CXCR4 and PD-1 Expression in Head and Neck Cancer with Perineural Spread

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Abstract

Keywords

- CXCR4
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- adenoid cystic carcinoma
- squamous cell carcinoma

Background Perineural spread (PNS) is a marker of aggressiveness and has been shown to occur in cranial nerves due to advanced mucosal and cutaneous head and neck cancer. Receptors CXCR4 chemokine receptor 4 (CXCR4) and programmed cell death-1 (PD-1) have been shown to be overexpressed in a variety of cancers with PNS, with the inhibition of these pathways offering a potential future treatment.

Methods Retrospective immunohistochemical staining for the CXCR4 and PD-1 receptors was performed on 28 head and neck specimens that demonstrated PNS from January 2017 to August 2017, at Royal Brisbane and Women's Hospital, Brisbane, Australia.

Results CXCR4 staining was positive in 52 and 60% of the squamous cell carcinoma (SCC) and adenoid cystic carcinoma PNS specimens, respectively. Cutaneous SCC tumors with no PNS stained positively in 33%. No significant staining for PD-1 in peritumoral lymphocytes or tumor specimens was seen.

Conclusion CXCR4 is overexpressed in advanced skin cancer and head and neck tumors that demonstrated PNS to large cranial nerves. Overall, these results provide strong support for using CXCR4 as a biomarker and further investigation of immunotherapeutic agents that could inhibit tumor progression via targeting CXCR4 expression.

Introduction

Queensland, Australia, has the highest incidence rates of cutaneous head and neck cancer in the world.¹ A small proportion of advanced cutaneous head and neck cancer cases are associated with clinical perineural spread (PNS) of cranial nerves, most commonly branches of the trigeminal and/or facial nerves which can spread proximally toward the

skull base.² Historically, these patients demonstrate higher rates of locoregional failure, distant metastases, and lower overall survival.^{3,4} In addition to cutaneous head and neck cancer, adenoid cystic carcinoma (ACC) typically demonstrates PNS, which makes surgical resection difficult and increases the risk of recurrence if resection is not taken to a clear pathological margin.⁵ Distant metastases rates for head and neck ACC have been estimated between 22 and 55%.^{6,7}

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CXC chemokine receptor 4 (CXCR4), a transmembrane G-protein-couple receptor, and its ligand C-X-C motif chemokine 12 (CXCL12), have been shown to play a central role in the neurotropism of cells.⁸ Overexpression in CXCR4 in tumor cells of head and neck cancer patients has been associated with higher rates of lymph node metastases, distant metastases, and a significantly worse 5-year survival rate.^{9–11} In particular, CXCR4 was highly expressed in ACC tumor cells, likely reflecting its inherent tumor cell neurotropism.⁵

The use of CXCR4 as a biomarker to identify head and neck cancer patients with tumors that have high metastatic potential has been the focus of contemporary research.^{12,13} However, CXCR4 expression in head and neck clinical PNS tumor cells has not been specifically investigated before.

Another potential biomarker or immunotherapy target is the cell surface receptor programmed cell death-1 (PD-1), and its ligand programmed death-ligand 1 (PD-L1). The PD-1/PD-L1 axis plays an integral role in the inhibition of T cell-mediated immune response to tumor cells.¹⁴ Overexpression of PD-L1 has been associated with poor clinical outcomes in many different types of cancer. Clinical trials in patients with melanoma, renal cell carcinoma, nonsmall-cell lung cancer, and bladder cancer have demonstrated improved outcomes with the use of monoclonal antibodies that target PD-1 or PD-L1 to stimulate T cell function.¹⁵

The aim of this study was to investigate whether CXCR4 and PD-1 overexpression occurs in the tumor cells of head and neck cancer patients that demonstrated PNS of cranial nerves.

Methods

Retrospective immunohistochemical staining for CXCR4 was performed on 28 head and neck cancer specimens that demonstrated PNS and were collected from January 2017 to August 2017, at Royal Brisbane and Women's Hospital (RBWH), Brisbane, Australia. PD-1 staining was performed on nine cases only. Nine cutaneous squamous cell carcinoma (SCC) with no PNS were used as negative controls. Ethics approval was provided by the RBWH Human Research Ethics Committee.

Formalin fixed paraffin-embedded tissue blocks were used to prepare 3 µm sections on coated slides. Immunostaining was performed with the BOND III, Leica Microsystems. Anti-CXCR4 antibody (UMB2 rabbit monoclonal, lot GR262216–27, ABCAM) dilution of 1:200 was used with BOND dilution buffer. The primary antibody was applied for 15 minutes at room temperature. BOND Epitope Retrieval Solution 1 was used as buffer with pH 5.9 to 6.1, citrate, and incubated the sections for 20 minutes in 100°C. PD-1 antibody (NAT105 mouse monoclonal, lot 1511215P, Cell Marque) dilution of 1:400 was used with BOND dilution buffer. The primary antibody was applied for 15 minutes at room temperature. BOND Epitope Retrieval Solution 2 was used as a buffer with pH 8.9 to 9.1, ethylenediaminetetraacetic acid, and incubated for 20 minutes at 100°C. Sections were exposed to BOND polymer refine detection DS9800 for 16 minutes at room temperature. DAB Leica microsystem DS9800 with 10-minute incubation time at room temperature and 1 minute at room temperature exposure to CuSO₄.

No counterstaining was performed. For negative control, the primary antibody was excluded. Positive control was obtained with human splenic tissue. Stromal tissue and normal epithelium served as internal positive control.

Staining was analyzed by two pathologists blinded to patients' details using a double-headed microscope with a consensus decision made in all cases. Staining analysis was based on a prevalidated H-score method. Staining intensity of 0, 1+, 2+, and 3+ was recorded for absent of staining, weak, intermediate, and strong staining, respectively. The following formula was used to calculate the score:

$[1 \times (\% \text{ cells } 1+) + 2 \times (\% \text{ cells } 2+) + 3 \times (\% \text{ cells } 3+)]$. H-score cutoff (greater than or equal to 150) allowed optimal discrimination (94% sensitivity and 98% specificity) and any H-score above 149 was viewed as positive.

Results

CXCR4 staining was positive in 52% (11/21) of the SCC PNS specimens (►Table 1). Histopathologically, these specimens demonstrated a dominant membranous and cytoplasmic staining pattern of CXCR4 (►Fig. 1).

ACC with PNS stained positively for CXCR4 in 60% of cases (4/6) and demonstrated a diffuse nuclear pattern of staining (►Fig. 2).

In a control group of SCC with no PNS, 33% (3/9) stained positive for CXCR4.

In contrast, no significant staining for PD-1 in peritumoral lymphocytes or tumor specimens was seen in the first nine cases so no further specimens were stained (0/9; ►Fig. 3).

Discussion

The current hypothesis is that CXCR4 expression correlates with metastasis, poor prognosis, and lower overall survival in multiple cancers.¹⁶ Supporting this is the evidence that activation of CXCR4 stimulates the production of matrix metalloproteases, which may facilitate tumor cell migration from the primary tumor to distant sites.¹⁷ In addition, CXCL12 signaling has been shown to upregulate integrin activity, which can facilitate tumor cell adhesion under flow conditions.¹⁸

Table 1 Specimen characteristics and immunohistochemistry results

Age (y)	Gender	Histo	CXCR4 staining	PD-1 staining
Median: 64	M: 24 (86%)	SCC: 21 (75%) BCC: 1 (4%)	SCC: 11 (52%)	SCC: 0 (0%) BCC: 0 (0%)
Range: 43–86	F: 4 (14%)	ACC: 6 (21%)	BCC: 0 (0%) ACC: 4 (67%)	ACC: 0 (0%)

Abbreviations: ACC, adenoid cystic carcinoma; BCC, basal cell carcinoma; CXCR4, CXC chemokine receptor 4; PD-1, programmed cell death-1; SCC, squamous cell carcinoma.

Fig. 1 Sections of a nerve infiltrated by squamous cell carcinoma, CXC chemokine receptor 4 (CXCR4) staining score 0 (A), score 1+ (B), and score 2+ (C). Full arrow pointing to tumor cells negatively stained. Hashed arrow pointing to perineural cells positively stained.

The results from this study indicate that CXCR4 may be overexpressed in advanced cutaneous and head and neck squamous cell carcinoma lesions that demonstrated PNS along large cranial nerves. This sort of spread is distinct from small nerve perineural infiltration which is a more common pathological entity and does not have the same increase in poor outcomes.^{19,20} Interestingly, the ACC tumors, which are inherently histologically aggressive, also showed a strong nuclear pattern of staining that was distinctly different to the SCC tumors. It could be postulated that this nuclear signal could be related to increased intracellular trafficking of CXCR4 and subsequent distant metastases often seen in ACC. Nuclear staining of CXCR4 has been found to be a significant predictor for survival in colorectal cancer patients, and strong nuclear localization was linked to poor prognosis.²¹

Fig. 2 Sections of a nerve infiltrated by adenoid cystic carcinoma, CXC chemokine receptor 4 (CXCR4) positive staining (score 2+), with inset $\times 400$ image. Full arrow pointing to the diffuse nuclear staining characteristic of adenoid cystic carcinoma (ACC).

PD-1 overexpression was not seen in this group of specimens. These results are in contrast to those of Linedale et al who demonstrated overexpression of PD-1 in T cell tumor infiltrates in a subset of patients with PNS of SCC.²² This difference may be potentially explained by the use of T cells extracted from freshly excised perineural tumors for flow cytometry, in comparison to immunohistochemistry with formalin-fixed specimens used in this study. Tumor cell expression of PD-1/PD-L1, in cutaneous SCC has been previously shown to be correlated with poor patient outcomes.²³

We acknowledge that this study does not have a large sample size to sufficiently allow for statistical analysis on other pathological parameters. However, this is the first study to report on the expression of CXCR-4 in head and neck cancer with PNS and also the first to exhibit evidence of overexpression of CXCR-4 in human nerves pathologically affected by ACC. This supplements findings in other studies that associate CXCR4 expression with histological patterns and metastatic potential in ACC.²⁴ Overall, these results provide strong support for further studies into the use of CXCR4 as a biomarker to predict survival and prognosis for head and neck patients with PNS along cranial nerves. Furthermore, these results also raise the question of whether immunotherapeutic agents targeting CXCR4 expression could be investigated with the aim of inhibiting tumor progression for patients with PNS in advanced head and neck cancer.

Fig. 3 Sections of a nerve infiltrated with squamous cell carcinoma (SCC) with peritumoral CD8 T cells (A, full arrow) present but negatively stained for programmed cell death-1 (PD-1) (B). A SCC-infiltrated lymph node demonstrates strong PD-1 staining (C, hashed arrow).

The CXCR4 antagonist, plerixafor (AMD3100), is Food and Drug Administration-approved for the mobilization of hematopoietic stem cells for autologous stem cell collection in lymphoma patients.^{25,26} The use of plerixafor in vitro and in a perineural invasion ACC mouse model, demonstrated inhibition of invasion.⁵ In head and neck SCC cells with overexpression of CXCR4, the use of a small interfering ribonucleic acid inhibitor of CXCR4 was found to decrease cell migration and proliferation while promoting cell cycle arrest and apoptosis.^{27,28} Targeted therapy with a CXCR4 antagonist to reduce PNS in a perioperative setting may have the potential to improve surgical resection results and overall patient outcomes.

Conclusion

Our study showed that over half of head and neck squamous cell carcinomas and ACCs that exhibited large nerve PNS also strongly expressed CXCR-4 receptors. Conversely, PD-1 expression was not evident in our specimens. Further testing on a larger cohort of patients is indicated, but if the results of this study are ratified, then the use of CXCR4 via targeted inhibitors in large nerve PNS in head and neck cancers via a multicenter clinical trial may be worth considering.

Conflict of Interest

None.

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