $CD8^+$ tumour-infiltrating lymphocytes mediate tumour rejection by recognising tumour antigens and directly killing transformed cells. Effector $CD8^+$ T cells in the tumour microenvironment produce interleukin (IL)-2, IL-12, and interferon gamma (IFNγ), which enhance the cytotoxic capacity of $CD8^+$ T cells and lead to targeted tumour cell killing.[48] Elevated levels of cytotoxic $CD8^+$ T cells in the tumour microenvironment are associated with improved antitumour effects and prognosis in various types of cancers.[49] Therefore, the poor prognosis of OSCC with high expression of *ITGA6* might be related to the low level of B cell infiltration.

By the reanalysis of gene expression data available in public databases, our study provides a new prognostic biomarker for OSCC. However, our study has some limitations: (1) The potential function of *ITGA6* in OSCC needs further exploration, and (2) the regulation of expression of *ITGA6* in OSCC remains to be investigated.

Conclusions

The autophagy-related gene *ITGA6* was identified and validated as an efficient biomarker for OSCC.

Author contributions

Churen Zhang: Conceptualisation, methodology, data curation, running software, preparation of original draft.

Qiaoling Cai: Visualisation, investigation, supervision, validation, manuscript revision.

Jianguo Ke: Conceptualisation, reviewing and editing of manuscript.

Acknowledgements

The authors made use of publicly available data from TCGA and GEO.

Funding

The study is funded by Start-up fund for introducing talents to the hospital of The First Affiliated Hospital of Xiamen University (XYJ2021006).

Conflict of interest

None disclosed.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.identj.2022.05.010.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7–34.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.
3. Singh S, Singh J, Chandra S, Samadi FM. Prevalence of oral cancer and oral epithelial dysplasia among North Indian population: A retrospective institutional study. J Oral Maxillofac Pathol 2020;24:87–92.
4. Takkem A, Barakat C, Zakaraia S, et al. Ki-67 prognostic value in different histological grades of oral epithelial dysplasia and oral squamous cell carcinoma. Asian Pac J Cancer Prev 2018;19:3279–86.
5. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002;52:195–215.
6. Winn DM, Lee YC, Hashibe M, Boffetta P, INHANCE Consortium. The INHANCE consortium: toward a better understanding of the causes and mechanisms of head and neck cancer. Oral Dis 2015;21:685–93.
7. Hu YJ, Chen J, Zhong WS, et al. Trend analysis of betel nut-associated oral cancer and health burden in China. Chin J Dent Res 2017;20:69–78.
8. Kumar M, Nanavati R, Modi TG, Dobariya C. Oral cancer: etiology and risk factors: a review. J Cancer Res Ther 2016;12:458–63.
9. Oral Cavity and Pharynx Cancer — Cancer Stat Facts section of Surveillance, Epidemiology, and End Results (SEER)

program. 2022. Available from: http://seer.cancer.gov. Accessed 1 July 2022.
10. Khanagar SB, Naik S, Al Kheraif AA, et al. Application and performance of artificial intelligence technology in oral cancer diagnosis and prediction of prognosis: a systematic review. Diagnostics (Basel) 2021:11.
11. Vallina C, Lopez-Pintor RM, Gonzalez-Serrano J, de Vicente JC, Hernandez G, Lorz C. Genes involved in the epithelial-mesenchymal transition in oral cancer: a systematic review. Oral Oncol 2021;117:105310.
12. Gawande M, Chaudhary M, Sharma P, Hande A, Patil S, Sonone A. Expression of p53 at invasive front of oral squamous cell carcinoma and negative histopathological surgical margins to establish correlation at 3-year survival. J Oral Maxillofac Pathol 2020;24:582.
13. Lorini L, Bescos Atin C, Thavaraj S, et al. Overview of oral potentially malignant disorders: from risk factors to specific therapies. Cancers (Basel) 2021;13.
14. Law ZJ, Khoo XH, Lim PT, et al. Extracellular vesicle-mediated chemoresistance in oral squamous cell carcinoma. Front Mol Biosci 2021;8:629888.
15. Ahn MY, Ahn SG, Yoon JH. Apicidin, a histone deaceylase inhibitor, induces both apoptosis and autophagy in human oral squamous carcinoma cells. Oral Oncol 2011;47:1032–8.
16. Amaravadi R, Kimmelman AC, White E. Recent insights into the function of autophagy in cancer. Genes Dev 2016;30:1913–30.
17. Levy JMM, Towers CG, Thorburn A. Targeting autophagy in cancer. Nat Rev Cancer 2017;17:528–42.
18. Huang L, Yu X, Jiang Z, Zeng P. Novel autophagy-related gene signature investigation for patients with oral squamous cell carcinoma. Front Genet 2021;12:673319.
19. Hou C, Cai H, Zhu Y, Huang S, Song F, Hou J. Development and validation of autophagy-related gene signature and nomogram for predicting survival in oral squamous cell carcinoma. Front Oncol 2020;10:558596.
20. Oghumu S, Knobloch TJ, Terrazas C, et al. Deletion of macrophage migration inhibitory factor inhibits murine oral carcinogenesis: potential role for chronic pro-inflammatory immune mediators. Int J Cancer 2016;139:1379–90.
21. Diboun I, Wernisch L, Orengo CA, Koltzenburg M. Microarray analysis after RNA amplification can detect pronounced differences in gene expression using limma. BMC Genomics 2006;7:252.
22. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 2014;8((Suppl 4)):S11.
23. Khan T, Relitti N, Brindisi M, et al. Autophagy modulators for the treatment of oral and esophageal squamous cell carcinomas. Med Res Rev 2020;40:1002–60.
24. Tan YQ, Zhang J, Zhou G. Autophagy and its implication in human oral diseases. Autophagy 2017;13:225–36.
25. Fei H, Chen X. A novel autophagy-related prognostic risk model and a nomogram for survival prediction of oral cancer patients. Biomed Res Int 2022;2022:2067540.
26. Liu C, Wu W, Xu M, Mi J, Xu L, Wang R. Development and validation of an autophagy-related signature for head and neck squamous cell carcinoma. Biomed Res Int 2021;2021:1028158.
27. Fang J, Yang Z, Xie J, et al. Identification and validation of autophagy-related prognostic signature for head and neck squamous cell carcinoma. Transl Oncol 2021;14:101094.
28. Huang GZ, Lu ZY, Rao Y, Gao H, Lv XZ. Screening and identification of autophagy-related biomarkers for oral squamous cell carcinoma (OSCC) via integrated bioinformatics analysis. J Cell Mol Med 2021;25:4444–54.
29. Li C, Wu ZH, Yuan K. Autophagy-related signature for head and neck squamous cell carcinoma. Dis Markers 2020;2020:8899337.
30. Lee EJ, Kim HJ, Choi MS, Chang JE. Crosstalk between autophagy and inflammatory processes in cancer. Life (Basel) 2021;11.
31. Wang Z, Zhou C, Yang S. The roles, controversies, and combination therapies of autophagy in lung cancer. Cell Biol Int 2022;46:3–11.
32. Hennig P, Fenini G, Di Filippo M, Karakaya T, Beer HD. The pathways underlying the multiple roles of p62 in inflammation and cancer. Biomedicines 2021;9.
33. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363:24–35.
34. Koneva LA, Zhang Y, Virani S, et al. HPV integration in HNSCC correlates with survival outcomes, immune response signatures, and candidate drivers. Mol Cancer Res 2018;16:90–102.
35. Duan X, Li H, Wang M, et al. PSMC2/ITGA6 axis plays critical role in the development and progression of hepatocellular carcinoma. Cell Death Discov 2021;7:217.
36. Cedano Prieto DM, Cheng Y, Chang CC, Yu J, Takada YK, Takada Y. Direct integrin binding to insulin-like growth factor-2 through the C-domain is required for insulin-like growth factor receptor type 1 (IGF1R) signaling. PLoS One 2017;12:e0184285.
37. Yu D, Hung MC. Overexpression of ErbB2 in cancer and ErbB2-targeting strategies. Oncogene 2000;19:6115–21.
38. Feng C, Jin X, Han Y, et al. Expression and prognostic analyses of ITGA3, ITGA5, and ITGA6 in head and neck squamous cell carcinoma. Med Sci Monit 2020;26:e926800.
39. Liu Z, Zhang K, Zhao Z, Qin Z, Tang H. Prognosis-related autophagy genes in female lung adenocarcinoma. Medicine (Baltimore) 2022;101:e28500.
40. Zhong H, Wang J, Zhu Y, Shen Y. Comprehensive analysis of a nine-gene signature related to tumor microenvironment in lung adenocarcinoma. Front Cell Dev Biol 2021;9:700607.
41. Mo BY, Pang JS, Dai WB, Su YS, Jiang W, Huang SN. A comprehensive evaluation of miR-144-3p expression and its targets in laryngeal squamous cell carcinoma. Comput Math Methods Med 2021;2021:6684186.
42. Pan F, Zhang D, Li N, Liu M. Circular RNA circFAT1(e2) promotes colorectal cancer tumorigenesis via the miR-30e-5p/ITGA6 Axis. Comput Math Methods Med 2021;2021:9980459.
43. Wu Y, Tan X, Liu P, et al. ITGA6 and RPSA synergistically promote pancreatic cancer invasion and metastasis via PI3K and MAPK signaling pathways. Exp Cell Res 2019;379:30–47.
44. Wang Y, Zhang H. Regulation of autophagy by mTOR signaling pathway. Adv Exp Med Biol 2019;1206:67–83.
45. Yang Y, Chen D, Liu H, Yang K. Increased expression of lncRNA CASC9 promotes tumor progression by suppressing autophagy-mediated cell apoptosis via the AKT/mTOR pathway in oral squamous cell carcinoma. Cell Death Dis 2019;10:41.
46. Alam M, Ali S, Mohammad T, Hasan GM, Yadav DK, Hassan MI. B cell lymphoma 2: a potential therapeutic target for cancer therapy. Int J Mol Sci 2021;22.
47. Tangye SG, Ma CS, Brink R, Deenick EK. The good, the bad and the ugly - TFH cells in human health and disease. Nat Rev Immunol 2013;13:412–26.
48. Kim A, Han CJ, Driver I, et al. Correction: LILRB1 blockade enhances bispecific T cell engager antibody-induced tumor cell killing by effector CD8(+) T cells. J Immunol 2019;203:2023–4.
49. Chen X, Wang D, Zhu X. Application of double-negative T cells in haematological malignancies: recent progress and future directions. Biomark Res 2022;10:11.