Expression profile and prognostic value of CXCR family members in head and neck squamous cell carcinoma

Yiming Shen¹²³†, Chongchang Zhou¹²†, Yujie Cao¹²³, Qun Li¹², Hongxia Deng¹², Shanshan Gu¹², Yidong Wu¹² and Zhisen Shen¹²³*

Abstract

Background: CXC chemokine receptor gene family consists of seven well-established members which are broadly involved in biological functions of various cancers. Currently, limited studies have shed light on the expression profile of CXCR family members (CXCRs), as well as their prognostic value, in head and neck squamous cells carcinoma (HNSCC).

Methods: The data for this study were retrieved from the Cancer Genome Atlas database and other publicly available databases, including gene expression, methylation profiles, clinical information, immunological features, and prognoses. The expression pattern and prognostic values of CXCRs were identified, and the potential mechanism underlying CXCRs function in HNSCC was investigated by gene set enrichment analysis (GSEA).

Results: CXCRs were differentially expressed in HNSCC. As shown by Kaplan–Meier analysis, high CXCR3-6 expression was significantly associated with better prognostic outcomes of HNSCC patients, including overall survival and progression-free survival. According to the results of univariate and multivariate Cox proportional risk regression analysis, it was demonstrated that upregulation of CXCR3-6 was an independent factor for better prognosis, while the two other clinical features, age and stage, were factors for worse prognosis. A significant positive correlation between CXCR3-6 and tumor-infiltrated immune cells was revealed by results from Tumor Immune Estimation Resource and CIBERSORT analysis database. The main involvement of CXCRs in immune and inflammatory responses was further confirmed by GSEA.

Conclusions: Overall, this study provided a rationale for targeting CXCRs as a promising therapeutic strategy of HNSCC.

Keywords: Head and neck squamous cell carcinoma, CXC chemokine receptor gene family, The Cancer Genome Atlas, Prognosis, Survival

Background

Head and neck squamous carcinoma (HNSCC) is the most common pathological subtype of head and neck cancer which is a highly aggressive malignancy [1]. The annual number of people diagnosed with head and neck cancer worldwide has reached nearly 500,000, and the mortality rate of the patients diagnosed with HNSCC has increased over the last few years [2]. Although prominent...
progress has been made in the treatment of HNSCC, the 5-year survival rate of HNSCC patients remains around 50% [3]. Therefore, further understanding of the complex cancer biology underlying this disease and developing more reliable predictive biomarkers for HNSCC prognosis are critical for facilitating the therapeutic efficacy of individualized treatment, which may ultimately help to enhance patients’ survival. With more than 50 members, vertebrate G protein-coupled chemokine receptors are comprised of four different subfamilies, including CC chemokine receptors, XC chemokine receptors, CXC chemokine receptors, and CX3C chemokine receptors. CXC chemokine receptors are a large subfamily of chemokine receptors, which consists of seven members known as CXCR1 to CXCR7 [4]. CXCR family members (CXCRs) are well-known to participate in both anti-tumor immune response and tumorigenesis [5]. CXC chemokine receptors 1 and 2 (CXCR1 and CXCR2), members of the receptors of ELR+ chemokines, are generally expressed simultaneously on neutrophils, fibroblasts, and vascular endothelial cells and have a relation to the activation of multiple downstream signaling pathways. A high affinity exhibited by both CXCR1 and CXCR2 has been observed toward the CXC motif chemokine ligand (CXCL) 8, a common ligand secreted by cancer cells and inflammatory cells. Binding of the ligand to these receptors promotes tumor invasion, angiogenesis, and metastasis [6, 7]. CXCR3-6 are the receptors of the ELR− chemokines [8]. CXCR3 can bind to CXCL9, CXCL10, and CXCL11, respectively, and its expression can be detected on various subtypes of natural killer (NK) cells and T cells [9–12]. CXCR3 exhibits diverse biological functions upon extracellular stimuli which activate intracellular signal transduction pathways that involved in survival, cell behavior, and cell identity. CXCR4, an important regulator of the homing and mobilization of hematopoietic cell, is widely expressed on different cell types like megakaryocytes, embryonic stem cells, vascular endothelial cells, and cancer cells [13, 14]. The interaction of CXCR4 with its ligand, CXCL12, has been shown to facilitate the migration of T and B lymphocyte. The CXCR5 is a receptor for CXCL13 which is a homeostatic chemokine. Upon contact with CXCL13, CXCR5 is capable of regulating cell migration [15]. The overexpression of CXCR5 and its cognate ligand CXCL13 has been implicated in many different types of cancer [16]. Expressed on CD8+ T, NK, and CD4+ T cells, CXCR6 could promote the recruitment of tumor-infiltrating lymphocytes in combination with CXCL16 expressed by tumor cells, thus contributing to a better prognosis for cancer patients [17]. CXCR7 is previously known as ACKR3. Although it is also a seven-transmembrane receptor, CXCR7 cannot mediate the classical Gi signaling pathway like CXCR3 and many other chemokine receptors [18]. CXCR7 has previously been regarded as a “decoy” and an atypical chemokine receptor for CXCL12. Moreover, CXCR7 is a promising therapeutic target with its involvement in tumorigenesis and autoimmune disorders. However, the mechanism by which CXCRs are activated or deactivated in the development and progression of HNSCC remains unclear until now. Here, we comprehensively analyzed the expression of the whole CXC chemokine receptor gene family and the potential correlation with prognostic value and clinical significance in HNSCC via different databases, including ONCOMINE and the Cancer Genome Atlas (TCGA), which provide detailed information about cancers. In addition, Tumor Immune Estimation Resource (TIMER) was employed to investigate the association between CXCRs and immune infiltration degree. To determine HNSCC-related risk factors, univariate/multivariate Cox regression models were utilized, and GSEA about CXCRs-related signaling pathways was performed.

Methods

Data resources

HNSCC transcriptomic sequencing data of 502 HNSCC and 44 normal samples were acquired from TCGA data (https://portal.gdc.cancer.gov/) [19]. The clinical features from the extracted clinical information of patients were included in the present study, including gender, age, stage, and tumor grade. Subsequently, the high-throughput sequencing (HTSeq) fragments per kilobase of transcript per million mapped reads (FPKM) data of CXCRs were download by utilizing Genomic Data Commons (GDC) Data Transfer Tool on the TCGA database. Besides, we also downloaded two HNSCC datasets (GSE41613 and GSE65858) from Gene Expression Omnibus (GEO) database to validate the association between CXCR members and overall survival. The basic characteristics of the HNSCC patients used in research are shown in Supplementary Table 1.

Expression data of CXCRs in HNSCC

Gene expression of CXCRs was analyzed across multiple datasets by comparing tumor tissues to the healthy tissues, using ONCOMINE (http://www.oncomine.org) [20]. And the thresholds were set as follows: gene rank was set in the top 10%, data type was limited to mRNA, p-value was set to 0.01, and folding change (FC) was defined as 1.5. Based on the HTSeq FPKM data, Perl 5.26 software was adopted to extract the mRNA expression levels of CXCRs in HNSCC. The limma package in R software was employed to analyze how CXCRs were expressed differentially in HNSCC tissues and normal
tissues. The **pheatmap** and **ggpubr** packages were utilized for the visualization of a heatmap and box plots to display analysis results.

**Relationship between methylation and mRNA expression of CXCRs in HNSCC**

Data on the methylation levels of cg sites, which were within the gene promoter regions of differentially expressed CXCRs in HNSCC tissues, was downloaded from Illumina Human Methylation 450 K through GDC Data Transfer Tool on the TCGA and then was annotated with the annotation file from the official Illumina website ([https://support.illumina.com/downloads/~infinium_humanmethylation450_product_files.html](https://support.illumina.com/downloads/~infinium_humanmethylation450_product_files.html)). After the methylation levels of cg sites were identified in CXCRs gene promoter regions, the **corrplot** package was utilized to understand how the methylation level and CXCR expression in HNSCC correlated.

**Prognostic value evaluation of CXCRs in HNSCC**

The Kaplan–Meier method was adopted for the analysis of the survival rate. R package survival analysis was implemented combined with gene expression profiles and survival information of HNSCC involving a total of 499 HNSCC patients, to evaluate how the mRNA expression of CXCR1-7 correlated with the prognoses of HNSCC patients. Then, the progression-free survival (PFS) was assessed along with overall survival (OS) time in search of the best optimal cutoff value. Moreover, for the estimation of the independent prognostic factors, we utilized univariate and multivariate Cox proportional hazards models. Forest plots were created to present the analysis results by using the **ggplot** package.

**Pearson correlation and protein-protein interaction (PPI) network of CXCRs**

To determine the correlation among CXCRs, a Pearson correlation analysis was carried out using the **corrplot** package of R software genes on the basis of the gene expression data obtained from the TCGA database. In order to retrieve interacting genes or proteins, we established the PPI networks of CXCRs by the search tool (STRING, [http://www.string-db.org/](http://www.string-db.org/)).

**Gene set enrichment analysis (GSEA)**

To further investigate how CXCRs participated in HNSCC carcinogenesis, GSEA was carried out (version 4.0.1; [http://software.broadinstitute.org/gsea/index.jsp](http://software.broadinstitute.org/gsea/index.jsp)) to identify the CXCRs-associated signaling pathways in the TCGA HNSCC tissues [21, 22]. The identification of significant enrichment pathways employed a false discovery rate (FDR) of < 0.05 and a random combination number of 1000 for each mutation, with the reference to the annotated gene set file from the Msig database (c2.cp.kegg.v7.0.symbols.gmt).

**Corrections between tumor immune-infiltrating cells (TIICs) and CXCRs**

During cancer progression, tumor cells interact with TIICs through a variety of genes and pathways. To explore the correlation of TIICs with CXCRs, the TIMER platform was adopted for analysis ([https://cistrome.shinyapps.io/timer/](https://cistrome.shinyapps.io/timer/)), which is an online database incorporating expression profiles of over 10,000 samples across 23 cancer types from TCGA [23, 24]. According to TIMER, macrophages, CD4+ T cells, neutrophils, dendritic cells, B cells, and CD8+ T cells are the main components of the TIICs. The purity-corrected partial Spearman’s correlation (partial-cor) provided by TIMER was presented together with p-value in scatterplots in the current study. On the basis of the median value of gene expression, we divided the samples into high expression group and low expression group. The **vioplot** package of R software was utilized to visualize the analysis results. In a mixed cell population, the specific proportions that 22 tumor-infiltrating lymphocyte subsets accounted for respectively were estimated by the analytical tool CIBERSORT with gene expression data ([https://cibersort.stanford.edu/](https://cibersort.stanford.edu/)).

**Results**

**Differential expression of CXCRs in HNSCC patients**

It has been implicated that the aberrant expression of CXCRs exists in various cancer types like leukemia, lymphomas, and lung cancer. To assess the expression pattern of CXCRs in HNSCC, the gene expression data from ONCOMINE was analyzed. ONCOMINE analysis results shown in Fig. 1A demonstrated that mRNA expression levels of CXCR4 and CXCR7 were upregulated in all subtypes of head and neck cancer, while CXCR2 and CXCR5 were downregulated (fold change > 1.5, p-value < 0.05). And the mRNA expression level of CXCR4 was notably upregulated in HNSCC tissue when compared with the healthy tissue (Table 1). In addition, 1.550-fold increase (p-value = 1.28E-10) and 2.378-fold increase (p-value **p** = 2.378E-10) were detected in CXCR4, while CXCR1 and CXCR2 were downregulated (p-value < 0.01; **p** = 0.001).

(See figure on next page.)

**Fig. 1** Expression of CXCR5s in different types of cancers. **A** Red and blue in the heatmap indicate the numbers of datasets with increased and decreased levels of CXCR family members, respectively (p < 0.05). **B** Differential expression of CXCRs in HNSCC samples and normal tissues represented by a heatmap. The tree diagram on the left side of heatmap showed the cluster analysis between CXCR family members. *p < 0.05, **p* < 0.01; ***p* < 0.001. **C** Differential expression of CXCRs in HNSCC samples and normal tissues represented by box plots.
Fig. 1 (See legend on previous page.)
Table 1 Transcriptional levels of CXCR family members between normal tissues and HNSCC (ONCOMINE)

| No. | Gene name | Fold change | p-value  | t-test  | References |
|-----|-----------|-------------|----------|---------|------------|
| 1   | CXCR4     | 3.447       | 2.75E-13 | 10.536  | [20]       |
| 2   | CXCR4     | 2.150       | 4.68E-6  | 5.002   | [21]       |
| 3   | CXCR7     | 2.185       | 3.24E-7  | 5.892   | [20]       |
| 4   | CXCR7     | 6.647       | 0.002    | 4.459   | [22]       |
| 5   | CXCR7     | 3.175       | 7.57E-4  | 3.497   | [23]       |
| 6   | CXCR7     | 1.550       | 1.28E-10 | 7.522   | [21]       |
| 7   | CXCR7     | 2.378       | 1.49E-4  | 3.937   | [24]       |

= 1.49E-4) in the CXCR6 expression in HNSCC tissues were found in two other studies [25, 26]. For the further understanding of the expression profile of CXCRs in HNSCC, based on 502 HNSCC samples and 44 healthy control samples from the TCGA database, Perl software and the limma package were utilized to obtain mRNA expression data of CXCR members (CXCR1–7) and analyze the differentially expressed CXCR members. The differential gene expression of CXCRs in the HNSCC samples, as well as normal tissues, was displayed in the heatmap generated by the pheatmap package (Fig. 1B). Furthermore, the aberrant expression levels of all CXCR members in HNSCC compared to the healthy tissues were revealed by the analysis results, especially the significant down-regulation of CXCR2 in HNSCC tissues, while the other CXCRs, namely CXCR1 and CXCR3 to CXCR7, were significantly upregulated in HNSCC tissues (Fig. 1C).

Methylation of the promoter regions of CXCR genes in HNSCC
In the progression of cancer, one of the most typical mechanisms having a critical impact on gene expression is methylation of gene promoter regions. As Pearson’s correlation analysis indicated, among the identified seven CXCR members in HNSCC, five of the CXCR members, including CXCR2 and CXCR4 to CXCR7, were negatively correlated with methylation level (Fig. 2 B–F). Moreover, of the 4 evaluated cg sites in the CXCR1 promoter region, only 2 were negatively associated with the expression of CXCR1 in HNSCC (Fig. 2A). Together, the expression of CXCR members was inversely correlated with their methylation level in HNSCC according to the existing data.

Prognostic value of CXCRs in HNSCC
To determine the correlation of CXCR family genes expression with the survival of HNSCC patients, how mRNA expression of CXCRs correlated with overall survivals on log-rank test was examined using Cutoff Finder (CXCR1: 0.255, CXCR2: 2.808, CXCR3: 1.205, CXCR4: 37.548, CXCR5: 0.0131, CXCR6: 1.390, CXCR7: 7.425). The overall survival and the progression-free survival in patients with CXCR1-7 expression were plotted by utilizing Kaplan–Meier curves (Fig. 3). A significant association was observed between high mRNA expression of CXCR3 to CXCR6 and favorable overall survival (Fig. 3 C–F). In addition, databases (GSE41613 and GSE65858) were used for survival analysis to validate the survival value of CXCR family genes (Supplementary Fig. 1). Furthermore, there was a trend toward improved relapse-free survival with the high expression level of CXCR3 to CXCR6, with statistical significance (log-rank test p < 0.01, Fig. 3 H–L). Subsequently, we aimed to determine the prognostic influences of CXCR members in HNSCC. To evaluate the predictive ability of differentially expressed CXCRs and their correlation with clinical characteristics, univariate Cox proportional hazards regression analysis was used. The association between the expression of four CXCR members (CXCR3 to CXCR6) and the favorable outcome of HNSCC patients was found, so as the association of two clinical features (age and stage) with poor outcome (hazard ratio [HR] for CXCR3: 0.649; HR for CXCR4: 0.529; HR for CXCR5: 0.623; HR for CXCR6: 0.650; HR for age: 1.024; and HR for stage: 1.448) (Table 2). In the evaluation of the independent prognostic values of CXCR3 to CXCR6 along with the controlling of prognostic effects of these clinical features, the independent prognostic biomarkers of HNSCC outcome were reported by multivariate Cox proportional hazards regression analysis, i.e., the expression of CXCR3 to CXCR6 and two clinical parameters (age and stage) (Fig. 4).

Potential molecular mechanism underlying the roles of prognostic CXCRs in HNSCC
On the basis of the gene expression data from TCGA, a PPI network was established by a Pearson correlation analysis combined with STRING database, aiming to determine the related protein interactions and possible correlation among CXCR family genes. A strong correlation was found between the CXCRs and interleukin-8 receptor activity (Fig. 5A), as well as the CXCR1 gene and CXCR2 (Fig. 5B). Besides, regarding the underlying biological mechanism of how the carcinogenesis of HNSCC is mediated by differential expression of CXCR3 to CXCR6, a GSEA was carried out on the differentially expressed CXCRs with statistical prognostic value. Ten common cell signaling pathways closely related to high expression of CXCR3 to CXCR6 were proposed by the GSEA (Fig. 6A). Pathway analysis identified many immune-related pathways including a pathway involved in natural killer cell-mediated cytotoxicity, a process that is important in inflammation and immune responses (Fig. 6 B–E).
Fig. 2  Correlation between methylation levels and expression of CXCRs in HNSCC
Associations between TIICs and CXCRs in HNSCC

Given the growing evidence on the association between cancer prognosis and immunological features, the potential relevance of CXCR members in TIICs in HNSCC was further explored. For the evaluation of the association between immune cells surrounding tumor cells and certain gene products, we made use of the public resource provided by the TIMER database. The scatterplots were shown here presenting the expression of CXCR members against tumor purity. It was reported that in stromal-immune cells surrounding tumor cells, the high expression of CXCRs was anticipated to be negatively correlated with tumor purity but positively correlated in tumor cells. Consistent with the results mentioned above, CXCR1 to CXCR6 were highly expressed in HNSCC tissues, which showed a negative correlation with tumor purity (Fig. 7) and suggested that there was a significant

Table 2 Univariate Cox proportional hazards regression analyses of CXCR members and clinical features in HNSCC

| Parameter | Univariate analysis | Hazard ratio | 95% CI | p  |
|-----------|---------------------|--------------|--------|----|
| Age       |                     | 1.024        | 1.010–1.038 | 0.001 |
| Gender    |                     | 0.776        | 0.566–1.063 | 0.114 |
| Grade     |                     | 1.152        | 0.917–1.449 | 0.225 |
| Stage     |                     | 1.448        | 1.203–1.743 | 9.15E-05 |
| CXCR1     |                     | 1.208        | 0.883–1.652 | 0.238 |
| CXCR2     |                     | 0.809        | 0.546–1.199 | 0.290 |
| CXCR3     |                     | 0.649        | 0.481–0.877 | 0.005 |
| CXCR4     |                     | 0.529        | 0.301–0.931 | 0.027 |
| CXCR5     |                     | 0.623        | 0.462–0.839 | 0.002 |
| CXCR6     |                     | 0.650        | 0.474–0.892 | 0.008 |
| CXCR7     |                     | 1.208        | 0.921–2.216 | 0.111 |

Bold means p < 0.05
correlation between TIICs and CXCR members, CXCR3-6 in particular. The relationship between CXCRs gene expression and the abundance of 22 immune cell types in HNSCC was revealed by CIBERSORT results (Fig. 8). High CXCR3 expression was significantly related with more regulatory T cells (Tregs), M0 macrophages, naive B cells, resting mast cells, follicular helper T cells, activated dendritic cells, activated mast cells, memory B cells, activated memory CD4+ T cells, M1 macrophages, CD8+ T cells, and less naive CD4+ T cells, and eosinophils infiltration. High CXCR4 expression was associated with gamma delta T cells, more naive B cells, activated NK cells, Tregs, activated memory CD4+ T cells, M2 macrophages, follicular helper T cells, activated dendritic cells, and less plasma cells, CD8+ T cells, resting NK cells, M0 macrophages, activated mast cells, and eosinophils infiltration. High CXCR5 expression was positively related with follicular helper T cells, naive B cells, Tregs, plasma cells, gamma delta T cells, CD8+ T cells, resting NK cells, resting mast cells, memory B cell, and negatively related with M0 macrophages, activated mast cells, eosinophils, activated memory CD4+ T cells, and activated NK cells. Moreover, it was also demonstrated that high CXCR6 expression was positively related with activated memory CD4+ T cells, naive B cells, Tregs, CD8+ T cells, resting NK cells, follicular helper T cells, and negatively related with activated mast cells, M1 macrophages, resting mast cells, eosinophils, activated dendritic cells, and M0 macrophages. Taking these findings together, CXCR family members may play a crucial role in the regulation of the immune microenvironment in HNSCC, with the potential to make promising targets for the treatment of HNSCC patients.

Discussion

CXC chemokine receptors represent a large subfamily of the G protein-coupled receptors superfamily. Chemokine receptors, as the name suggests, have the primary function of orchestrating cell trafficking, in particular the mobilization of immune cells to the sites of inflammation. Although the role of the CXCR gene family in the onset and progression of many human cancers has been highlighted by accumulating evidence, including breast cancer, bladder cancer, acute lymphoblastic leukemia (ALL), and acute myeloid leukemia (AML) [27–29], the exact prognostic values of CXCRs in HNSCC remain to
Fig. 5  A PPI network among CXCRs. B Correlations between CXCRs
Fig. 6 A Venn diagram showed CXCR3-6 related to 10 common enriched pathways (KEGG). Cancer-related Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with B CXCR3, C CXCR4, D CXCR5, and E CXCR6 based on a GSEA.
be further explored. With increasing published or publicly available genomic data and a variety of online platforms, it is possible to investigate the expression profiles and clinical practice value of families of genes in human cancers. In the current study, the distinct expression profile, methylation, and prognostic values of CXCRs were clarified, as well as their involvement in various biological processes in HNSCC.
We carried out the evaluation of the CXCRs expression levels in different malignancies and found the high expression of CXCR4 and CXCR7 in HNSCC samples from ONCOMINE. And a previous study found that CXCR4 was shown to be overexpressed in HNSCC tissues in comparison with the healthy tissues, with a fold change (FC) of 3.447 ($p$-value = 2.75E-13) [30]. Also, in another study, the mRNA level of CXCR4 was found to have a 2.150-fold increase in HNSCC tissue ($p$-value = 4.68E-6) [25]. Moreover, a significantly high level of CXCR7 mRNA expression was previously found in HNSCC tissues in other studies. For example, the FC of the CXCR7 expression in HNSCC tissue was 2.185 ($p$-value = 3.24E-7) and 6.647 ($p$-value = 0.002), respectively, in the study by Matthew et al. and Gokce et al. [30]. The significant difference between the transcription level of CXCR7 in HNSCC and that in healthy head and neck tissues was revealed in another study [31]. Moreover, TCGA data analysis implicated the high expression of CXCR1/3/4/5/6/7 in HNSCC compared to the healthy tissues. Although CXCRs have been confirmed to be highly expressed in various tumor tissues by previous research, this study is the first to present the expression of CXCR1 to CXCR7 in HNSCC. The methylation levels in promoter areas were further analyzed to explore the underlying mechanism of abnormal CXCRs expression in HNSCC. Genes are involved in apoptosis, cell proliferation, and cell cycle through gene silencing and reactivation, which can be caused by demethylation and methylation of cg sites in promoter regions. The regional context along with neighboring sites is concerned with methylation changes at individual cg site. According to Pearson’s correlation analysis, among the six differentially expressed CXCR members (CXCR1, CXCR2 to CXCR7), the expression level of CXCR4 is particularly affected by its methylation level, suggesting the possible crucial role of abnormal methylation in the aberrant expression of these genes. Yet, the abnormal expression of the CXCR gene family may also result from other epigenetic or genetic alterations like gene mutations and copy number changes.

No significant correlation of mRNA expression levels of CXCR1 and CXCR2 with OS and RFS was found in this study, while high expression of CXCR3/4/5/6...
turned out to be related to favorable survival outcomes in patients with HNSCC. Univariate and multivariate Cox analyses were carried out combined with parameters of age, stage, and the 7 identified hub genes, in the hope of establishing a relatively accurate prognostic model for patients with HNSCC. Our analysis data suggested the predictive potential of CXCR as four CXCR members (CXCR3, CXCR4, CXCR5, and CXCR6) were significantly related to better clinical outcomes in HNSCC, as reported in acute myeloid leukemia [32].

The underlying molecular mechanisms of abnormal expression of CXCRs regulating HNSCC carcinogenesis were further studied. The significant correlation among CXCRs and their vital role in the interaction with CXC motif chemokine 8/9/10/12/13 were clarified by Pearson’s correlation analysis and the PPI network. CXCRs expression was also concerned with some signaling pathways involved in cancer progression, as indicated by the GSEA, thus leading to the potential mechanism of CXCR carcinogenicity. The significant correlation between CXCR3 to CXCR6 and cytokine-cytokine receptor interaction as well as cell adhesion molecules (CAMs) was also worth noting, which indicated an interaction with CXC motif chemokine. The association between CXCR family genes and T1ICs was observed by the TIMER and CIBERSORT analyses, offering favorable proof for the connection between CXCRs expression and the immune microenvironment in HNSCC. Taken together, our data supported the regulatory function of CXCRs family members in the HNSCC immune microenvironment, which deserves further research.

It is already known that CXCR3 is primarily expressed on vascular cells, NK cells, tumor cells, and activated T lymphocytes and binds to CXCL9 to CXCL11 [10, 11]. CXCR3 has three different isoforms in humans, CXCR3A, CXCR3B, and CXCR3Alt, and its dual role in immune response and cancer has been reported. For example, in a previous study, CXCR3 was found to be the key mediator of tumorigenesis and the main cause of poor response and early recurrence in multiple cancers in multiple cancers [33]. Conversely, another study reported that CXCR3-CXCL9/10/11 signaling pathway contributed to the chemotactic movement of immune cells activated by CXCR3 to the tumor site, thereby promoting the antitumor immune response [34]. The data presented here verified that CXCR3 expression was positively associated with immune cell infiltration, suggesting the overexpression of CXCR3 could probably increase the infiltration of immune cells of all sorts into the tumor microenvironment.

The crystal structures of CXCR4 reveal the way chemokine receptors are regulated by various modulators binding to different or sometimes overlapping binding pockets. CXCR4 has been implicated in the onset of a great number of tumors, due to the reason that this receptor is considered to be crucial in immune cells chemotaxis, tumor cell proliferation, invasion, metastasis, and angiogenesis [35]. Widely expressed in physiological conditions, CXCR4 and its ligand CXCL12 are essential regulators of hematopoiesis, cardiogenesis, and neurogenesis [36-39]. In addition, there were some strong CXCR4 inhibitors which as adjuvant treatments for anticancer therapies against human cancer [40, 41].

As a specific receptor of CXCL13, CXCR5 mediates cancer functions regulated by CXCL13 [42]. According to Singh et al., the expression of CXCR5 was increased significantly only in squamous cell carcinoma (SCC) with lung node metastasis, but not in other subtypes of lung cancer [43]. Moreover, Ma et al. reported the contribution of CD4 + CXCR5 + T cells to antitumor immunity and its relation to better outcomes in non-small cell lung cancer [44].

CXCR6 expression was discerned in different hepatoma cell lines with various metastatic properties. Also, CXCR6 contributes to the microenvironment by inducing inflammatory cytokines, leading to the invasion and metastasis of hepatoma cells [45]. The high expression of CXCR6 in HNSCC was revealed by TCGA database analysis in this study, especially in HNSCC patients at the advanced stage or the elder. Furthermore, high expression of CXCR6 was proven to be connected to improve OS in HNSCC, and meanwhile, 5 CpGs of CXCR6 make a difference to favorable survival. CXCR6-CXCL16 axis also recruits immune cells to cancer sites just like the way of other CXCRs, thereby affecting the progression of cancer. However, the cell-specific functions of CXCR6 remain unclear to a large extent [46]. Currently, there were no known drugs that specifically target CXCR5 and CXCR6.

Overall, this study performed a comprehensive and thorough analysis of the expression and prognostic values of the CXCRs in HNSCC using publicly available TCGA databases. This study found that some of the CXCR family members could serve as clinical biomarkers of HNSCC. Our findings highlighted the vital function of CXCR3 to CXCR6 in HNSCC progression, as well as their clinical significance in HNSCC. Furthermore, we provided an insight into the role of CXCR3 to CXCR6 in mediating different signaling pathways in HNSCC. Our study also suggested that CXCR3-6 mediated inhibition of HNSCC progression may correlate with T-cell receptor signaling pathway, leukocyte trans-endothelial migration, and natural killer cell cytotoxicity. Nevertheless, the specific role of CXCR3 to CXCR6 in HNSCC requires future exploration.
Conclusions
In general, despite the promising results achieved, there are still some limitations in the present study. We performed all analyses and obtained the results on the sole basis of data from the TCGA, without verifying the expression levels of CXCRs protein and mRNA in HNSCC as well. Nevertheless, as suggested by our findings, targeting CXCR family members could be a promising therapeutic approach for treating patients with HNSCC.

Abbreviations
HNSCC: Head and neck squamous carcinoma; CXCRs: CXC chemokine receptor gene family members; GSEA: Gene set enrichment analysis; CXCL: CXC motif chemokine ligand; NK: Natural killer cells; TCGA: The Cancer Genome Atlas; HTseq: High-throughput sequence; FPKM: Fragments per kilobase of transcript per million mapped reads; GDC: Genomic Data Commons; PFS: Progression-free survival; OS: Overall survival; PPI: Pearson correlation and protein-protein interaction; FDR: False discovery rate; TIICs: Corrections between tumor immune-infiltrating cells; FC: Fold change; ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; Tregs: Regulatory T cells.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12957-022-02713-z.

Additional file 1: Supplementary Figure 1. The survival value of CXCR family genes in GSE41613 and GSE65858.

Additional file 2: Supplementary Table 1. Clinical characteristics of the patients from multiple institutions.

Acknowledgements
We thank Medina Abdulla for editing the manuscript.

Authors’ contributions
YMS, CCZ, and ZSS conceived and designed the study: YMS, CCZ, YJC, and QL performed the analyses. HXD, SSG, and YDW elaborated all figures and tables. YMS, CCZ, and ZSS wrote the main manuscript. The authors read and approved the final manuscript.

Funding
The Zhejiang Provincial Natural Science Foundation of China (LY19H160014; LQ21H130001), Ningbo “Technology Innovation 2025” Major Special Project (20202097, 2018B10015), and Medical and Health Research Project of Zhejiang Province (2019ZD018, 2021KY307)

Availability of data and materials
The gene expression data and clinical features used for bioinformatics analyses in this study are publicly available on the Cancer Genome Atlas (TCGA) program website (https://portal.gdc.cancer.gov). The authors are solely responsible for interpreting and reporting these data.

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Department of Otolaryngology, Head and Neck Surgery, The Affiliated Lihuili Hospital, Ningbo University, Ningbo, China. 2 Department of Otolaryngology, Head and Neck Surgery, Ningbo Medical Center Lihuili Hospital, Ningbo, China. 3 Medical School of Ningbo University, Ningbo 315000, China.

Received: 14 November 2021 Accepted: 22 July 2022
Published online: 17 August 2022

References
1. 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.
2. Peter F, Wittke J, Cinkensieper M, Kehnhofst M, Guntinas-Lichius O. Prognostic impact of pretherapeutic laboratory values in head and neck cancer patients. J Cancer Res Clin Oncol. 2013;139:171–8.
3. Lin SS, Massa ST, Varvaes MA. Improved overall survival and mortality in head and neck cancer with adjuvant concurrent chemoradiotherapy in national databases. Head Neck. 2016;38:208–15.
4. Sun M, Sun L, Sun D, Zhang C, Li M. Targeted delivery of immuno-RNase may improve cancer therapy. Cancer Cell Int. 2018;18:58.
5. Ben-Baruch A. The multifaceted roles of chemokines in malignancy. Cancer Metastasis Rev. 2006;25:357–71.
6. Ha H, Debnath B, Neamati N. Role of the CXCL8-CXCR1/2 axis in cancer and inflammatory diseases. Theranostics. 2017;7:1543–88.
7. Huang W, Chen Z, Zhang L, Tian D, Wang D, Fan D, et al. Interleukin-1β induces expression of FOXo1 to promote transactivation of CXCR1 and CCL2 in hepatocellular carcinoma cell lines and formation of metastases in mice. Gastroenterology. 2015;149:1053–1067.e1014.
8. Zhu Q, Han X, Peng J, Qin H, Wang Y. The role of CXC chemokines and their receptors in the progression and treatment of tumors. J Mol Histol. 2012;43:699–713.
9. El-Sherebny YM, Kelly RJ, Hill A, Doody GM, Hillmen P, Cook GP. Altered natural killer cell subset homeostasis and defective chemotactic responses in paroxysmal nocturnal hemoglobinuria. Blood. 2013;122:1887–90.
10. Lima M, Leander M, Santos M, Santos AH, Lau C, Queirós ML, et al. Chemokine receptor expression on normal blood CD56(+) NK-cells elucidates cell partners that coaggregate during the innate and adaptive immune responses and identifies a transitional NK-cell population. J Immunol. 2015;2015:839684.
11. Gallust F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med. 1998;187:875–83.
12. Chow MT, Ozga AJ, Servis RL, Frederick DT, Lo JA, Fisher DE, et al. Intratumoral activity of the CXCR3 chemokine system is required for the efficacy of anti-PD-1 therapy. Immunity. 2019;50:1498–1512.e1495.
13. Chatterjee S, Behnam Azad B, Nimmagadda S. The intricate role of CXCR4 in cancer. Adv Cancer Res. 2014;124:31–82.
14. Gagliardi F, Narayan An, Reni M, Franzin A, Mazza E, Boani N, et al. The role of CXCR4 in highly malignant human gliomas biology: current knowledge and future directions. Glia. 2014;62:1015–23.
15. Liu JF, Lin LC, Chao CC, Chang TM, Han CK, et al. CXCL13/CXCR5 interaction facilitates VCAM-1-dependent migration in human osteosarcoma. Int J Mol Sci. 2020;21(17):6095.
16. Han B, Shao-long E, Luan L, Li N, Liu X. CXCR4/PK3 promotes clear cell renal cell carcinoma (ccRCC) cells proliferation and metastasis via altering of miR-508-3p/CXCL13 signal. Onco Targets Ther. 2020;13:6051–62.
17. Deng L, Chen N, Li Y, Zheng H, Lei Q. CXCR6/CXCL16 functions as a regulator in metastasis and progression of cancer. Biochim Biophys Acta Cancer. 2010;1806:42–9.
18. Adleire C, Caspar B, Arimont M, Dekkers S, Visser K, Stuut J, et al. Modulators of CXCR4 and CXCR7/ACKR3 function. Mol Pharmacol. 2019;96:737–52.
19. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pol). 2015;19:66–77.
20. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally S, Ghosh D, et al. Oncomine: a cancer microarray database and integrated data-mining platform. Neoplasia. 2006;8:4–6.

21. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102:15545–50.

22. Mootha VK, Lindgren CM, Eriksson FF, Subramanian A, Sihag S, Lehar J, et al. PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet. 2003;34:267–73.

23. Li T, Fan J, Wang R, Traugh N, Chen Q, Liu JS, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77:e108–10.

24. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res. 2020;48:W509–14.

25. Peng CH, Liao CT, Peng SC, Chen YJ, Cheng AJ, Juang JL, et al. A novel molecular signature identified by systems genetics approach predicts prognosis in oral squamous cell carcinoma. PLoS One. 2011;6:e23452.

26. Estilo CL, O-charoenrat P, Talbot S, Socci ND, Carlson DL, Ghosein R, et al. Oral tongue cancer gene expression profiling: identification of novel potential prognosticators by oligonucleotide microarray analysis. BMC Cancer. 2009;9:11.

27. Del Barrio IDM, Wilkins GC, Meeson A, Ali S, Kirby JA. Breast cancer: an examination of the potential of ACKR3 to modify the response of CXCR4 to CXCL12. Int J Mol Sci. 2018;19(11):3592.

28. Nazari A, Khorramdolazad H, Hassanshahi G. Biological/pathological functions of the CXCL12/CXCR4/CXCR7 axes in the pathogenesis of bladder cancer. Int J Clin Oncol. 2017;22:991–1000.

29. Cancilla D, Rettag MP, DiPersio JF. Targeting CXCR4 in AML and ALL. Front Oncol. 2020;10:584766.

30. Ginos MA, Page GP, Michalowicz BS, Patel KJ, Volk E, Pambuccian SE, et al. Identification of a gene expression signature associated with recurrent disease in squamous cell carcinoma of the head and neck. Cancer Res. 2004;64:55–63.

31. Ye H, Yu T, Temam S, Ziober BL, Wang J, Schwartz JL, et al. Transcriptional dissection of tongue squamous cell carcinoma. BMC Genomics. 2008;9:69.

32. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77:e108–10.

33. Peng CH, Liao CT, Peng SC, Chen YJ, Cheng AJ, Juang JL, et al. A novel molecular signature identified by systems genetics approach predicts prognosis in oral squamous cell carcinoma. PLoS One. 2011;6:e23452.

34. Estilo CL, O-charoenrat P, Talbot S, Socci ND, Carlson DL, Ghosein R, et al. Oral tongue cancer gene expression profiling: identification of novel potential prognosticators by oligonucleotide microarray analysis. BMC Cancer. 2009;9:11.

35. Del Barrio IDM, Wilkins GC, Meeson A, Ali S, Kirby JA. Breast cancer: an examination of the potential of ACKR3 to modify the response of CXCR4 to CXCL12. Int J Mol Sci. 2018;19(11):3592.

36. Nazari A, Khorramdolazad H, Hassanshahi G. Biological/pathological functions of the CXCL12/CXCR4/CXCR7 axes in the pathogenesis of bladder cancer. Int J Clin Oncol. 2017;22:991–1000.

37. Cancilla D, Rettag MP, DiPersio JF. Targeting CXCR4 in AML and ALL. Front Oncol. 2020;10:584766.

38. Ginos MA, Page GP, Michalowicz BS, Patel KJ, Volk E, Pambuccian SE, et al. Identification of a gene expression signature associated with recurrent disease in squamous cell carcinoma of the head and neck. Cancer Res. 2004;64:55–63.

39. Ye H, Yu T, Temam S, Ziober BL, Wang J, Schwartz JL, et al. Transcriptional dissection of tongue squamous cell carcinoma. BMC Genomics. 2008;9:69.

40. Lu C, Zhu J, Chen X, Hu Y, Xie W, Yao J, et al. Risk stratification in acute myeloid leukemia using CXCR4 gene signatures: a bioinformatics analysis. Front Oncol. 2020;10:584766.

41. Li Y, Reader JC, Ma X, Kundi N, Kochel T, Fulton AM. Divergent roles of CXCR3 isoforms in promoting cancer stem-like cell survival and metastasis. Breast Cancer Res Treat. 2015;149:403–15.

42. Shahabuddin S, Ji R, Wang P, Brailoiu E, Dun N, Yang Y, et al. CXCR3 isoforms in promoting cancer stem-like cell survival and metastasis and poor patient outcomes in hepatocellular carcinoma. Cancer Res. 2012;72:3546–56.

43. Singh R, Gupta P, Kloecker GH, Singh S, Lillard JW Jr. Expression and clinical significance of CXCR5/CXCL13 in human non-small cell lung carcinoma. Int J Oncol. 2014;45:2232–40.

44. Ma QT, Huang DY, Zhang HJ, Chen J, Miller W, Chen XF. Function of follicular helper T cell is impaired and correlates with survival time in non-small cell lung cancer. Int Immunopharmacol. 2016;41:1–7.

45. Gao Q, Zhao YJ, Wang XY, Qiu SJ, Shi YH, Sun J, et al. CXCR6 upregulation contributes to a proinflammatory tumor microenvironment that drives metastasis and poor patient outcomes in hepatocellular carcinoma. Cancer Res. 2012;72:3546–56.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.