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Julius Vahl, Stephanie Weissinger, Claudia Welke, Thomas Hoffmann, Johannes Döscher

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Chemokine Receptor Expression in Squamous Cell - and Adeno-Subtypes of Sinonasal Carcinoma

Original Article

Julius Malte Vahl[1], Stephanie Ellen Weissinger[2], Claudia Welke[3], Thomas Karl Hoffmann[1], Johannes Doescher[1,4]

Department of [1]Otorhinolaryngology and Head & Neck Surgery, [2]Pathology, [3]Clinical Cancer Registry, Comprehensive Cancer Center Ulm, University Medical Center Ulm, [4]Department of Otorhinolaryngology, University Hospital Augsburg, Germany.

ABSTRACT

Background: Chemokines are involved in the chemotaxis of immune cells, angiogenesis, and cancer progression. For several tumor entities, it has been shown that the expression of chemokine receptors CCR7 and CXCR5 is of prognostic relevance. However, their role in the development of sinonasal cancer is currently not understood.

Patients and Methods: This work aimed to examine the relevance of chemokine receptor expression of CXCR5 and CXCR7 in sinonasal squamous cell carcinoma and adenocarcinoma and investigate a possible prognostic role. Tissue sections of 56 patients suffering from sinonasal cancers (squamous cell carcinoma and adenocarcinoma n = 41/15) were immunohistochemically stained for CCR7 and CXCR5. Afterwards, slides were evaluated by an immune scoring model.

Results: CCR7 expression was more abundant in squamous cell carcinoma than in adenocarcinoma (5.43 points vs. 2.38 points; $p < 0.001$). There was no significant correlation between CCR7 or CXCR5 expression and age, histological type, grading, TNM status, UICC classification, recurrence, and event of distant metastasis. We neither detected any effect of CCR7 nor CXCR5 on survival.

Conclusion: CXCR5 and CCR7 may play an important role in the tumor microenvironment signaling in various tumors including those of the head and neck region and are mostly associated with an impaired prognosis. However, this does not seem to be the case in the investigated subtypes of sinonasal cancer.

Key Words: cc chemokine receptor 7, cxc chemokine receptor 5, head and neck cancer, mean survival time, metastases.

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Corresponding Author: Vahl Julius, Department of Otorhinolaryngology and Head & Neck Surgery, University Medical Center Ulm, 89077 Ulm, Germany. **Tel.:** +4973150059570, **E-mail**: julius.vahl@uniklinik-ulm.de

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INTRODUCTION

Sinonasal cancers (SNC) are heterogenous and account for approximately 5 % of head and neck cancers, with an incidence of 1/100.000[1]. The majority of malignancies consists of squamous cell carcinoma (SNSCC) and adenocarcinoma (SNAC)[2]. Internationally, they account for around 0.2 % of all cancers among males and 0.1 % among females (excluding non-melanoma skin cancer). The nasal cavity, paranasal sinuses and their surrounding anatomical structures may be involved. Complete resection of small tumors is associated with a more favorable outcome. On the contrary, in advanced cancers the respectability is often limited because of adjacent structures like the orbit, optic nerve, internal carotid artery and brain. Adjuvant radiotherapy is therefore commonly applied, if a close margin situation or infiltrated margins remain after surgery[3]. Although 5-year overall survival is poor (53.7 %), especially for overlapping lesions[4], its locoregional recurrence is rare, when local control can be achieved[5]. In summary, it is a heterogenous group of tumors which is quite complex in treatment and could greatly benefit from the development of new disease-specific treatment modalities. These require a deeper understanding of the tumor biology and its interaction within the tumor microenvironment.

Molecular-biologically, proliferation, dedifferentiation, invasion, metastasis and immune escape of head and neck cancers are linked to the liberation of a variety of messengers like growth factors, hormones and cytokines by the tumor microenvironment and the tumor itself. Cytokines thereby play a significant role. They are basically divided into interferons, interleukins, lymphokines, tumor necrosis factors and chemokines. Chemokines, in turn, are involved in chemotaxis of immune cells, angiogenesis and even cancer progression[6]. The chemokine receptors CCR7 and CXCR5 are mainly expressed by lymphocytes in healthy individuals[7]. CXCR5 expressing cells are attracted towards a gradient of its ligand CXCL13 which so mediates regulation of cell migration within various regions of secondary lymphoid organs. CXCL13 especially attracts B

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cells and is involved in B-cell differentiation, maintenance of lymphoid tissue microarchitecture, and the development of B- and T-cell-mediated immune responses[8]. The ligands of CCR7 are CCL19 and CCL21. It is known to play a role in migration, activation and survival of many cell types including dendritic cells, T cells, eosinophils, B cells, and endothelial cells. In conclusion, CCR7 signaling is important for lymph node homeostasis, T cell activation, immune tolerance and inflammatory response[9]. In cancers, CCR7[6, 7, 9-12] and CXCR5[6, 8, 11, 13, 14] were frequently found to be associated with invasion and metastasis.

As a basis for new targeted treatment approaches, we analyzed the expression of CCR7 and CCR5 in a cohort of patients with sinonasal cancers and correlated immunohistochemical stainings with the clinical outcome.

PATIENTS AND METHODS:

Cohort details and data collection. Patients with histopathological proven sinonasal carcinoma between 2006 and 2015 (n = 56) who were treated at the Head and Neck Tumor Center of the Comprehensive Cancer Center Ulm (CCCU) were retrospectively enrolled in the study after approval by the local ethics committee. Key inclusion criteria were histology of squamous cell carcinoma and adenocarcinoma. Cases with insufficient material were excluded. Data collection of the patient clinical courses was done over a period of 16,25 years (January of 2006 – March of 2022). General health data was hereby collected from electronic patient records in cooperation with the Clinical Cancer Registry of the Comprehensive Cancer Center Ulm and collected in an anonymized format. Patient registration to Clinical Cancer Registries is required by German Federal Law. Patients signed informed consent for data analysis at hospital admission.

Staining and immune scoring. Cancer tissue was obtained during diagnostic or therapeutic surgery, formalin-fixed and paraffin embedded. Archival tissue sections were stained using antibodies for specific antigens after adapted manufacturers protocol: anti-CCR7 antibody ([Y59] ab32527 (abcam)) and anti-CXCR5 antibody ([MM0225-8C17] ab89259 (abcam)) after antigen retrieval with sodium citrate dihydrate. Antibodies were then visualized using chromogen Diaminobenzidin (DAB); hematoxylin (Dako Denmark) was used for counterstaining. Positive controls were performed on spleen and lymph node tissue. Evaluation of stainings was done by two independent researchers (JD and SEW). The percentage of positive tumor cells was analyzed and categorized into four groups (< 25 %, 25 – 50 %, 51 – 75 %, > 75 %). Staining intensity (from 1 for low to 3 for high intensity; 0 = negative) of stained tumor cells was determined. Adding the values of quantity and intensity resulted in a score ranging from 2 to 7 points, previously described as immunoreactive score[15]. Staining examples can be found in (Figure 1a).

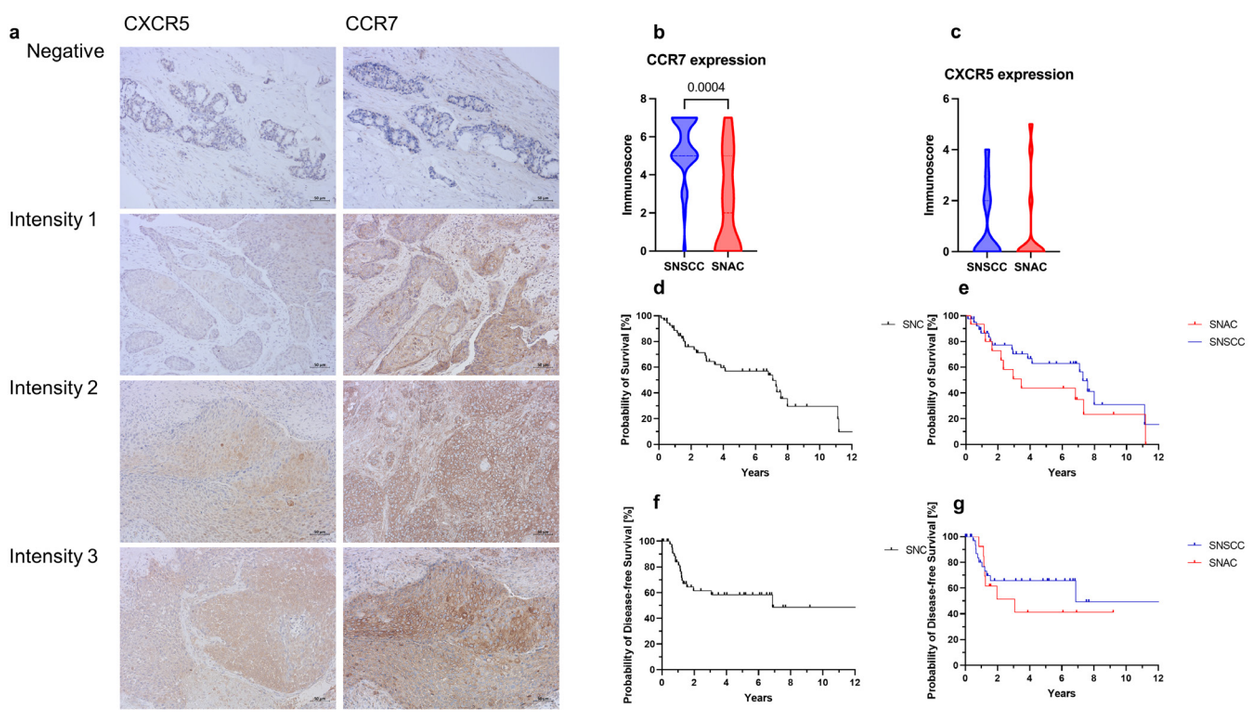

Fig. 1: Histological sections of SNSCC and SNADC patients were immunohistochemically stained and representative ones are shown for each intensity grade, magnification 10x (a). Furthermore, chemokine expression of CCR7 (b) and CXCR5 (c) is presented in dependence of the histological SNC subtype. Finally, the overall and disease-free survival of all SNC patients (d, f), also in relation to their histological subtype (e, g), is presented.

Statistical evaluation:

Overall and disease-free survival analysis was performed by Kaplan Meier method, differences among groups were analyzed by the log rank test. Cox regression was performed to question association between various parameters and survival. The presence of a normal distribution in the investigated samples was tested with the Shapiro-Wilk test. Mann-Whitney-U-Test was then conducted to investigate differences in central tendencies of two independent, not normally distributed samples. Furthermore, correlation analysis of ordinally scaled parameters was done and given with Spearman's rank correlation coefficient. All statistical computation was done with SPSS v26 (IBM). For visualization Prism v9 (Graphpad) was employed.

RESULTS:

Study population. The patient cohort consisted of 56 head and neck cancer patients with SNC of the nasal cavity or paranasal sinus. 34 men and 22 women were included. The mean follow-up time was 4.07 years; SD = 3.28 years. Histologically, adenocarcinomas (n = 15) and squamous cell carcinomas (n = 41) were included. The majority was diagnosed with UICC-stage I cancer (50 %). Their mean age was 62 years (SD = 14.32). All patients were treated with standard of care (surgery and/or radio- or chemoradiotherapy) according to their disease status referring to a consensus decision of a multidisciplinary team. Patient characteristics are summarized in (Table 1).

CXCR5 and CCR7 expression by immunochemistry. The chemokines of interest were only expressed on tumor cells in the analyzed cohort. Patients with SNSCC scored on average 5.43 immune points (SD = 1.67) for CCR7 and 0.89 points (SD = 1.41) for CXCR5. Patients with SNAC reached on average 2.38 points (SD = 2.63) for CCR7 and 1.00 points (SD = 1.73) for CXCR5 (Figure 1b, c). The expression of CCR7 was significantly higher for SNSCC as compared to SNAC ($p < 0.001$). CXCR5 being generally expressed on a low frequency was not significantly different between the two entities ($p = 0.65$). CCR7 and CXCR5 expression correlated weakly with each other (Spearman $r^2 = 0.25$, $p = 0.01$).

Overall and disease-free survival analysis. The median survival time in our cohort (Figure 1 d) was 7.08 years (SD = 1.58). There was no significant difference (Figure 1e) between patients with SNAC and SNSCC ($p = 0.23$). Furthermore, Cox regression revealed that CCR7 and CXCR5 scoring did not significantly influence survival of SNC patients when using age as control parameter (CCR7 $b = 0.03$; $p = 0.74$ and CXCR5 $b = 0.01$; $p = 0.96$). The median disease-free survival time in our cohort was 3.88 years (SD = 2.18). It accounted 6.88 years (SD =1.24) for SNSCC and 3.07 years for SNAC (SD = 1.32), there was no significant difference (Figure 1f, g) detected ($p = 0.48$). Furthermore, CCR7 and CXCR5 scoring did not influence disease-free survival in cox regression model (CCR7 $b = 0.13$; $p = 0.25$ and CXCR5 $b = 0.10$; $p = 0.96$).

Correlation analysis of CXCR5 and CCR7. Furthermore, we looked for relevant correlations between chemokine expression and age, histological type, grading, TNM status, UICC classification, recurrence and event of distant metastasis during observation period. There were no significant correlations detectable ($p > 0.05$).

Table I: Patient characteristics concerning gender, TNM status, histological type, grading and age are demonstrated.

Patient characteristics (n = 56)	Number	Proportion [%]
Male	34	60.71
Female	22	39.29
cT1	12	21.43
cT2	17	30.36
cT3	8	14.29
cT4	19	33.93
cN0	47	83.93
cN+	9	16.07
cM0	54	96.43
cM1	2	3.57
Adenocarcinoma	15	26.79
Squamous cell carcinoma	41	73.21
GI	8	14.29
GII	36	64.29
GIII	12	21.43
Mean Age: 62 years (SD = 14.32)	Not applicable	Not applicable

DISCUSSION

In the present work we aimed to examine the impact of CXCR5 and CCR7 expression on tumor cells of currently poorly studied sinonasal cancers on overall and recurrence-free survival. The chemokine receptors CXCR5 and CCR7 are usually expressed by lymphocytes and are important for cell trafficking[7]. There are many reports that also cancer cells are able to express chemokine receptors like CCR7 and CXCR5, which is generally thought to promote tumor cell proliferation, invasion, metastasis, and angiogenesis[7, 16]. Although no significant effect on overall survival or recurrence-free survival was seen in our investigations, our data suggests a role of CCR7 in the formation of lymph node metastasis of sinonasal carcinoma. One of the major differences in the clinical behavior of SNSCC and SNAC is the very low rate of lymph node metastases of SNAC (0.8 % vs. 9.1 %)[17]. This matches our observation of a significantly higher CCR7 expression in SNSCC enabling tumor cells exit to lymph node tissue via high endothelial venules.

Besides, there are also some reports, that on the contrary high CXCR5 and CCR7 expression could be associated with an improved overall survival, also in HNC (head and neck cancer) patients[18, 19]. CCR7 signaling itself is indeed known to play two opposing roles in cancer. On the one hand, it is involved in migration of tumor cells, but on the other hand this axis is engaged in lymphocyte trafficking and therefore may support antitumoral immune response[19, 20]. A similar explanation could be the fundament of the conflictive results concerning CXCR5, meaning, that in case of a positive correlation of CXCR5 and patients' prognosis, a higher amount of CD4+ T cells, CD8+ T cells and CD38+ plasma cells can be detected[18]. Therefore, it may not be as easy to say in general what effect the detection of an increased expression of these receptors has. It might more likely be a matter of which cells precisely have these receptors expressed on their surface to predict whether the antitumoral immune response or the tumor cell migration process is dominating[8].

The resulting rational could therefore be to specifically block CCR7 and CXCR5 on tumor cells and promote the expression of antitumoral tumor infiltrating immune cells via immune modulation methods. There is already some evidence that targeted anti-CCR7 and anti-CXCR5 immune therapy is beneficial in lymphoma[8, 21-23]. In addition to biologicals or customized equivalents, there are various known inhibitors of CCR7 like triptolide (epoxide of the thunder god vine, used in Traditional Chinese Medicine)[24] or cosalane (anti-HIV medication)[25]. Interestingly, researchers even identified endogenous microRNA (hsa-let-7e-5p), which was found to be down-regulated in head and neck squamous cancer (HNSCC) patients (n = 15) and may act as a tumor suppressor by targeting CCR7 expression[26]. Besides, signal pathways interfering with CCR7 signaling, such as COX-2/PGE2, which is known to upregulate CCR7 expression, might be of interest for therapeutic immune modulation[19]. Similar considerations can be made for CXCR5, too. A known inhibitor of CXCR5 (except antibodies or similar) is Navarixin, which is a multitarget antagonist, especially examined in the context of asthma and COPD[27]. The challenge of all these agents would be to specifically inhibit CCR7 on tumor cells, but not on circulating immune cells, which would interrupt the lymph node homing and maturation of T cells after antigen contact. Particular methods of patient adapted galenics and nanotechnology could be useful[28].

In the end, it has to be noticed, that there is only little evidence on the role of CXCR5 and CCR7 in sinonasal cancer until now. Further on, our results might be biased by the fact, that our population only enclosed 56 patients with a fairly large proportion of low UICC stages at primary diagnosis. Besides, the validity of our immunohistochemical testing could be enhanced by further processing the tissue sections microscopically classified as tumor tissue by our pathologists by simultaneous immunofluorescence analysis to clearly differentiate immune and epithelial cells. Nevertheless, chemokines are a promising group of possible anti-cancer targets.

CONCLUSION

CXCR5 and CCR7 play an important role in the tumor microenvironment signaling in various tumors and are mostly associated with poor prognosis of cancer patients. Therefore, they might be potential targets of individualized tumor therapy. However, further research is needed, especially in head and neck cancer to further characterize their ambivalent role in cancers and to deviate treatment approaches.

ABBREVIATIONS

Sinonasal cancers (SNC)

Sinonasal squamous cell carcinoma (SNSCC)

Sinonasal adenocarcinoma (SNAC)

Comprehensive Cancer Center Ulm (CCCU).

CONFLICT OF INTEREST

There are no conflicts of interest.

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