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Exostoisns (EXT1/2) in Head and Neck Cancers: An In Silico Analysis and Clinical Correlates

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SUMMARY

Objectives: The exostosins (EXT), which are responsible for heparan sulfate backbone synthesis and play a vital role in tissue homeostasis, have been reported to be correlated with prognosis of various cancers. However, the expression, prognostic value, and immune infiltration of EXT1 and EXT2 in head and neck squamous cell carcinoma (HNSC) remain uncertain.

Methods: GEPIA, UALCAN, and Xiantao bioinformatics tools were used to explore the EXT1 and EXT2 expression level in HNSC. GEPIA and Sangerbox were utilised to obtain the prognostic value of EXT1 and EXT2 in HNSC. Genetic alterations, immune cell infiltration, and single-cell analysis were conducted in cBioPortal, TIMER, and TISCH2. In addition, the expressions of EXT1 and EXT2 were validated by real-time polymerase chain reaction (PCR) in HNSC samples.

Results: EXT1 and EXT2 were highly expressed in HNSC, especially in malignant cells. Only EXT2 was significantly negatively correlated to the prognosis of patients with HNSC. EXT1 and EXT2 were found to be associated with focal adhesin and cell adhesin molecule binding. EXT1 expression levels were considerably connected with CD8+ T cell infiltrating levels, whilst EXT2 expression levels were considerably negatively connected with infiltrating levels of CD4+ T cells, macrophages, neutrophils, and dendritic cells in HNSC. The gene mutation rates of EXT1 and EXT2 in HNSC were 7% and 2.8%, respectively. Moreover, EXT2 was validated to be highly expressed in HNSC samples by real-time PCR.

Conclusion: EXT2 was highly expressed and presented negative correlation with the prognosis and immune infiltration of HNSC, which might be a potential biomarker for HNSC.

Introduction

About 5% of all malignancies are head and neck squamous cell carcinomas (HNSC), which include cancers of the mouth, salivary glands, and oropharynx.[1] With 890,000 new cases and 450,000 fatalities each year, HNSC is the seventh most prevalent malignancy and the sixth highest cause of cancer-related deaths in the world.[2] The surgical, chemotherapy, and radiation treatments for this type of cancer have advanced dramatically over the past few decades, increasing the 5-year survival rate in the United States to more than 65%.[3] However, the majority of patients continue to receive advanced diagnoses, skipping necessary treatment, and making surgery challenging due to the demands of intrinsic,

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life-essential functions like speaking and swallowing.[4] Patients still have a 40% to 60% likelihood of experiencing HNSC recurrence even after tumour removal and clinical treatment.[5] Chemotherapy, surgery, and radiotherapy continue to be the main HNSC treatment modalities today. These methods may have severe unfavourable outcomes and lower patient quality of life. In addition, recurrence or distant metastases will occur in 15% to 40% of patients with locally progressed HNSC.[6] The median overall survival for patients with recurrence or distant metastases is 10 to 13 months, whilst the 5-year survival rate is approximately 4%.[7]

Since the discovery of tumour heterogeneity, it has been increasingly clear that the complicated heterogeneous structure of tumours may be to blame for the delayed advancement in the treatment of malignant tumours such as HNSC.[8] The 2 main elements of tumour heterogeneity are the microenvironment in which the tumour develops and the heterogeneity of the tumour cells themselves, for example, the presence of distinct populations of cancer cells with various gene expressions and biological properties within the tumour.[9] This variation is brought on by the altered expression of oncogenes that take place during the process of cell proliferation. For the creation of more precise diagnostic devices and therapeutic strategies, it is vital to understand the molecular underpinnings underlying HNSC progression and metastasis.

The exostosin family members EXT1 and EXT2 are responsible for heparan sulfate (HS) backbone synthesis, which adds glycosaminoglycan residues to the core protein to produce complex polysaccharides.[10] HS proteoglycans are abundant extracellular matrix components that play a vital function in tissue homeostasis.[11] The EXT1/EXT2 complex has significantly higher glycosyltransferase activity than either EXT1 or EXT2 alone. Endoplasmic reticulum transmembrane proteins EXT1 and EXT2 are frequently mutated in skeletal abnormalities including numerous osteochondromas. The EXT1 gene is overexpressed in breast cancer.[12] Furthermore, EXT1 mRNA levels are higher in squamous cell lung cancer than in normal tissue, and EXT1 is linked to a poor prognosis in squamous cell lung carcinoma.[13] In terms of extracellular matrix assembly, EXT2 is thought to play a key role in the invasive nature of colorectal cancer.[14] However, the link between EXT1 and EXT2 and HNSC remains to be investigated.

Bioinformatic analysis was conducted for the exploration of the link between EXT1 and EXT2 and HNSC, as in our previous study.[15,16] The current study examines EXT1 and EXT2 mRNA expression in HNSC using the GEPIA database, the UALCAN database, and the Xiantao bioinformatics tool. The HNSC survival analysis was carried out using the GEPIA database and the Sangerbox platform. The Tumor Immune Assessment Resource (TIMER) database was used to investigate the link between EXT1 and EXT2 expression and immune infiltration. Furthermore, the Gene Ontology (GO) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analyses of EXT1 and EXT2 were carried out using the Xiantao bioinformatics tool. The objective of this study was to investigate the role of EXT1/2 in HNSC.

Materials and methods

Gene expression profiling interactive analysis (GEPIA) dataset

GEPIA[17] (http://gepia.cancer-pku.cn/) integrates gene expression profiling data from the Cancer Genome Atlas Program (TCGA) and Genotype-Tissue Expression (GTEx) projects, providing a variety of data analysis and visualisation capabilities. TCGA was initiated jointly by the National Cancer and Oncology Institute and the National Human Genome Research Institute in 2006, including clinical and genetic information of more than 11,000 patients with more than 30 cancers. GTEx collected multiple human tissues (brain, heart, lung, breast, skin and whole blood, etc) for genetic sequencing from approximately 960 donors and more than 30,000 samples. GEPIA is used for pancancer analysis and prognostic analysis of HNSC for EXT1 and EXT2.

UALCAN

UALCAN[18] (http://ualcan.path.uab.edu/index.html) is an effective website for online analysis and exploration of cancer data, mainly based on relevant cancer data in TCGA database, which can help medical scientists to carry out biomarker identification, expression profile analysis of relevant genes, and survival analysis. It is used for evaluation of the mRNA expression of EXT1 and EXT2 in different subgroups of patients with HNSC.

Sangerbox

Sangerbox (http://vip.sangerbox.com), a web-based tool platform, provides interactive graphical analysis tools, including correlation analysis tools, pathway enrichment analysis, mRNA expression analysis, and other common tools and functions. The platform integrates databases such as GEO, TCGA, and ICGC and performs fast batch processing of these data. It is used for analysing the relationship between the mRNA expression of EXT1 and EXT2 with cancer prognosis.

Xiantao bioinformatics tool

The Xiantao bioinformatics tool (https://www.xiantao.love/) is a free online analytics platform that collects and standardises data from TCGA. It is used to analyse the expression of EXT1 and EXT2 between the HNSC group and the compared control group. The top 50 co-expressed genes of EXT1 and EXT2 are obtained through the Xiantao bioinformatics tool, and GO/KEGG analysis is simultaneously conducted.

TIMER

TIMER (cistrome.org) uses RNA-Seq expression profiling data to detect the infiltration of immune cells in tumour tissues. Gene module is selected to visualise the correlation of EXT1 and EXT2 expression with immune infiltration level in HNSC. The SCNA module provides the comparison of tumour infiltration levels amongst tumours with different somatic copy number alterations for EXT1 and EXT2.

cBio cancer genomics portal (cBioPortal)

The cBioPortal database (http://cbioportal.org/) includes somatic mutations, DNA copy number changes, mRNA and microRNA expression, DNA methylation, protein abundance, and phosphoprotein abundance. cBioPortal now incorporates the results of TCGA, the Broad Institute's Genome Data Analysis Center, and Cancer Genomics Browser. The database allows you to download each tumour sample and data. At the genomic level, cBioPortal unifies and simplifies these complicated data, and each sample may be queried for specific biologic traits such as mutations, purex deletions, and gene amplifications. We selected 6 datasets of HNSC in the cBioPortal database to analyse the gene mutations of EXT1 and EXT2.

Tumor immune single-cell hub 2 (TISCH2)

TISCH2 (comp-genomics.org) is a single-cell RNA-seq database focusing on the tumour microenvironment (TME). TISCH2 provides detailed cell-type annotation at the single-cell level, enabling the exploration of TME across different cancer types.

Clinical samples and real-time polymerase chain reaction (PCR)

Three patients with HSNC were recruited from the Stomatological Hospital of Xiamen Medical College. The information of the patients is shown in Supplement 1. This study was conducted based on the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of the Stomatological Hospital of Xiamen Medical College (HS20220720004). This as an in silico study with a small patient sample to determine any correlations. A total of 12 samples were obtained from the patient's tumour tissue and adjacent healthy tissue. Then the samples were used to extract total RNA. The mRNA expressions of EXT1 and EXT2 were validated through real-time PCR by a fluorescence kit (SYBR Green, Roche) with a real-time thermocycler. With GAPDH as the internal reference, the relative mRNA levels of EXT1 and EXT2 were calculated by the $2^{-\Delta\Delta Ct}$ method. The primer sequences are showed in Supplement 2. Shapiro–Wilk test was applied to analyse the data's normality of the results of real-time PCR. If the data were normally distributed, Student *t* test was used, and if the data were not normally distributed, Mann–Whitney *U* test was used. $P < .05$ was considered statistically significant when using GraphPad Prism 8.3.0 (GraphPad Software).

Results

The expression of EXT1 and EXT2 in HNSC

In the UALCAN database, samples from patients with HNSC had a significantly higher level of EXT1 and EXT2 compared with normal tissues (**Figure 1A–D**). Furthermore, analysis of numerous clinicopathologic features of HNSC samples in the TCGA showed that EXT1 and EXT2 mRNA expression mainly

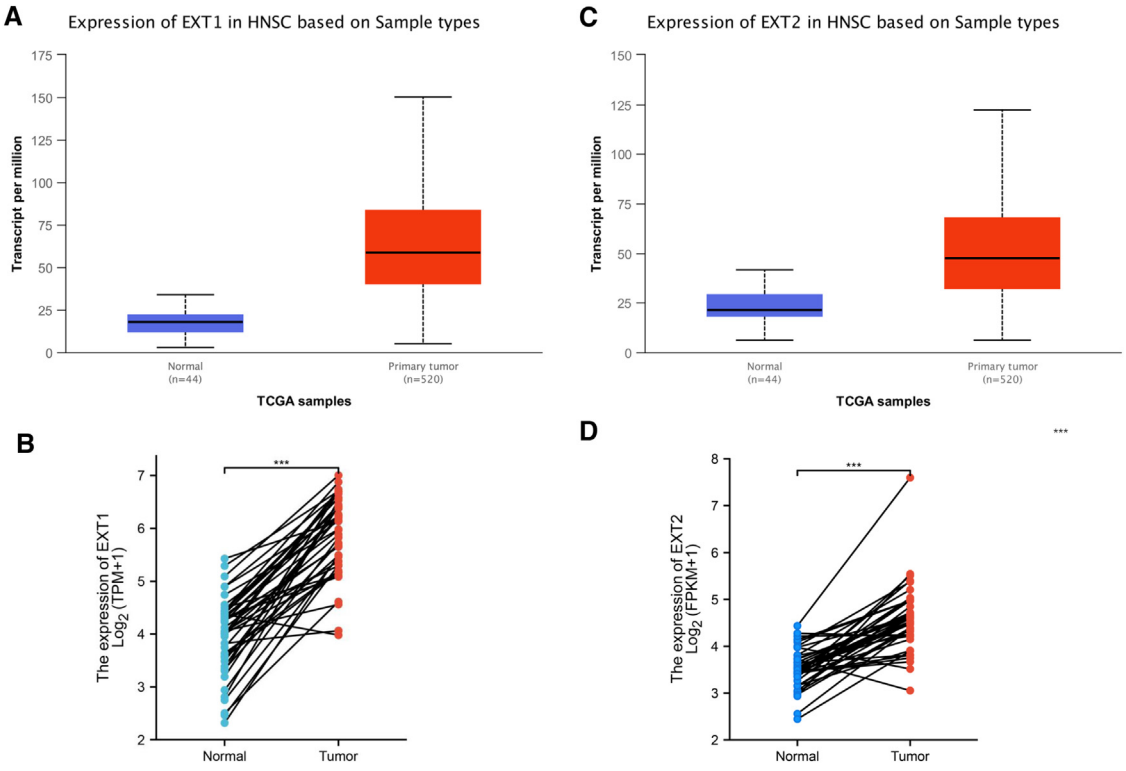

Fig. 1 – The expression of EXT1 and EXT2 in head and neck squamous cell carcinoma (HNSC). A, The expression of EXT1 in HNSC in UALCAN database. B, The expression of EXT1 in paired HNSC samples. C, The expression of EXT2 in normal tissues and primary HNSC tissues in UALCAN database. D, The expression of EXT2 in paired HNSC samples.

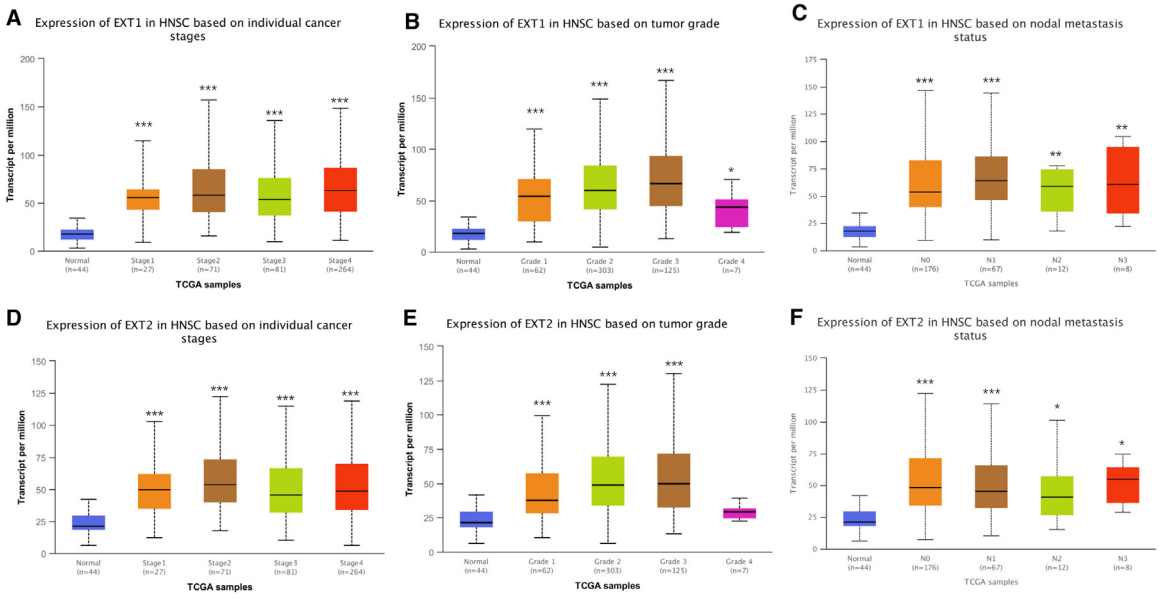

Fig. 2 – The expression of EXT1 and EXT2 in subgroups of head and neck squamous cell carcinoma (HNSC). The expression of EXT1 in (A) individual cancer stages, (B) tumour grade, and (C) nodal metastasis status. The expression of EXT2 in (D) individual cancer stages, (E) tumour grade, and (F) nodal metastasis status.

correlates with cancer stage (Figure 2A, 2D), tumour grade (Figure 2B, 2E), and nodal metastasis status (Figure 2C, 2F). EXT1/2-related baseline information sheets of HNSC from TCGA are shown in Supplement 3 and 4.

EXT2 was negatively correlated to prognosis of HNSC

By using GEPIA, we investigated the effect of EXT1 and EXT2 expression on overall survival and disease-free survival of patients with HNSC. Patients with HNSC and elevated EXT2 expression had a better prognosis, but disease-free survival rate was not affected. The expression of EXT1 had no correlation with the prognosis of HNSC (Figure 3).

The GO/KEGG analysis of EXT1 and EXT2 in HNSC

In the GEPIA database, the top 50 genes with a positive correlation between EXT1 and EXT2 expression in HNSC were obtained (Figure 4A, 4B). Then the Xiantao bioinformatics tool was used to conduct GO/KEGG analysis of the 2 gene clusters. The EXT1 gene cluster was found to be associated with focal adhesin, cell adhesin molecule binding, hemidesmosome assembly, and so on (Figure 4C). The EXT2 gene cluster was found to be associated with Extracellular matrix (ECM)-reporter interaction, cell adhesin molecule binding, and cell-substrate adhesin (Figure 4D).

The links between EXT1 and EXT2 and immune infiltration of HNSC

The relationship between EXT1 and EXT2 expression and immune infiltration level in HNSC was investigated using the TIMER database. EXT1 expression levels were considerably negatively connected with CD8+ T cell infiltrating levels and significantly positively correlated with CD4+ T cell, neutrophil, and dendritic cell infiltrating levels in HNSC, but not with B cells and macrophages (Figure 5A). EXT2 expression levels were clearly connected with tumour purity and with infiltrating levels of CD4+ T cells, macrophages, neutrophils, and dendritic cells in HNSC, but not with B cells, CD8+ T cells, or CD4+ T cells (Figure 5B). Furthermore, EXT1 copy number mutations could partially limit immune infiltration of B cells, CD8+ T cells, CD4+ T cells, and dendritic cells, but not macrophages and neutrophils (Figure 5C). EXT2 copy number mutations could reduce immunologic infiltration of CD8+ T cells, neutrophils, and dendritic cells, but not B cells, macrophages, or CD4+ T cells (Figure 5D).

Gene mutations of EXT1 and EXT2 in HNSC

The gene mutation rates of EXT1 and EXT2 in HNSC were 7% and 2.8%, separately (Supplement 5A). The distribution of EXT gene mutations in different datasets was shown in Supplement 5B. EXT gene mutations did not influence the prognosis of patients with HNSC (Supplement 5C).

Correlation between EXT1 and EXT2 and the TME

The TME played an important role in tumour occurrence. We used one dataset (GSE103322) in the TISCH2 database to obtain the expression of EXT1 and EXT2 in TME-related cells (Supplement 6A). Low expression of EXT1 and EXT2 was found in CD4+ T cells, CD8+ T cells, plasma, mast. The highest expressions of EXT1 and EXT2 were found in malignant cells (Supplement 6B–E).

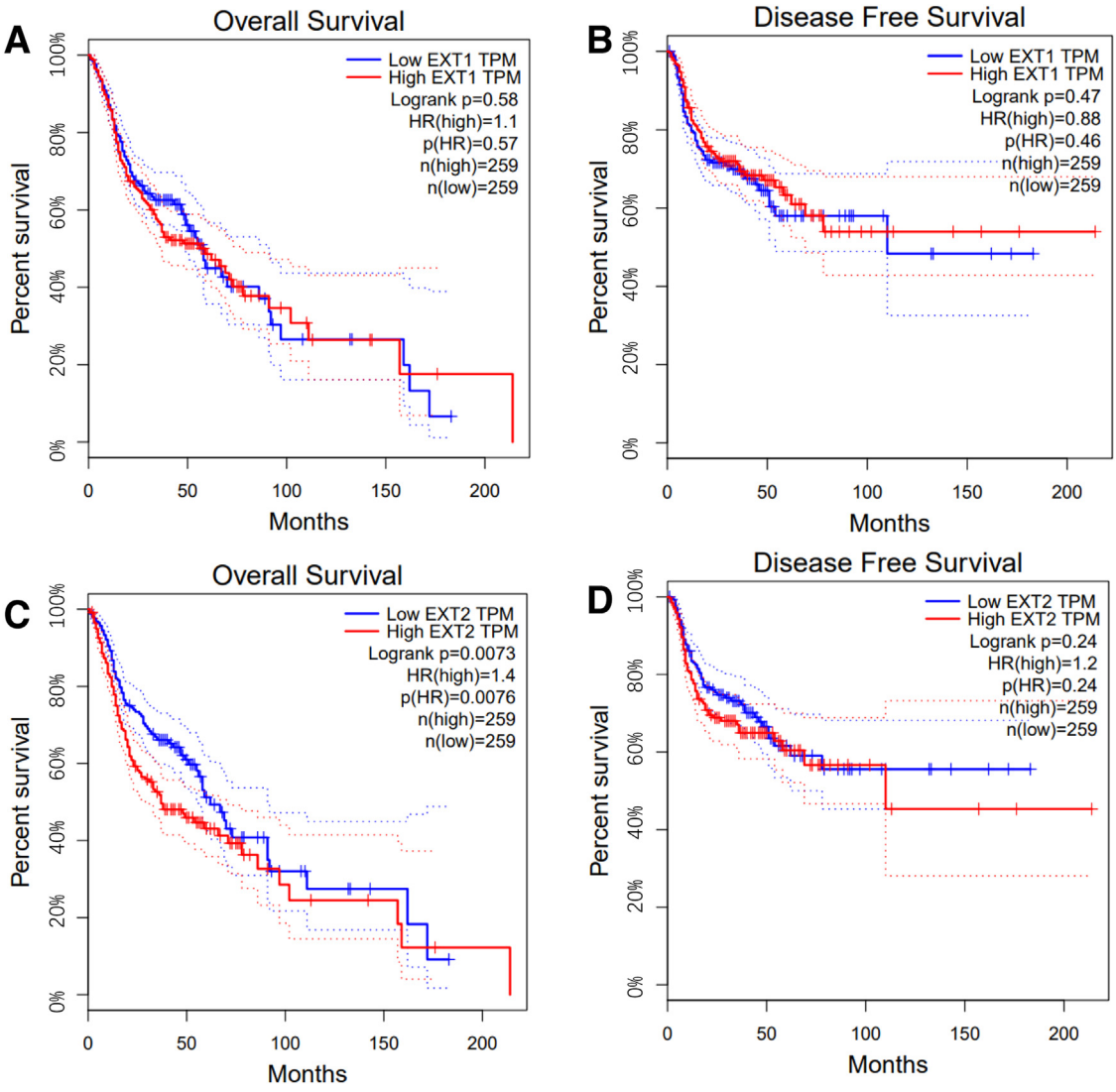

Fig. 3 – The effect of EXT1 and EXT2 expression on overall survival and disease-free survival of patients with head and neck squamous cell carcinoma (HNSC). A, The effect of EXT1 on overall survival of HNSC patients. B, The effect of EXT1 on disease-free survival of patients with HNSC. C, The effect of EXT2 on overall survival of patients with HNSC. D, The effect of EXT2 on disease-free survival of patients with HNSC.

The EXT1 and EXT2 mRNA expression level in HNSC samples

The EXT1 mRNA expression level in the HNSC group was decreased in HNSC compared with the paracancerous tissue (Supplement 7A). The EXT2 mRNA expression level in the HNSC group was about 1.9-fold compared to that in the control group (Supplement 7B).

Discussion

The function of HS-related genes (EXT1 and EXT2) in many diseases, including multiple osteochondroma and hepatocellular carcinoma had been discussed. However, the expression, prognostic value, and immune infiltration influence of EXT1 and EXT2 in HNSC have not been comprehensively explored.

HS influences tumour growth by regulating a number of pathologic mechanisms, including apoptosis, invasion, migration, and tumour cell immune evasion.[19] HS has been demonstrated to increase cell-cell and cell-ECM adhesion as well as to prevent invasion and metastasis, and decreased HS levels in some malignancies lead to more aggressive malignant cells. The association between HS and HNSC had not been discussed. Both EXT1 and EXT2 have glucuronosyltransferase and acetylglucosaminyltransferase activities that are essential for HS production. The glucuronosyltransferase structural domain was discovered to be located at the N-terminus of the EXT1 protein in ovarian cells of Chinese hamster mutants lacking HS.[20]

Different data platforms based on TCGA were applied to reveal upregulated expression of EXT1 and EXT2 in HNSC. Subgroup analysis indicated that EXT1 and EXT2 mRNA expression mainly correlates with cancer stage, tumour

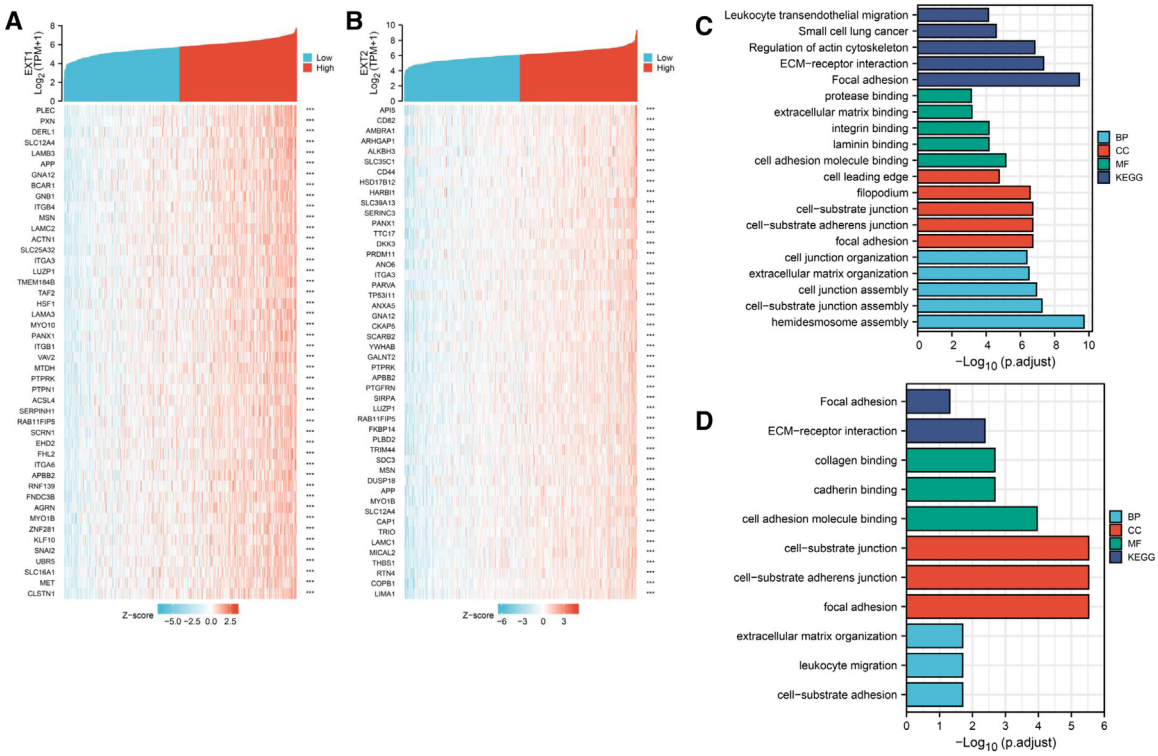

Fig. 4 – The GO/KEGG analysis of EXT1 and EXT2 in head and neck squamous cell carcinoma (HNSC). A, Top 50 genes with positive correlation between EXT1 expression in HNSC in the GEPIA database. B, Top 50 genes with positive correlation between EXT2 expression in HNSC in the GEPIA database. C, The GO/KEGG analysis of EXT1 and the above 50 genes in HNSC; the EXT1 gene cluster was found to be associated with focal adhesin, cell adhesin molecule binding, and hemidesmosome assembly. D, The GO/KEGG analysis of EXT2 and the above 50 genes in HNSC; the EXT2 gene cluster was found to be associated with Extracellular matrix (ECM)-reporter interaction, cell adhesin molecule binding, and cell-substrate adhesin.

grade, and nodal metastasis status.[14] The expression levels of EXT1 and EXT2 were significantly greater in cancer than that in adjacent normal tissues. Highly expressed EXT2 was correlated with the poor prognosis of patients with HNSC. In squamous cell lung carcinoma, EXT1 and EXT2 expression was also upregulated, and high expression of EXT1 was

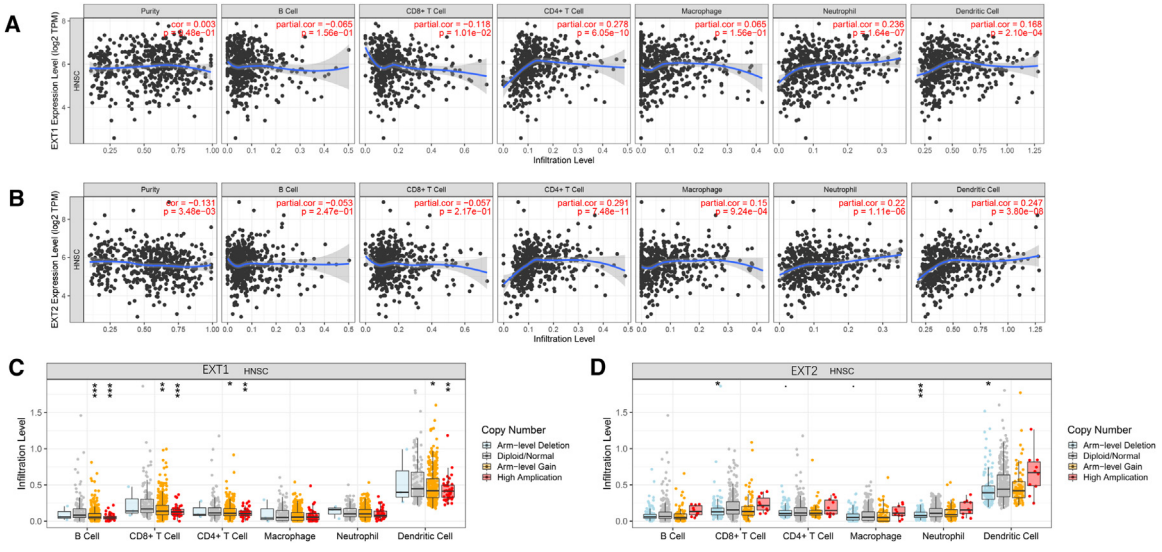

Fig. 5 – The links between EXT1 and EXT2 and immune infiltration of head and neck squamous cell carcinoma (HNSC). A, The correlation between EXT1 expression and immune cell infiltrating levels. B, The correlation between EXT2 expression and immune cell infiltrating levels. C, EXT1 copy number mutations and immune cell infiltration. D, EXT2 copy number mutations and immune cell infiltration.

related to unfavourable prognosis.[13] In acute lymphoblastic leukemia, EXT1 expression is downregulated and related to poor patient survival.[21] Oncogenic mutation of EXT2 was found in thyroid cancer, and EXT2 could be the potential treatment target of thyroid cancer.[22]

We selected the top 50 genes co-expressed with EXT1 and EXT2 to conduct GO/KEGG analysis in HNSC. The results showed that EXT1 and EXT2 were related to cell adhesin molecule binding and cell–substrate adhesin. Tumour invasion and metastasis are key causes of therapy failure and patient death in cancer.[23,24] It is a complex and ongoing active process in which tumour cells proliferate and detach from the primary site, penetrate the vasculature and enter the vasculature or lymphatics, survive in the vasculature by evading platelets and host immunologically active cells, and remain distant and extravasate out of the vasculature by interacting with the vascular or lymphatic endothelium or/and subendothelial basement membrane vasculature.[25] Many associated genes are activated and expressed as a result of this process. Cell adhesion molecules are essential for tumour cell attachment to the basal lamina away from the main location, as well as cell adhesion to each other. Many adhesion molecules have been implicated in the invasion and metastasis of HNSC, including integrins, cadherins, selectins.[26] The present study indicated that EXT1 and EXT2 might be related to HNSC through cell adhesin molecule binding.

EXT1 expression levels were considerably connected with CD8 + T cell, CD4+ T cell, neutrophil, and dendritic cell infiltrating levels in HNSC. EXT2 expression levels were clearly connected with tumour purity and with infiltrating levels of CD4+ T cells, macrophages, neutrophils, and dendritic cells in HNSC. CD8+ T cell infiltration score was reported to be an independent prognostic marker of HNSC.[27] A high level of CD8+ T cell infiltration was positively associated with longer survival of patients with oral squamous cell carcinoma.[28] High neutrophil counts predicted a poor prognosis in patients with oral squamous cell carcinoma.[16,29] Hence, it was probable that the increased neutrophil infiltration correlated to highly expressed EXT2 in HNSC cases in the present study was one of multiple factors related to the poor prognosis.

The gene mutation rates of EXT1 and EXT2 in HNSC was low and not related to prognosis. However, EXTs were closely associated with hereditary multiple exostoses, which was an autosomal dominant disorder involved in the formation of exostoses. To present, more 50 different EXT1 and 30 different EXT2 mutations were already discovered in patients with hereditary multiple exostoses, with indications suggesting that mutations in these 2 genes account for more than 70% of hereditary multiple exostoses cases.[30]

Overall, we found that EXT1 and EXT2 were highly expressed in HNSC, and this was validated by real-time PCR using HNSC samples. The expression of EXT2 was associated with immune infiltration and prognosis of HNSC. There was a limitation in that the sample size was small. More studies are needed to explore the function of EXT2 in the carcinogenesis of HNSC.

Conclusion

EXT1 and EXT2 were highly expressed in HNSC. Overexpression of EXT2 was associated with poor prognosis in HNSC. This was largely an in silico and bioinformatic-based analysis.

Conflict of interest

None disclosed.

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Author contributions

Yiping Wang and Yan Huang: methodology, data curation, software, writing–original draft preparation. Houwei Zhu, Zhenzhen Guo, and Jun Cheng: visualisation, investigation, supervision. Churen Zhang and Ming Zhong: conceptualisation, visualisation, investigation, supervision, and article revision.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.identj.2023.10.017.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–49.
2. Ringash J, Bernstein LJ, Devins G, et al. Head and neck cancer survivorship: learning the needs, meeting the needs. Semin Radiat Oncol 2018;28:64–74.
3. Peltanova B, Raudenska M, Masarik M. Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: a systematic review. Mol Cancer 2019;18:63.
4. Krentowska A, Sierko-Nobis E, Strzalka A, Pietruszewska W, Hempel D, Sierko E. Awareness of head and neck cancer - a multicentre survey among young respondents in Poland. Int Dent J 2018;68:441–9.
5. Posner MR, Hershock DM, Blajman CR, et al. Cisplatin and fluorouracil alone or with docetaxel in head and neck cancer. N Engl J Med 2007;357:1705–15.
6. Cohen EEW, Bell RB, Bifulco CB, et al. The Society for Immunotherapy of Cancer consensus statement on immunotherapy for the treatment of squamous cell carcinoma of the head and neck (HNSCC). J Immunother Cancer 2019;7:184.
7. Beckham TH, Leeman JE, Xie P, et al. Long-term survival in patients with metastatic head and neck squamous cell

carcinoma treated with metastasis-directed therapy. Br J Cancer 2019;121:897–903.
8. Ogawa H, McKenna G. Kettratad-Pruksapong M. Prevention of oral functional decline. Int Dent J 2022;72:S21–6.
9. Carlisle JW, Steuer CE, Owonikoko TK, Saba NF. An update on the immune landscape in lung and head and neck cancers. CA Cancer J Clin 2020;70:505–17.
10. D'Arienzo A, Andreani L, Sacchetti F, Colangeli S, Capanna R. Hereditary multiple exostoses: current insights. Orthop Res Rev 2019;11:199–211.
11. Hayes AJ, Melrose J. HS, an ancient molecular recognition and information storage glycosaminoglycan, equips hs-proteoglycans with diverse matrix and cell-interactive properties operative in tissue development and tissue function in health and disease. Int J Mol Sci 2023:24.
12. Manandhar S, Kim CG, Lee SH, Kang SH, Basnet N, Lee YM. Exostosin 1 regulates cancer cell stemness in doxorubicin-resistant breast cancer cells. Oncotarget 2017;8:70521–37.
13. Wu D, Huo C, Jiang S, et al. Exostosin1 as a novel prognostic and predictive biomarker for squamous cell lung carcinoma: a study based on bioinformatics analysis. Cancer Med 2021;10:2787–801.
14. Nagarajan A, Malvi P, Wajapeyee N. Heparan sulfate and heparan sulfate proteoglycans in cancer initiation and progression. Front Endocrinol (Lausanne) 2018;9:483.
15. Zhang C, Xue P, Ke J, Cai Q. Development of ferroptosis-associated ceRNA network in periodontitis. Int Dent J 2023;73:186–94.
16. Zhang C, Cai Q, Ke J. Poor prognosis of oral squamous cell carcinoma correlates with ITGA6. Int Dent J 2023;73:178–85.
17. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017;45:W98–102.
18. Chandrashekar DS, Karthikeyan SK, Korla PK, et al. UALCAN: an update to the integrated cancer data analysis platform. Neoplasia 2022;25:18–27.
19. Onyeisi JOS, Ferreira BZF, Nader HB, Lopes CC. Heparan sulfate proteoglycans as targets for cancer therapy: a review. Cancer Biol Ther 2020;21:1087–94.
20. Busse M, Feta A, Presto J, et al. Contribution of EXT1, EXT2, and EXTL3 to heparan sulfate chain elongation. J Biol Chem 2007;282:32802–10.
21. Liu NW, Huang X, Liu S, Lu Y. EXT1, regulated by MiR-665, promotes cell apoptosis via ERK1/2 signaling pathway in acute lymphoblastic leukemia. Med Sci Monit 2019;25:6491–503.
22. Pitt SC, Hernandez RA, Nehs MA, et al. Identification of novel oncogenic mutations in thyroid cancer. J Am Coll Surg 2016;222:1036–43 e1032.
23. Han S, Wang R, Zhang Y, et al. The role of ubiquitination and deubiquitination in tumor invasion and metastasis. Int J Biol Sci 2022;18:2292–303.
24. Kalyanyama BM, Matee MI, Vuhahula E. Oral tumours in Tanzanian children based on biopsy materials examined over a 15-year period from 1982 to 1997. Int Dent J 2002;52:10–4.
25. Bian X, Yin S, Yang S, et al. Roles of platelets in tumor invasion and metastasis: a review. Heliyon 2022;8:e12072.
26. Yang Y, Zheng H, Zhan Y, Fan S. An emerging tumor invasion mechanism about the collective cell migration. Am J Transl Res 2019;11:5301–12.
27. Zhang H, Li Z, Zheng Y. Identifying the therapeutic and prognostic role of the CD8+ T cell-related gene ALDH2 in head and neck squamous cell carcinoma. Cancer Inform 2022; 21:11769351221139252.
28. Fang J, Li X, Ma D, et al. Prognostic significance of tumor infiltrating immune cells in oral squamous cell carcinoma. BMC Cancer 2017;17:375.
29. Ding M, Song Y, Jing J, et al. The ratio of preoperative serum biomarkers predicts prognosis in patients with oral squamous cell carcinoma. Front Oncol 2021;11:719513.
30. Bukowska-Olech E, Trzebiatowska W, Czech W, et al. Hereditary multiple exostoses-a review of the molecular background, diagnostics, and potential therapeutic strategies. Front Genet 2021;12:759129.