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Comprehensive Analysis of CXCL6 Biological Significance in Head and Neck Squamous Cell Carcinoma

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Abstract: This study investigates the role of *CXCL6* in head and neck squamous carcinoma (HNSC) through comprehensive expression and methylation analyses, genetic mutation analysis, and prognostic assessment. Utilizing the UALCAN dataset, *CXCL6* expression analysis revealed a significant overexpression in HNSC cells compared to normal control samples, indicating its role in HNSC proliferation. Furthermore, an analysis of *CXCL6* expression across different clinical parameters showed substantial up-regulation in various cancer stages, racial groups, gender, and age groups, underscoring its fundamental role in cancer progression. Validation of *CXCL6* expression using the GEPIA2.0 online tool confirmed that *CXCL6* was highly expressed in HNSC development compared to control samples. An analysis of *CXCL6* expression across different stages of cancer revealed dysregulation in all four stages, with the highest expression in stage II and the lowest in stage III. This study also explored the promoter methylation levels of *CXCL6*, establishing a significant association between HNSC samples and normal controls. Examining promoter methylation across different clinical parameters revealed considerable variations, with distinct methylation patterns observed across cancer stages, racial groups, gender, and age. Overall survival (OS) and disease-free survival (DFS) analyses using the KM plotter tool demonstrated that high *CXCL6* expression was associated with poorer OS compared to low expression levels. Similarly, DFS analysis showed that patients with low *CXCL6* expression experienced better DFS outcomes compared to those with high *CXCL6* expression. Finally, mutational analysis using cBioPortal revealed no significant mutations in HNSC samples. These findings highlight the complex involvement of *CXCL6* in HNSC pathogenesis, underscoring its potential as a prognostic biomarker and therapeutic target in HNSC management.

Keywords: Head and neck squamous carcinoma; Diagnosis; Treatment; Biomarker

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1. Introduction

Head and neck cancers, with over 800,000 new cases annually, are among the most prevalent malignancies worldwide [1]. Head and neck malignancies rank as the seventh most common cancer globally, accounting for 3% of all tumors, with approximately 900,000 new cases and half a million deaths each year [2]. Head and neck squamous cell carcinoma (HNSC) is a group of tumors arising from the squamous epithelium of the oral cavity, oropharynx, larynx, and hypopharynx. Among all cancers in the head and neck region, including the oral cavity, oropharynx, hypopharynx, and larynx, squamous cell carcinoma accounts for around 90% [3,4]. The major risk factors for HNSC are tobacco use, heavy alcohol consumption, and human papillomavirus (HPV) infection [5-8]. Continuous exposure to tobacco and alcohol is known to increase the risk of HNSC [9]. In recent decades, there has been a significant decline in smoking-related HNSC in high-income countries due to reduced smoking rates [10,11]. However, the incidence of HPV-positive HNSC has markedly increased worldwide [12-18]. The primary treatment strategies for HNSC include surgery, radiotherapy, and chemotherapy. However, once distant metastasis is diagnosed, the median survival time is only 3.3–3.9 months, and the mortality rate remains high [19,20]. Cancer recurrence following standard therapy occurs in 15%–50% of cases [20]. Early diagnosis is a critical factor in achieving less invasive and more successful treatment, leading to improved patient outcomes [21]. Advances in bioinformatics and the identification of biomarkers have facilitated significant progress in diagnosing and treating cancers like HNSC [22,23]. In addition to HPV status, biomarkers for targeted treatment of HNSC remain underexplored [24].

CXCL6, also known as granulocyte chemotactic protein 2 (GCP-2), is a small cytokine belonging to the CXC chemokine family. It was first identified in the MG-63 osteosarcoma cell line [25]. Chemokines are chemotactic and inducible small-molecule peptides that play a crucial role in acute and chronic inflammation [26]. Among the chemokines, CXCL6 is a significant inflammatory cytokine that recruits inflammatory cells to inflammation sites by binding to CXCR1 and CXCR2 receptors [27]. In a cigarette-induced CXCL6-deficient mouse inflammation model, macrophage recruitment to the lungs was significantly reduced [28]. CXCL6 is not only highly expressed in inflammatory bowel disease but also significantly upregulated in patients with periodontitis. Immunohistochemistry has confirmed that CXCL6 is primarily distributed in gingival vascular endothelial cells [29]. Additionally, CXCL6 levels have been found to be elevated in the serum of osteosarcoma patients, and recombinant CXCL6 promotes the proliferation of osteosarcoma cells [30]. CXCL6 is widely recognized for promoting the development and metastasis of various tumors, including non-small cell lung cancer [31], colon cancer [30], and melanoma [32]. The angiogenesis-promoting effects of CXCL6 on tumor growth and metastasis have also been confirmed [33].

The present study aimed to investigate *CXCL6* mutations, expression levels, and their prognostic implications on survival, as well as functional aspects within the context of HNSC through bioinformatics analysis. Additionally, the relationship between *CXCL6* expression and promoter methylation levels was examined. Various databases, including The Cancer Genome Atlas (TCGA), UALCAN, Kaplan-Meier, GEPIA2.0, and cBioPortal, were used to achieve this. The primary objective of this study was to evaluate *CXCL6* expression patterns in HNSC and elucidate its potential significance in cancer treatment and prognosis.

2. Materials and methods

2.1. Kaplan-Meier plotter analysis

The Kaplan-Meier (KM) plotter is an essential tool for survival analysis [34]. This online portal includes extensive clinical data to assess the impact of specific genes on patient survival across various cancer types. The

KM plotter provides Kaplan-Meier survival curves, offering insights into how gene expression correlates with patient outcomes. In this study, the KM plotter tool was used to analyze the effect of *CXCL6* dysregulation on the overall survival (OS) and disease-free survival (DFS) of cancer patients.

2.2. UALCAN analysis

To elucidate the expression and methylation of *CXCL6* in HNSC, the UALCAN interactive online tool was used [35]. UALCAN allows users to identify biomarkers, perform in silico validation of potential genes of interest, evaluate epigenetic regulation via promoter methylation, and assess gene expression in cancer progression. In this study, *CXCL6* expression in normal and HNSC samples was analyzed by extracting data from the TCGA platform. Additionally, expression was examined across clinical parameters such as age, gender, and race using the UALCAN database.

2.3. GEPIA2.0 analysis

GEPIA2.0 offers features to process user-uploaded expression data and compare it with the large datasets from TCGA and GTEx [36]. The differences between CXCL6 expression and prognosis (OS and DFS) in HNSC patients were obtained from the GEPIA2.0 database. In this study, GEPIA2.0 was employed to analyze the association between *CXCL6* expression and patient prognosis in HNSC.

2.4. cBioPortal analysis

cBioPortal is a critical database for cancer genomics research [37]. It provides an intuitive platform to explore large-scale cancer genomic datasets, enabling researchers to investigate genetic alterations, pathways, and clinical relevance across different cancer types. With user-friendly visualization tools, it simplifies the analysis of complex genomic data, making it accessible to a broad range of researchers. In this study, cBioPortal was used to perform mutational analysis of *CXCL6* across various tumors.

3. Results

3.1. Expression analysis of CXCL6 in HNSC based on sample types

CXCL6 expression in HNSC and normal control samples was examined using the UALCAN dataset (**Figure 1**). The analysis revealed significant overexpression of *CXCL6* in HNSC cancer cells compared to normal control samples. This overexpression suggests a strong association between *CXCL6* expression and the proliferation of HNSC cancerous cells.

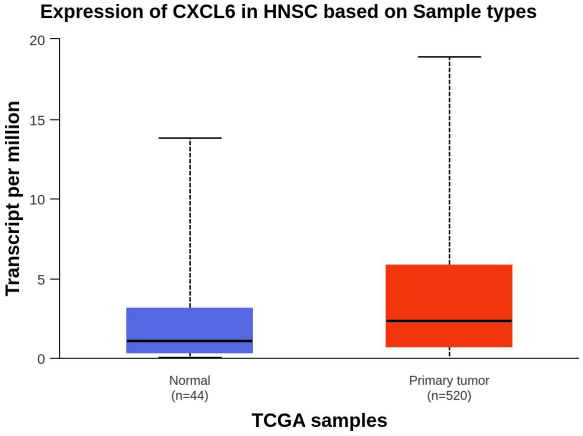

Figure 1. Expression profiling of *CXCL6* in HNSC and normal tissue samples

3.2. Expression analysis of CXCL6 in HNSC across different clinical parameters

CXCL6 expression in HNSC samples was evaluated across various clinical parameters, including cancer stages, race, gender, and age (**Figure 2**). Initially, *CXCL6*

expression was analyzed across different stages of cancer progression, revealing significant overexpression in all stages compared to normal controls (**Figure 2A**). *CXCL6* expression was then assessed in HNSC patients of different racial groups, showing significant overexpression in Caucasian and African American patients, while downregulation was observed in the Asian group (**Figure 2B**). Additionally, *CXCL6* expression was significantly upregulated in both male and female patients compared to normal controls (**Figure 2C**). Finally, *CXCL6* expression was examined across various age groups, revealing overexpression in all age categories among HNSC patients (**Figure 2D**).

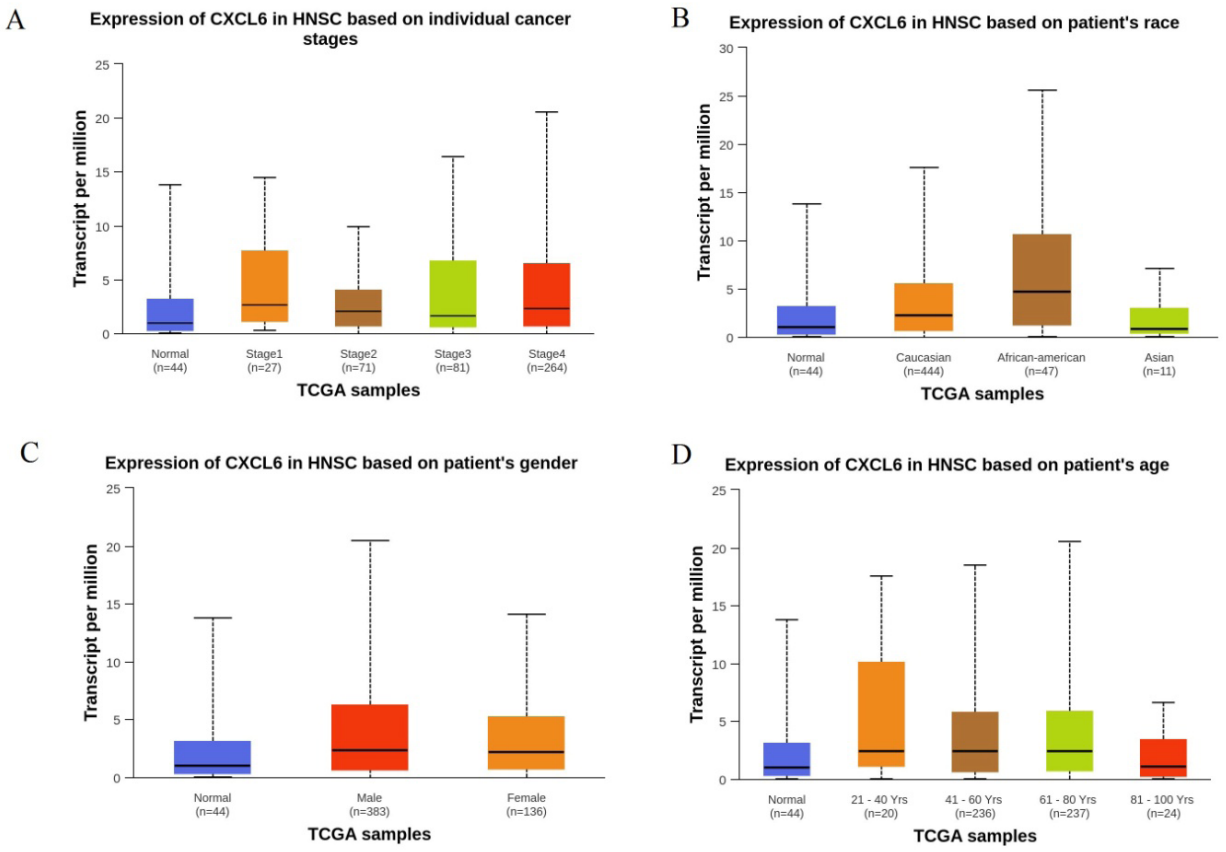

Figure 2. Expression of *CXCL6* across different clinical parameters

3.3. Validation of CXCL6 expression in HNSC

CXCL6 expression was validated using the GEPIA2.0 tool, which compared expression levels in HNSC and normal tissues. The results confirmed that *CXCL6* was highly expressed in HNSC compared to normal control samples (**Figure 3A**). The relationship between *CXCL6* expression and cancer stages was also analyzed, showing that *CXCL6* expression was significantly associated with different stages of HNSC. Notably, *CXCL6* had the highest expression in stage II and the lowest in stage III (**Figure 3B**).

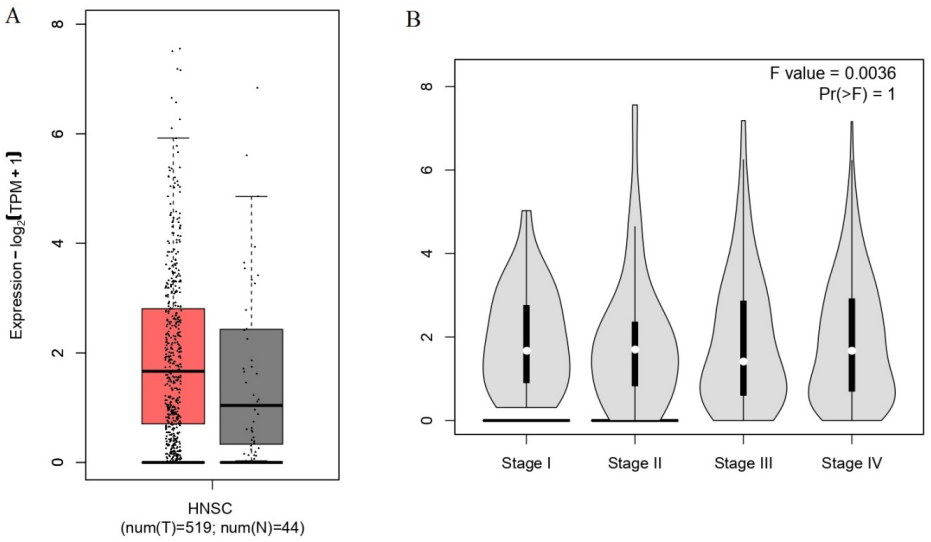

Figure 3. Validation of *CXCL6* expression across different stages of HNSC

3.4. Promoter methylation of CXCL6 in HNSC and normal tissues

The promoter methylation levels of *CXCL6* in HNSC and normal control samples were analyzed using the UALCAN dataset (**Figure 4**). The analysis revealed significant variation, particularly hypermethylation, in the promoter methylation levels of *CXCL6* in HNSC compared to normal controls. This suggests potential epigenetic dysregulation of *CXCL6*, highlighting its involvement in HNSC pathogenesis. These findings contribute to the understanding of the molecular mechanisms underlying HNSC progression and the potential role of *CXCL6* as a biomarker or therapeutic target.

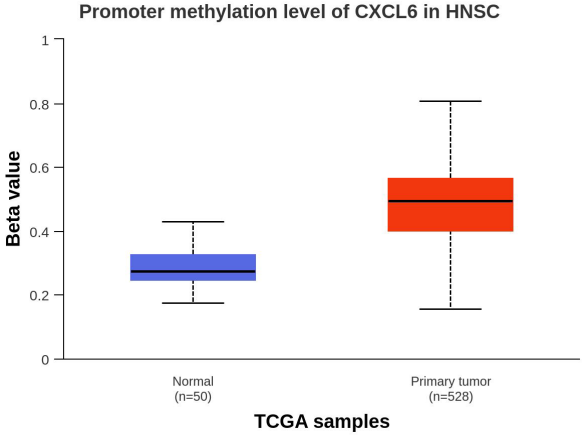

Figure 4. Promoter methylation pattern of *CXCL6* in HNSC and normal control samples

3.5. Promoter methylation of CXCL6 in HNSC across different clinical parameters

Further analysis of *CXCL6* promoter methylation was conducted across various clinical parameters (**Figure 5**). *CXCL6* promoter methylation was examined across different stages of HNSC, revealing significant hypermethylation in all stages compared to normal controls (**Figure 5A**). Additionally, *CXCL6* promoter methylation was assessed based on race, showing hypermethylation across all racial groups, including

Caucasian, African American, and Asian patients (**Figure 5B**). Methylation analysis by gender revealed significant hypermethylation in both male and female patients (**Figure 5C**). Finally, age-based analysis indicated varying levels of methylation across different age groups (**Figure 5D**). These findings highlight the complex relationship between *CXCL6* promoter methylation and clinical parameters in HNSC, providing insights into *CXCL6* expression regulation.

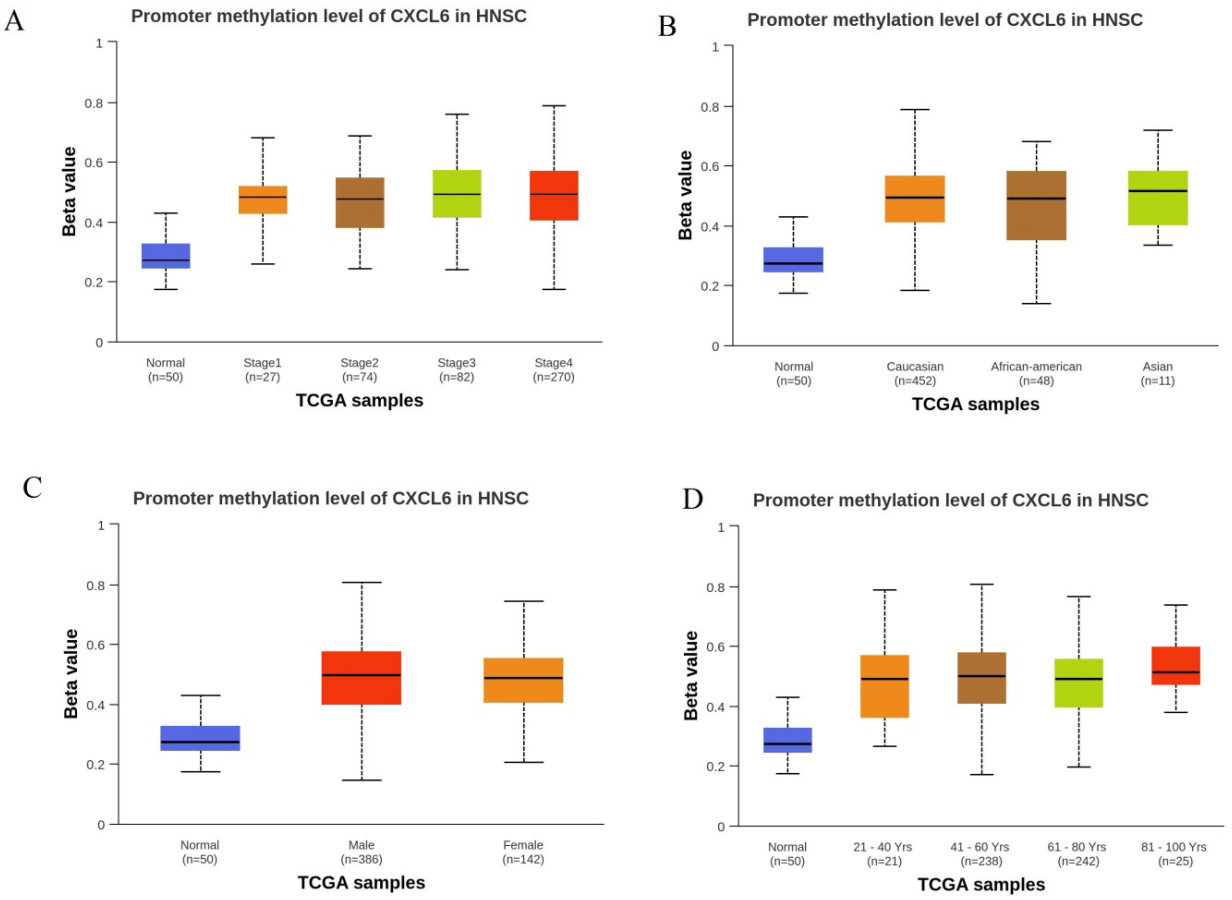

Figure 5. *CXCL6* promoter methylation pattern across different clinical parameters

3.6. Survival analysis of CXCL6

To assess the impact of *CXCL6* gene expression on survival outcomes in HNSC, OS and DFS were evaluated using the KM plotter platform. A significant association was observed between *CXCL6* expression and patient survival outcomes. Specifically, patients with high *CXCL6* expression experienced worse OS compared to those with low expression levels (**Figure 6A**). Furthermore, DFS analysis revealed that patients with low *CXCL6* expression had better DFS outcomes compared to those with high expression (**Figure 6B**). These findings emphasize the crucial role of *CXCL6* in influencing survival outcomes, underscoring its potential clinical significance as a prognostic marker in HNSC management.

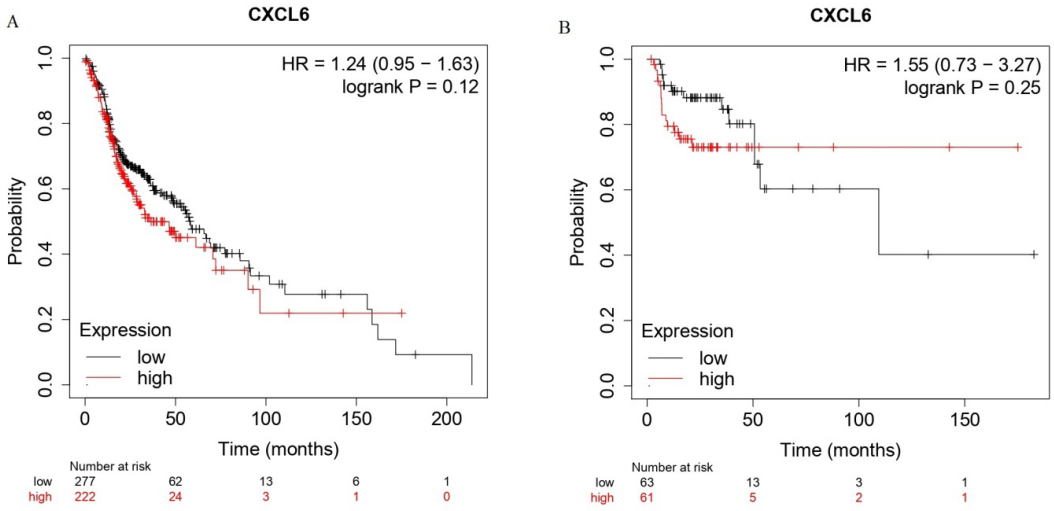

Figure 6. KM survival curve (OS and DFS) of *CXCL6* in HNSC patients

3.7. Validation of CXCL6 survival in HNSC

The GEPIA2.0 dataset was used to further evaluate the prognostic value of *CXCL6* expression in HNSC. Patients were categorized into low and high *CXCL6* expression groups. The analysis revealed that low *CXCL6* expression was associated with better OS compared to high expression (**Figure 7A**). Similarly, low *CXCL6* expression was linked to improved DFS in HNSC compared to high expression groups (**Figure 7B**).

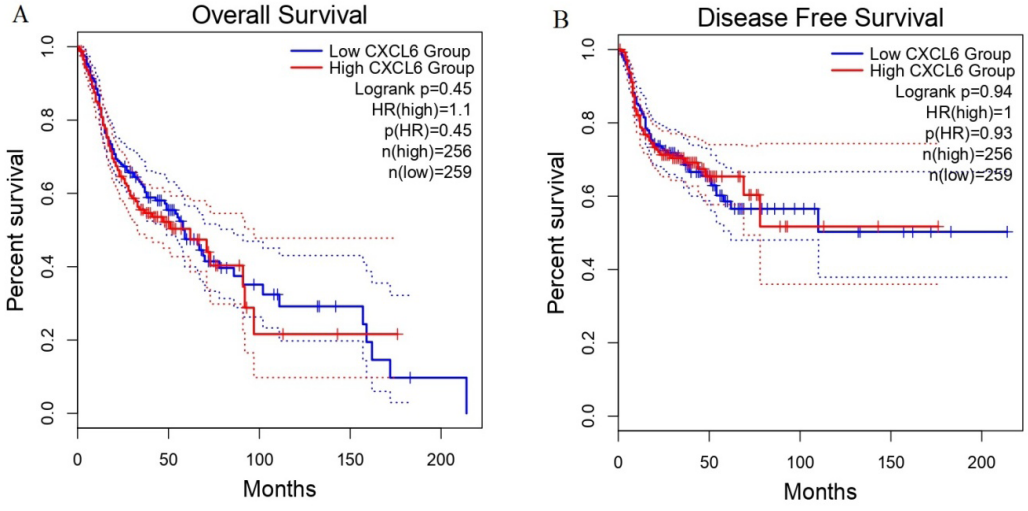

Figure 7. Survival curve (OS and DFS) of CXCL6 in HNSC patients

3.8. Mutational analysis of CXCL6 in HNSC

Mutational analysis of *CXCL6* in HNSC was conducted using the cBioPortal dataset. The analysis revealed no significant mutations in *CXCL6* within HNSC samples (**Figure 8**).

Figure 8. Oncoplot of *CXCL6* mutations in HNSC

4. Discussion

This study investigated *CXCL6* expression, prognosis, methylation, survival, and mutation, evaluating its role in HNSC using various bioinformatics tools. OS and DFS were used to validate the differentially expressed features of HNSC. The results suggest that *CXCL6* expression plays a significant role in human health and is likely associated with the proliferation of HNSC, proposing *CXCL6* as a potential regulator in HNSC pathogenesis.

Head and neck cancers are heterogeneous, involving the oral cavity, oropharynx, hypopharynx, larynx, and other subregions [4]. Approximately 30%–40% of HNSC patients are diagnosed with early-stage disease (stage I/II) and are typically treated with surgery or radiotherapy (RT) alone [38]. Over 60% of HNSC patients are diagnosed with locally advanced (LA) disease [39]. Previous studies have confirmed that concurrent chemoradiotherapy (CCRT) increases survival in LA-HNSCC patients, with a 6.5% increase in 5-year survival and significantly reduced local failure rates [40,41]. High-dose platinum combined with RT is the accepted standard treatment regimen for improving OS in LA-HNSCC patients [42]. Induction chemotherapy (IC) has been reported to reduce the risk of distant metastases [43] and has also been proposed to enhance OS in LA-HNSCC patients [44]. IC may be considered a screening strategy, with additional treatment options, such as RT or CCRT, depending on the patient's response to IC [45].

CXCL6, a member of the chemokine family, plays a crucial role in experimental bleomycin-induced pulmonary fibrosis [46]. Chemokines are secreted proteins involved in numerous physiological and pathological processes, including immunity, homeostasis, and oncogenesis [47]. The pro-angiogenic ELR+CXCL family, which includes CXCL1, 2, 3, 5, 6, 7, and 8, has been shown to promote cancer progression [48,49]. High glucose concentrations, a risk factor in diabetes, are known to stimulate the expansion, differentiation, and survival of certain cell types [50]. CXCL6 and CXCR1 expression are significantly induced by high glucose in a dose-dependent manner, suggesting that CXCL6 may play a key role in high glucose-induced renal fibrosis. CXCL6 can be secreted by various tumor cells, including osteosarcoma cells, and plays a pivotal role in cancer development [51-53]. Like other ELR+CXCLs, CXCL6 has been confirmed to have angiogenic properties in cancers [54]. Plasma levels of CXCL6 were found to be elevated in osteosarcoma patients, correlating with poor outcomes [30]. Previous studies have shown that increased CXCL6 secretion enhances the metastatic potential of colon cancer [33], while inhibition of CXCL6 expression restricts the migration and invasion of hepatocellular carcinoma cells [55]. Other studies have highlighted the significant role of the CXCL16/CXCR6 chemokine axis in the metastasis of prostate, liver, and ovarian cancers [56–58]. These findings demonstrate CXCL6's involvement in cancer cell metastasis. Based on these results, inhibition of endogenous CXCL6 significantly reduces the migration and invasion of osteosarcoma cells, while the addition of exogenous recombinant human CXCL6 (rhCXCL6) produces the opposite effect, suggesting CXCL6's role in metastasis.

In this study, the UALCAN dataset was used to analyze *CXCL6* expression in HNSC. The analysis showed

upregulation of *CXCL6* in various stages, cancer types, age groups, gender, and racial groups. The findings also revealed that *CXCL6* expression levels were significantly higher in HNSC tissues compared to normal control samples. Moreover, survival analysis using the KM plotter revealed that HNSC patients with high *CXCL6* expression experienced shorter OS and worse DFS compared to those with lower expression levels. The study indicated that *CXCL6* expression in tissue serves as a poor prognostic factor. Further research is required to investigate the prognostic value of *CXCL6* in cancer progression.

5. Conclusion

Compared to adjacent normal tissues, *CXCL6* expression levels were elevated in HNSC tissues. Higher *CXCL6* expression levels were associated with poor overall survival, disease-free survival, and clinical features, including promoter methylation levels and genetic alterations. It is speculated that *CXCL6* promotes cancer development through its involvement in the cell cycle. Furthermore, *CXCL6* may play a therapeutic role in HNSC-related resistance. Therefore, *CXCL6* may serve as a potential biomarker for early HNSC diagnosis and prognostic prediction.

Disclosure statement

The authors declare no conflict of interest.

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