CCR5 as a prognostic biomarker correlated with immune infiltrates in head and neck squamous cell carcinoma by bioinformatic study

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Abstract

Background: C-C chemokine receptor 5 (CCR5) has recently been recognized as an underlying therapeutic target for various malignancies. However, the association of CCR5 with prognosis in the head and neck squamous cell carcinoma (HNSC) patients and tumor-infiltrating lymphocytes (TILs) is unclear.

Methods: In the current experiment, methods such as the Tumor Immune Estimation Resource Analysis (TIMER), Gene Expression Profiling Interactive Analysis (GEPIA), UALCAN, and Kaplan-Meier plotter Analysis were used to comprehensively evaluate the expression of CCR5 in human various malignancies and the clinical prognosis in HNSC patients. Subsequently, we used the TIMER database and the TISIDB platform to investigate the correlation between CCR5 expression levels and immune cell infiltration in the HNSC tumor microenvironment. Furthermore, immuno-modulatory and chemokine profiling were performed using the TISIDB platform to analyse the correlation between CCR5 expression levels and immunomodulation in HNSC patients.

Results: We found that CCR5 expression in HNSC tumor tissues was significantly upregulated than in normal tissues. In HNSC, patients with high CCR5 expression levels had worse overall survival (OS, HR = 0.59, p = 0.00015) and worse recurrence-free survival (RFS, HR = 3.27, p = 0.00098). Upregulation of CCR5 expression is closely associated with immunomodulators, chemokines, and infiltrating levels of CD4+ T cells, neutrophils, macrophages, and myeloid dendritic cells. Furthermore, upregulated CCR5 was significantly associated with different immune markers in the immune cell subsets of HNSC.

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Conclusions: High expression of CCR5 plays an important prognostic role in HNSC patients and may serve as a prognostic biomarker correlated with immune infiltration, and further studies are still needed to investigate therapeutic targeting HNSC patients in the future.

Keywords: Head and neck squamous cell carcinoma, C-C chemokine receptor 5, Tumor-infiltrating lymphocytes, Prognostic biomarker, Immune infiltration

Introduction
Head and neck squamous cell carcinoma (HNSC) are the sixth most common cancer in the world. It is highly aggressive and locally progressive, with lymph node metastases, and approximately 900,000 new patients are diagnosed each year [1–3]. It originates from the mucosal epithelium of the lips, mouth, nasal passages, sinuses, nasopharynx, mid-pharynx, larynx, and hypopharynx, and is mainly correlated with smoking, hard-drinking, and the human papillomavirus infection (HPV). Most patients with HNSCs are treated with surgery, standard cytotoxic chemotherapy, radiation therapy, and anticancer drugs, but the overall response remains insufficient, and local recurrence or distant metastasis is common [4, 5]. China is undergoing rapid social and economic changes that may affect the incidence of cancer, especially lifestyle-related cancers such as HNSC. Its incidence has been on the rise in recent years. Furthermore, patients with recurrent or metastatic HNSC (R/M) have a poor prognosis. Immunotherapy is currently an effective treatment option for a variety of malignancies, including HNSC patients with R/M disease [6]. However, in the absence of specific immune-related biomarkers, the five-year survival rates of HNSC patients remains low.

As a seven-transmembrane G protein-coupled receptor (GPCR), CC chemokine receptor 5 (CCR5) can regulate the transport and the effector functions of a variety of immune cells [7]. CCR5 promotes angiogenesis by promoting endothelial cell migration, proliferation, angiogenesis and VEGF secretion. In situ expression of the CCR5 can activate the Ca$^{2+}$ signaling pathway, which facilitates the differentiation and metastasis of the regulatory T cells (Tregs) to the part of inflammation, and is involved in stimulating tumor cell proliferation, infiltration, and migration through various mechanisms [8, 9]. Besides being a co-receptor of HIV, CCR5 is closely related to breast cancer, colon cancer, pancreatic cancer, glioblastoma multiforme, liver cancer and other cancers, and has been identified as a potential therapeutic target for various malignant tumors [10–14]. However, the functional role and immunomodulatory mechanisms of CCR5 in HNSC are unclear.

In this study, methods such as the TIMER database analysis, GEPIA platform analysis, UALCAN database analysis, and Kaplan-Meier plotter analysis were used to comprehensively evaluate the expression levels of CCR5 in HNSC patients and its correlation with prognosis. Subsequently, we researched the relevance of CCR5 expression levels and the immune infiltration in HNSC tumor microenvironment using the TIMER database and the TISIDB platform. Furthermore, immunomodulatory and chemokine profiling was performed using the TISIDB platform resources to analyse the relevance between CCR5 expression levels and immunomodulation for HNSC patients. Our findings suggested that the CCR5 is highly expressed in in HNSC patients, and possible links and mechanisms of CCR5 regulation in TILs.

Materials and methods
CCR5 expression analysis in various human malignancies
The expression levels of CCR5 in the various malignancies were analyzed using the TIMER 2.0 (http://timer.cistrome.org/), which is web server provides comprehensive analysis and visualization functions of tumor infiltrating immune cells [15]. Among them, 520 were HNSC patients and 44 were healthy controls in the TIMER 2.0 database. The expression data of CCR5 in the tumors without normal tissue samples in the Cancer Genome Atlas (TCGA) database and the reciprocal normal specimens of the Genotype-Tissue Expression database (GTEx) were acquired and analyzed using the GEPIA analysis (http://gepia2.cancer-pku.cn/#index), which is an enhanced web server for large-scale expression profiling and interactive analysis [16]. To further validate the expression levels of CCR5 in different cancer types, we performed the UALCAN database analysis (http://ualcan.path.uab.edu/), which is a web portal for facilitating tumor subgroup gene expression and survival analyses [17] on different tumor types and the reciprocal normal specimens.

Clinical-pathological parameters analysis
Kaplan-Meier plotter analysis (https://kmplot.com/analysis/) that is web-based survival analysis tool tailor for medical research was adopted to analyze the relevance between CCR5 expression levels and the survival situation in HNSC patients [18]. Specifically, the relevance between the expression levels of CCR5 and clinical prognostic significance in HNSC patients was explored using the UALCAN and Kaplan-Meier plots in
key clinicopathological parameters (e.g., patient’s gender, patient’s races, cancer stages, cancer grades, and lymph node stage). We employed the Kaplan-Meier plots to analyze the clinical prognostic significance of CCR5 in HNSC patients, including overall survival (OS) profiling and recurrence-free survival (RFS) profiling. Hazard ratios (HR) with 95% confidence intervals were estimated, along with log-rank p-values. p-values < 0.05 was defined as significant difference.

Immune cell infiltration analysis
To investigate the relevance between the expression levels of CCR5 and immune infiltration in HNSC patients, the immune cell infiltration profiling was explored using the TIMER and TISIDB platform resources (http://cis.hku.hk/TISIDB/index.php), which is an integrated repository portal for tumor–immune system interactions [19]. Specifically, we examined the relevance between CCR5 expression levels and TILs levels, including CD8+ T cells, B cells, monocytes, TAMs, M1 macrophages, M2 macrophages, neutrophils, and dendritic cells. According to the relevant control modules, the relevance between CCR5 expression levels with immunogenic markers of TILs has been further investigated, including immunological biomarkers of CD8+/CD4+ T cells, B cells, monocytes, natural killer cells, dendritic cells, TAMs, M1 macrophages, M2 macrophages, neutrophils, T cells, and other related cell subtypes.

Immunomodulators and chemokine analysis
To further explore the relevance between CCR5 expression levels and immune regulation in HNSC patients, immunomodulators and chemokine analysis was performed using the TISIDB platform. Specifically, we investigated the relevance of CCR5 expression with 45 immunostimulators, 24 immunoinhibitors, 41 chemokines, and 18 receptors in HNSC patients.

Statistical analysis
Kaplan-Meier plots were used to structure survival curves. For the Kaplan-Meier, GEPIA and TISIDB plots, HR and p-values were detected by log-rank test. Spearman correlation coefficients were analyzed to explore the relationship between CCR5 expression levels and immune cell infiltration, immunomodulators and chemokine levels. Relevance strength was assessed as follows: < 0.2 for a very weak relevance, < 0.4 for a weak relevance, < 0.6 for a moderate relevance, < 0.8 for a strong relevance, and < 1.0 for a very strong relevance. p-values < 0.05 was defined as significant difference.

Correlation between upregulated CCR5 and clinical pathology in HNSC patients
We further investigated the expression levels of CCR5 in several clinicopathological parameters using the TCGA HNSC dataset (Fig. 3). A study showed that the expression levels of CCR5 were significantly increased in the early-stage cancers than in the middle and late-stage cancers, indicating an underlying effect for CCR5 lies in the early detection of early-stage tumors. The expression levels of CCR5 in the patient’s race was significantly increased in Caucasians than in the other two races, suggesting that the expression levels of CCR5 is related to...
race. As a result, females had much higher CCR5 expression levels than males, and the expression levels of CCR5 in TP53 non-mutant types were also increased than that in TP53 mutant types. In particular, the expression levels of CCR5 were significantly increased at the lymph node stage than at all stages of tumor development. This suggests that CCR5 is existing in malignancies (Fig. 3).

Next, to further discover the underlying molecular mechanism of CCR5 in tumor development, the Kaplan-Meier plotter analysis was applied to dissect
the relevance CCR5 expression levels with clinical prognosis (Table 1). In particular, we found that the significant upregulation of CCR5 was correlated with worse OS and RFS in male (OS, HR = 0.5, \( p = 2.4 \times 10^{-5} \); RFS, HR = 3.58, \( p = 0.0304 \)). Furthermore, we observed that OS at stage 3 (HR = 0.32, \( p = 0.002 \)) and stage 4 (HR = 0.67, \( p = 0.0268 \)) correlated with CCR5 expression (Table 1). These findings manifested that the prognostic importance of CCR5 in HNSC patients depends on its clinical characteristics.

Correlation between upregulated CCR5 and prognosis in HNSC patients

Then, the clinical prognostic significance of CCR5 in HNSC patients was determined using the KM plotter. The results of KM plotter analysis showed that
the increased expression levels of CCR5 were dramatically correlated with a poorer prognosis in HNSC patients (OS, HR = 0.59, p = 0.00015; RFS, HR = 3.27, p = 0.00098), suggesting that CCR5 can serve as an underlying prognostic biomarker (Fig. 4).

Correlation between upregulated CCR5 and immune infiltration in HNSC patients

Immune infiltration around tumors has been shown to be closely related to clinical outcomes in tumor patients. Therefore, we tested the relevance between the expression levels of CCR5 and immune infiltration in HNSC patients by TISIDB and TIMER platforms, determined whether the expression levels of CCR5 interrelated with immune infiltration level, and found that the upregulated CCR5 was significantly negatively interrelated with tumor purity (rho = −0.279, P = 3.04e-10). Our data also found a strong relevance between the upregulated CCR5 and the abundance of TILs. For example, the upregulation of CCR5 in HNSC patients was positively associated with the infiltrating levels of CD4+ T cells (rho = 0.472), neutrophils (rho = 0.758), macrophages (rho = 0.227), and the myeloid dendritic cells (rho = 0.393). Besides, the upregulated CCR5 was inversely correlated with CD8+ T cells (rho = −0.092) and B cells (rho = −0.213) infiltration levels (Fig. 5). As a result, our data indicated that CCR5 plays a significant function in the immune infiltration for HNSC patients.

Considering the important role of CCR5 in regulating immune infiltration in HNSC, we examined the relevance of the upregulated CCR5 with immunogenic biomarkers of TILs in HNSC patients using the TIMER and GEPIA databases. The data showed that the upregulated CCR5 positively correlated with the most immunogenic biomarkers of TILs in HNSC patients, particularly in CD8+ T cells (CD8A and CD8B), T cells (CD3D, CD3E, and CD2), B cells (CD19 and CD79A) and Monocytes (CD86 and CD115) (Table 2).
Table 1  Correlation of CCR5 mRNA expression and clinical prognosis in head and neck squamous cell carcinoma with different clinicopathological factors by Kaplan-Meier plotter

| Clinicopathological characteristics | Overall survival (n = 7462) | Recurrence free survival (n = 4420) |
|------------------------------------|-----------------------------|------------------------------------|
|                                    | N  | Hazard ratio  | p       | N  | Hazard ratio  | p       |
| Gender                             |    |              |        |    |              |        |
| Female                             | 133| 0.65 (0.36–1.18) | 0.153  | 46 | 2.03 (0.71–5.85) | 0.1794  |
| Male                               | 366| 0.5 (0.36–0.69)  | 2.4e-5 | 78 | 3.04 (1.06–8.75) | 0.0304  |
| Stage                              |    |              |        |    |              |        |
| 1                                  | 25 | 4.28 (0.44–41.33) | 0.172  | 23 | 8.92 (1.54–51.79) | 0.0041  |
| 2                                  | 69 | 0.65 (0.29–1.46)  | 0.2945 | 43 | 5.77 (1.06–31.58) | 0.022   |
| 3                                  | 78 | 0.32 (0.15–0.68)  | 0.002  | 42 | 0.4 (0.11–1.46)  | 0.155   |
| 4                                  | 259| 0.67 (0.47–0.96)  | 0.0268 | /  | /              | /       |
| Race                               |    |              |        |    |              |        |
| white                              | 426| 0.62 (0.46–0.83)  | 0.0012 | 110| 3.97 (1.76–8.89) | 0.0004  |
| asian / / / / / / / /              | /  | /              | /      | /  | /              | /       |
| black/african america              | 47 | 0.51 (0.21–1.24)  | 0.131  | /  | /              | /       |
| Grade                              |    |              |        |    |              |        |
| 1                                  | 61 | 0.52 (0.19–1.44)  | 0.1991 | 26 | 6.65 (0.78–55.14) | 0.0471  |
| 2                                  | 298| 0.59 (0.42–0.84)  | 0.0027 | 69 | 2.37 (0.8–7.01)  | 0.1077  |
| 3                                  | 119| 0.59 (0.33–1.07)  | 0.0776 | 25 | 5.91 (0.91–27.57) | 0.0408  |
| 4                                  | /  | /              | /      | /  | /              | /       |
| Mutation burden                    |    |              |        |    |              |        |
| high                               | 251| 0.58 (0.4–0.83)  | 0.0025 | 50 | 5.58 (0.88–14.69) | 0.0592  |
| low                                | 234| 0.57 (0.35–0.93)  | 0.024  | 73 | 3.05 (1.17–7.94) | 0.016   |

Note. Bold values indicate p < 0.05

Fig. 4  Kaplan-Meier survival curves comparing the high and low expression of CCR5 in HNSC in Kaplan-Meier plotter databases. (A) Survival curves of overall survival (OS, HR = 0.59, p = 0.00015) in the HNSC cohort. (B) Survival curves of recurrence-free survival (RFS, HR = 3.27, p = 0.00098) in the HNSC cohort.
The upregulated CCR5 was significantly associated with most immunogenic biomarkers of monocytes, TAMs, M1 macrophages, M2 macrophages, and T cell exhaustion in HNSC patients (Table 2). In particular, the results indicated that the expression levels of CCR5 was dramatically interrelated with monocytes (CD86 and CSF1), TAMs (CCL2, CD68, and IL10), M1 macrophages (NOS2 and IRF5), M2 macrophages (CD163, VSIG4, and MS4A4A), and T cell exhaustion (PDCD1, PDCD1LG2, CTLA4, LAG3, HAVCR2, and GZMB) (Fig. 6). In addition, based on the GEPIA database, we further investigated the relationship between the expression levels of CCR5 and immunogenic biomarkers such as monocytes, TAMs, M1 macrophages, M2 macrophages, and T cell exhaustion, which was similar to the TIMER platforms (Table 3).

**Correlation between upregulated CCR5 and chemokines in HNSC patients**

This study demonstrated the correlation of the expression levels of CCR5 with chemokines in HNSC patients. In particular, we found that the upregulated CCR5 was positively interrelated with chemokines in HNSC patients ($p<2.2e^{-16}$), containing ADORA2A ($\rho=0.727$), BTLA ($\rho=0.837$), CD244 ($\rho=0.702$), CD274 ($\rho=0.542$), and CD96 ($\rho=0.901$). The increased expression levels of CCR5 were also dramatically interrelated with immunostimulators in HNSC patients ($p<2.2e^{-16}$), containing CD27 ($\rho=0.832$), CD28 ($\rho=0.839$), CD40 ($\rho=0.454$), CD40LG ($\rho=0.777$), and CD48 ($\rho=0.92$) (Fig. 7). These data shown that CCR5 is closely involved in the modulation of immune system interactions and may control tumor immune microenvironment (TME).

**Correlation between upregulated CCR5 and immunomodulators in HNSC patients**

Significantly, we found that the upregulated CCR5 was positively correlated with immunoinhibitors in HNSC patients ($p<2.2e^{-16}$), including ADORA2A ($\rho=0.727$), BTLA ($\rho=0.837$), CD244 ($\rho=0.702$), CD274 ($\rho=0.542$), and CD96 ($\rho=0.901$). The increased expression levels of CCR5 were also dramatically interrelated with immunostimulators in HNSC patients ($p<2.2e^{-16}$), containing CD27 ($\rho=0.832$), CD28 ($\rho=0.839$), CD40 ($\rho=0.454$), CD40LG ($\rho=0.777$), and CD48 ($\rho=0.92$) (Fig. 7). These data shown that CCR5 is closely involved in the modulation of immune system interactions and may control tumor immune microenvironment (TME).
| Description       | Gene markers | HNSC          |               | Purity          |               |
|-------------------|--------------|---------------|---------------|-----------------|---------------|
|                   |              | Cor          | p             | Cor             | p             |
| CD8+ T cell       | CD8A         | 0.896        | 9.19e-186***  | 0.887           | 1.58e-166***  |
|                   | CD8B         | 0.846        | 5.64e-144***  | 0.832           | 2e-127***     |
| T cell            | CD3D         | 0.87         | 1.71e-161***  | 0.859           | 2.3e-144***   |
|                   | CD3E         | 0.939        | 1.51e-242***  | 0.933           | 5.57e-220***  |
|                   | CD2          | 0.938        | 4.26e-242***  | 0.932           | 1.49e-217***  |
| B cell            | CD19         | 0.588        | 6.64e-50***   | 0.558           | 1.52e-41***   |
|                   | CD79A        | 0.582        | 1.26e-48***   | 0.553           | 8.95e-41***   |
| Monocyte          | CD86         | 0.772        | 2.16e-104***  | 0.749           | 1.37e-89***   |
| TAM               | CD115 (CSF1R)| 0.841        | 7.1e-141***   | 0.828           | 2.87e-125***  |
| TAM               | CCL2         | 0.587        | 1e-49***      | 0.553           | 1.01e-40***   |
| TAM               | CD68         | 0.473        | 1.85e-30***   | 0.457           | 9.69e-27***   |
| TAM               | IL6          | 0.618        | 2.29e-56***   | 0.58            | 1.28e-45***   |
| TAM               | INOS (NOS2)  | 0.314        | 1.99e-13***   | 0.341           | 7.91e-15***   |
| TAM               | IRF5         | 0.358        | 3.2e-17***    | 0.378           | 3.71e-18***   |
| TAM               | COX2 (PTGS2)| −0.147       | 7.7e-04***    | −0.132          | 3.4e-03***    |
| M2 Macrophage     | CD163        | 0.71         | 3.85e-81***   | 0.701           | 8.05e-74***   |
| M2 Macrophage     | VSIG4        | 0.642        | 4.64e-62***   | 0.638           | 1.43e-57***   |
| M2 Macrophage     | M544A        | 0.746        | 5.93e-94***   | 0.73            | 3.89e-83***   |
| Neutrophils       | CD66b (CEACAM8) | 0.075       | 8.81e-02      | 0.059           | 1.9e-01       |
| Neutrophils       | CD11b (ITGAM) | 0.633       | 9.83e-60***   | 0.614           | 2.08e-52***   |
| Neutrophils       | CCR7         | 0.749        | 3.41e-95***   | 0.726           | 1.37e-81***   |
| Natural killer cell| KIR2DL1      | 0.398        | 2.86e-21***   | 0.4             | 2.39e-20***   |
| Natural killer cell| KIR2DL3      | 0.555        | 1.38e-43***   | 0.549           | 4.7e-40***    |
| Natural killer cell| KIR2DL4      | 0.639        | 2.37e-61***   | 0.639           | 7.05e-56***   |
| Natural killer cell| KIR3DL1      | 0.509        | 9.34e-36***   | 0.508           | 1.16e-33***   |
| Natural killer cell| KIR3DL2      | 0.651        | 2.58e-64***   | 0.644           | 5.71e-55***   |
| Natural killer cell| KIR3DL3      | 0.261        | 1.52e-09***   | 0.253           | 1.33e-08***   |
| Natural killer cell| KIR2DS4      | 0.409        | 1.85e-22***   | 0.386           | 6.8e-19***    |
| Natural killer cell| HLA-DPB1     | 0.862        | 2.47e-155***  | 0.848           | 4.71e-137***  |
| Natural killer cell| HLA-DQB1     | 0.698        | 2.36e-77***   | 0.678           | 1.42e-67***   |
| Natural killer cell| HLA-DRA      | 0.858        | 1.69e-152***  | 0.844           | 2.04e-134***  |
| Natural killer cell| HLA-DPA1     | 0.864        | 7.54e-157***  | 0.851           | 5.61e-139***  |
| Natural killer cell| HLA-DPA2     | 0.054        | 7.3e-41***    | 0.489           | 6.57e-31***   |
| Natural killer cell| BDCA-1(CD1C)| 0.431        | 4.4e-25***    | 0.415           | 6.67e-22***   |
| Natural killer cell| BDCA-4(NRP1)| 0.696        | 6.05e-77***   | 0.674           | 2.02e-66***   |
| Th1               | T-bet (TBX21)| 0.896        | 5.71e-185***  | 0.888           | 2.99e-167***  |
| Th1               | STAT4        | 0.681        | 1.74e-72***   | 0.652           | 7.99e-61***   |
| Th1               | STAT1        | 0.623        | 1.97e-57***   | 0.605           | 1.95e-50***   |
| Th1               | IFN-γ (IFNG)| 0.759        | 4.45e-99***   | 0.743           | 2.28e-87***   |
| Th2               | TNF-α (TNF)  | 0.227        | 1.62e-07***   | 0.2             | 7.51e-06***   |
| Th2               | GATA3        | 0.472        | 2.81e-30***   | 0.431           | 1.19e-23***   |
| Th2               | STAT6        | 0.323        | 3.55e-14***   | 0.371           | 1.87e-17***   |
| Th2               | STAT5A       | 0.661        | 9.69e-67***   | 0.654           | 2.84e-61***   |
| Th2               | IL13         | 0.481        | 1.47e-31***   | 0.456           | 1.09e-26***   |
| Tfh               | BCL6         | 0.122        | 5.09e-03      | 0.185           | 3.61e-05***   |
| Tfh               | IL21         | 0.572        | 1.24e-46***   | 0.545           | 1.82e-39***   |
Table 2 (continued)

| Description       | Gene markers | HNSC None | Purity Cor | Purity Purity |
|-------------------|--------------|-----------|------------|--------------|
|                   |              | Cor       | p          | Cor          | p            |
| Th17              | STAT3        | 0.395     | 6.09e-21***| 0.399        | 2.85e-20***  |
|                   | IL17A        | 0.362     | 1.23e-17***| 0.337        | 1.5e-14***   |
| Treg              | FOXP3        | 0.853     | 9.12e-149***| 0.839        | 1.05e-131*** |
|                   | CCR8         | 0.752     | 4.31e-96*** | 0.728        | 1.89e-82***  |
|                   | STAT5B       | 0.431     | 5.72e-25*** | 0.43         | 1.43e-23***  |
|                   | TGFβ (TGFB1) | −0.012    | 7.89e-01   | −0.028       | 5.42e-01    |
| T cell exhaustion | PD-1 (PDCD1)| 0.895     | 4.3e-184*** | 0.887        | 9.24e-167*** |
|                   | PDL1 (PDCD1LG2) | 0.593 | 6.31e-51*** | 0.567        | 3.74e-43***  |
|                   | CTLA4        | 0.823     | 1.06e-129***| 0.808        | 1.07e-114*** |
|                   | LAG3         | 0.817     | 2.22e-126***| 0.807        | 2.88e-114*** |
|                   | TIM-3 (HAVCR2)| 0.879 | 2.91e-169***| 0.873        | 6.53e-155*** |
|                   | GZMB         | 0.776     | 3.73e-106***| 0.759        | 2.31e-93***  |

Note. HNSC Head and neck squamous cell carcinoma, TAM Tumor-associated macrophage, Th T helper cell, Tfh Follicular helper T cell, Treg Regulatory T cell, Cor R value of Spearman’s correlation, None Correlation without adjustment, Purity Correlation adjusted by purity. *p < 0.01; **p < 0.001; ***p < 0.0001

Fig. 6 CCR5 expression correlated with monocyte, macrophages, and T cell exhaustion in HNSC patients. Immunogenic biomarkers include CD86 and CSF1R of monocytes; CCL2, CD68, and IL10 of TAMs; NOS2, IRF5, and PTGS2 of M1 macrophages; and CD163, VSG4, and MS4A4A of M2 macrophages. Scatterplots of correlations between CCR5 expression and immunogenic biomarkers of monocytes (A), TAMs (B), and M1 (C), M2 macrophages (D) and T-cell exhaustion (E) in HNSC patients.
that the upregulated CCR5 was significantly interrelated with chemokine receptors in HNSC patients \( (p < 2.2 \times 10^{-16}) \), containing CCR1 \( (\rho = 0.84) \), CCR2 \( (\rho = 0.805) \), CCR4 \( (\rho = 0.691) \), CCR6 \( (\rho = 0.707) \), and CCR7 \( (\rho = 0.721) \) (Fig. 8). These findings further revealed that CCR5 may serve as an immunoregulatory element in HNSC patients.

**Discussion**

HNSC is a very heterogeneous group of tumors with a poor prognosis. Despite major progresses in the diagnostic scheme and therapeutic approach of HNSC, prognosis remains difficult. It is increasingly clear that the TME plays a crucial function in HNSC tumorigenesis by promoting aggressive tumor development and therapy resistance [20]. However, in the absence of specific immune-related biomarkers, the five-year survival rates of HNSC patients remains low. In the current research, the clinical significance and the expression levels of CCR5 in HNSC patients were systematically analyzed by a global bioinformatics study. Our data suggested that the poor prognosis of HNSC is associated with the upregulation of CCR5 expression. Moreover, our results demonstrated that the upregulated CCR5 is closely related to the infiltrating levels of various immune cells, immunoinhibitors, immunostimulators, chemokines and receptors in HNSC. Therefore, our study provides a brand-new perspective on the basic effect of CCR5, and it may serve as a prognostic biomarker in HNSC patients.

As a seven-transmembrane G protein-coupled receptor, CCR5 can bind to a variety of ligands CCL3 (MIP1α), CCL3L1, CCL4 (MP-1β), CCL5 (RANTES), CCL8 (MCP2), CCL11 (Eotaxin), CCL13 (MCP-4), and CCL16, which are reported to be highly expressed in most malignant tumors [9]. With the new focus on tumorigenesis and persistent atypical signaling, CCR5 is currently being used as an emerging therapeutic target for most metastatic cancer, with current clinical experiments in breast and colon cancers [21, 22]. However, the underlying molecular mechanisms of action of CCR5 in HNSC tumors is unclear. Therefore, we detected CCR5 mRNA expression levels in various malignancies tissue specimens and normal control tissue specimens using the TIMER and GEPIA databases, and validated them with the UALCAN database. We discovered that CCR5 was expressed in both tumor tissue specimens and normal tissue specimens in several cancer types. The expression levels of CCR5 were significantly up-regulated in HNSC tissue specimens compared to paracancerous tissue specimens. The results are consistent with those of the TCGA database and other studies [23, 24]. We also found that the CCR5 mRNA levels were upregulated in most HNSC

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**Table 3** Correlation analysis between CCR5 and related genes and markers of monocyte, macrophages, and T-cell exhaustion in Gene Expression Profiling Interaction Analysis (GEPIA)

| Description | Gene markers | HNSC | Tumor | Normal |
|-------------|--------------|------|-------|--------|
| Monocyte    | CD86         | 0.77 | 4.7e-101*** | 0.67    | 7.6e-07*** |
|             | CD115 (CSF1R)| 0.84 | 1.3e-136*** | 0.69    | 2.4e-07*** |
| TAM         | CCL2         | 0.58 | 4.9e-48***  | 0.12    | 0.45     |
|             | CD68         | 0.43 | 4.9e-25***  | 0.35    | 0.02     |
|             | IL10         | 0.63 | 2.8e-59***  | 0.56    | 7.9e-05*** |
| M1 Macrophage| INOS (NOS2)  | 0.31 | 6.3e-13***  | 0.38    | 0.011    |
|             | IRF5         | 0.33 | 1.5e-14***  | 0.29    | 0.052    |
|             | COX2 (PTGS2) | −0.14| 0.0017*    | 0.08    | 0.6      |
| M2 Macrophage| CD163        | 0.67 | 5.5e-70***  | 0.19    | 0.23     |
|             | VSG4         | 0.63 | 3.9e-59***  | 0.2     | 0.18     |
|             | M5A4A        | 0.74 | 1.7e-89***  | 0.22    | 0.15     |
| T cell exhaustion | PD-1 (PDCD1) | 0.9  | 7.1e-184*** | 0.76    | 2.2e-09*** |
|                         | PDL1 (PDCD1LG2)| 0.59 | 7.6e-50***  | 0.51    | 0.00038***|
|                         | CTLA4        | 0.83 | 1.8e-133*** | 0.64    | 2.9e-06***|
|                         | LAG3         | 0.79 | 8.7e-113*** | 0.67    | 5.4e-07***|
|                         | TIM-3 (HAVCR2)| 0.88 | 9.9e-167*** | 0.72    | 2.9e-08***|
|                         | GZMB         | 0.75 | 1.6e-94***  | 0.56    | 7.5e-05***|

Note. HNSC Head and neck squamous cell carcinoma, TAM Tumor-associated macrophages; Tumor correlation analysis in tumor tissue of TCGA; Normal correlation analysis in normal tissue of TCGA. *\( p < 0.01 \); **\( p < 0.001 \); ***\( p < 0.0001 \)
Fig. 7 The expression of CCR5 is associated with immunomodulators in HNSC patients. A Correlation between CCR5 expression and immunoinhibitors in HNSC cancer available at TISIDB database. B Correlation between CCR5 expression and immunostimulators in HNSC available at TISIDB database
tissue specimens compared with matched paracancerous tissue specimens. Significantly, we found that the expression levels of CCR5 were consistent with PD1, and the AUC was 0.638 (95% CI: 0.571–0.705) for CCR5 in HNSC patients. In addition, to notarized whether CCR5 can serve as a prognostic biomarker, we explored the prognostic significance of CCR5 in HNSC patients using the Kaplan-Meier plotter analysis. The results indicated that the upregulated CCR5 was dramatically interrelated with a worse prognosis in HNSC patients, suggesting that CCR5 can serve as a potential prognostic biomarker for HNSC.

As immunotherapies primarily target the TME, we investigated the influence of CCR5 on immune
cell infiltration of HNSC in the current research. Our results showed that the upregulated CCR5 in HNSC was positively interrelated with infiltrating levels of CD4+ T cells, neutrophils, macrophages, and myeloid dendritic cells. On the other hand, the upregulated CCR5 was associated with immunoinhibitors, immunostimulators, chemokines, and receptors. Furthermore, the present study also confirmed the correlation between the expression levels of CCR5 and the immunogenic markers of immune cells of HNSC. We clearly observed that the upregulated CCR5 was significantly positively interrelated with the immunogenic markers of monocytes (CD86 and CSF1), TAMs (CCL2, CD68, and IL10), M1 macrophages (NOS2 and IRF5), M2 macrophages (CD163, VSG4, and MS4A4A), and T cell exhaustions (PDCD1, PDCD1LG2, CTLA4, LAG3, HAVCR2, and GZMB). Furthermore, the findings demonstrated that the upregulated CCR5 activates Treg and B cells, induces T cell exhaustion, promotes Treg responses, and suppresses T cell-mediated immunity, thereby modulating T cell responses in HNSC patients. The upregulated CCR5 can promote the polarization of macrophages towards M1 and M2 phenotypes. Overall, CCR5 plays a vital function in the recruitment and modulation of TILs in HNSC patients, and the underlying molecular mechanisms of action and function of CCR5 in regulating the TME deserve further study. In addition, further studies are still needed to investigate therapeutic targeting HNSC patients in the future.

Conclusion

The upregulated CCR5 is strongly associated with worse prognosis and the infiltrating levels of various immune cells in HNSC patients. In addition, it helps to regulate the polarization of macrophages, and the exhaustion of T cells. Therefore, the present research demonstrates that CCR5 may serve as a prognostic biomarker, highlighting its underlying function in regulating TME in HNSC patients.

Abbreviations

CCR5: C-C chemokine receptor 5; HNSC: Head and neck squamous cell carcinoma; TILs: Tumor-infiltrating lymphocytes; TIMER: Tumor Immune Estimation Resource; GEPIA: Gene Expression Profiling Interactive Analysis; TCGA: The Cancer Genome Atlas; OS: Overall survival; RFS: Recurrence-free survival; HPV: Human papillomavirus; R/M: Recurrent or metastatic squamous cell carcinoma; GPCR: G protein-coupled receptor; Tregs: Regulatory T cells; GTEx: Genotype-Tissue Expression; BRCA: Breast invasive carcinoma; ESCA: Esophageal carcinoma; GMB: Glioblastoma; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; PAAD: Pancreatic cancer; STAD: Stomach adenocarcinoma; GBM: Glioblastoma; KIRP: Kidney renal papillary cell carcinoma; PAAD: Pancreatic cancer; STAD: Stomach adenocarcinoma; HNSCC: Head and neck squamous cell carcinoma; HNSC: Head and neck squamous cell carcinoma. 

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Code availability

None.

Authors’ contributions

Li CH, Ou ML, Hou XL and Tang JH conceived and designed the experiments. Li CH, Liu CY, Mo CE, Gong WW, Hu JH, He M, and Xie L performed the analysis of the data. Li CH, Chen SL, and Tang JH wrote the manuscript. Li CH, Ou ML, and Hou XL critically reviewed the manuscript. Ou ML, Tang JH and He M obtained the funding. All authors read and approved the final manuscript.

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Availability of data and materials

All the datasets were retrieved from the publishing literature, so it was confirmed that all written informed consent was obtained. And all of other materials are available by the corresponding authors.

Declarations

Competing of interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors are consent for the publication of this work.

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References

1. Marur S, Forastiere AA. Head and neck squamous cell carcinoma: update on epidemiology, diagnosis, and treatment. Mayo Clin Proc. 2016;91(3):386–96.
2. Johnson DE, Burtness B, Leemans CR, Lu VWY, Bauman JE. Grandis JR. Neoantigen immunotherapy for the treatment of squamous cell carcinoma of the head and neck (HNSCC). J Immunother Cancer. 2019;7(1):184.
3. Huang C, Chen L, Savage SR, Egeuz RV, Dou Y, Li Y, 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(3):209–49.
4. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, 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(3):209–49.
5. Cohen EEW, Bell RB, Bifulco CB, Burtness B, Gillison ML, Harrington KJ, 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(1):184.
6. Yokota T, Homma A, Kiyota N, Tahara M, Hanai N, Asakage T, et al. Immuno-
    notherapy for squamous cell carcinoma of the head and neck. Jpn J Clin
    Oncol. 2020;50(10):1089–96.
7. Oppermann M. Chemokine receptor CCR5: insights into structure, func-
    tion, Regulation. Cell Signal. 2004;16(11):1201–10.
8. Hemmatzad H, Berger MD. CCR5 is a potential therapeutic target for
    cancer. Expert Opin Ther Targets. 2021;25(4):311–27.
9. Jiao X, Nawab O, Patel T, Kosenkov AV, Halama N, Jaeger D, et al. Recent
    advances targeting CCR5 for cancer and its role in immune-oncology.
    Cancer Res. 2019;79(19):4801–7.
10. Véласко-Vélazquez M, Jiao X, De La Fuente M, Pestell TG, Ertel A, Lisanti
    MP, et al. CCR5 antagonist blocks metastasis of basal breast cancer cells.
    Cancer Res. 2012;72(15):3839–50.
11. Nishikawa G, Kawada K, Nakagawa J, Toda K, Ogawa R, Inamoto S, et al.
    Bone marrow-derived mesenchymal stem cells promote colorectal
    cancer progression via CCR5. Cell Death Dis. 2019;10(4):1–13.
12. Singh SK, Mishra MK, Eltouni I-EA, Bae S, Lillard JW, Singh RJ. CCR5/CCL5
    axis interaction promotes migratory and invasiveness of pancreatic
    cancer cells. Sci Rep. 2018;8(1):1–12.
13. Kranjc MK, Novak M, Pestell RG, Lah TJJR. Cytokine CCL5 and receptor
    CCR5 axis in glioblastoma multiforme. Radiol Oncol. 2019;53(4):397.
14. Singh SK, Mishra MK, Rivers BM, Gordesky JB, Bae S, Singh RJ. Biological
    and clinical significance of the CCR5/CCL5 axis in hepatocellular carcino-
    noma. Cancers (Basel). 2020;12(4):883.
15. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, et al. TIMGER: 0 for analysis of
    tumor-infiltrating immune cells. Nucleic Acids Res. 2020;48(W1):W509–14.
16. Tang Z, Li C, Kang B, Gao G, Li C, Zhang ZJ. GEPIA: a web server for cancer
    and normal gene expression profiling and interactive analyses. Nucleic
    Acids Res. 2017;45(W1):W98–W102.
17. Chandrashekar DS, Karikeyan SK, Korla PK, Patel H, Shovon AR, Athar M,
    et al. UALCAN: an update to the integrated cancer data analysis platform.
    Neoplasia. 2022;25:18–27.
18. Lándczyk A, Győrffy BJ. Web-based survival analysis tool tailored for medi-
    cal research (KMplot): Development and implementation. J Med Internet
    Res. 2021;23(7):e27633.
19. Ru B, Wong CN, Tong Y, Zhong JX, Zhong SS, Wu WC, et al. TISIDB: an
    integrated repository portal for tumor–immune system interactions.
    Bioinformatics. 2019;35(20):4200–2.
20. Elmusrati A, Wang J, Wang CY. Tumor microenvironment and immune
    evasion in head and neck squamous cell carcinoma. Int J Oral Sci.
    2021;13(1):1–11.
21. Haag GM, Halama N, Springfeld C, Grün B, Apostolidis L, Zschaebitz S,
    Dietrich M, Berger A-K, Weber TF, Zoenig I. Combined PD-1 inhibition
    (Pembrolizumab) and CCR5 inhibition (Maraviroc) for the treatment of
    refractory micrometastatic stable (MSS) metastatic colorectal cancer
    (mCRC); first results of the PICCASSO phase I trial. American Society of
    Clinical Oncology; 2020.
22. Cristofanilli M, Dolezal M, Lalezari J, Rui H, Patterson B, Tang C-M, Adams
    D, Zhang Q, Kazempour K, Pourhassan N. Abstract CT233: phase Ib/
    II study of leronlimab (PRO 140) combined with carboplatin in CCR5+
    mTNBC patients. AACR; 2020.
23. González-Arriagada WA, Lozano-Burgos C, Zúñiga-Moreta R, González-
    Díaz P, Coletta RD. Clinicopathological significance of chemokine
    receptor (CCR 1, CCR 3, CCR 4, CCR 5, CCR 7 and CXCR 4) expres-
    sion in head and neck squamous cell carcinomas. J Oral PatholMed.
    2018;47(8):755–63.
24. Liu Y, Qi W, Gong N, Zhu F, Xu R, Teng Z, et al. Identification of the
    immune-related genes in tumor microenvironment that associated with
    the recurrence of head and neck squamous cell carcinoma. Front Cell
    Dev Biol. 2021;2340.