

Loss of protective CD169 macrophages in lymph nodes with metastatic SCC with preservation in adjacent negative draining lymph nodes

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Abstract

Importance: With regards to antitumor immunity, regional lymph nodes that drain malignant primary tumors are thought to be the first site where the immune system interacts with tumor cells and their products. It has not been clearly defined why some lymph nodes are more susceptible to regional metastatic spread, while others in the same drainage pattern are spared from tumor infiltration. Lymph node macrophages are important cells in anti-tumor immunity with CD169+ macrophages acting as a sentinel barrier to the influx of soluble mediators.

Objective: Examine the macrophage phenotype based on CD169 expression in the subcapsular sinus of positive lymph nodes and nearby negative draining lymph nodes (dLN) in patients with oral cavity squamous cell carcinoma (SCC).

Methods: CD169 staining was performed on seven oral cavity SCC patients with nodal disease. CD169 staining of the positive lymph nodes was compared to the same patient's nearby negative dLN.

Results: The subcapsular sinus of negative dLN showed an intensely staining, high density of CD169+ macrophages. Cancer containing lymph nodes had significantly fewer sentinel CD169+ macrophages when compared to negative dLN (164.6 vs. 417.3 CD169+ macrophages/mm², 252.6 CD169+ macrophages/mm², 95% CI 67.4 to 437.9 CD169+ macrophages/mm²).

Conclusions and Relevance: Sentinel CD169+ macrophages are more prevalent in the subcapsular sinus of negative draining lymph nodes when compared to nearby lymph nodes with SCC metastases. Further research is necessary to understand the mechanism by which CD169+ macrophages prevent SCC from metastasizing and its clinical significance related to prognosis and response to treatment.

Introduction

Lymph node metastases are considered the single most important prognostic determinant in head and neck squamous cell carcinoma (HNSCC) decreasing survival by approximately 50%.¹ Despite the prognostic implications of lymph node metastasis and the fact that lymph nodes are one of the initial organs that dominate the immune response to immunogenic antigens, little is known about the immune microenvironment within regional lymph nodes (RLN) in HNSCC. With regards to antitumor immunity in RLN, sinus macrophages, which specifically express CD169, have emerged as a cell population with particular prognostic significance.²⁻⁴ When fragmented dead tumor cells flow into the sinus area of RLN via lymphatic vessels, they are endocytosed by sinus macrophages.⁵ Sinus macrophages subsequently process and present these antigens on MHC I complexes inducing activation of tumor antigen-specific T- and B-cells.⁶ Recent research has demonstrated the prognostic significance of CD169+ macrophages in endometrial cancer, breast cancer, and malignant melanoma.²⁻⁴ To date, no studies have been performed on the prognostic significance of CD169+ sinus macrophages in HNSCC.

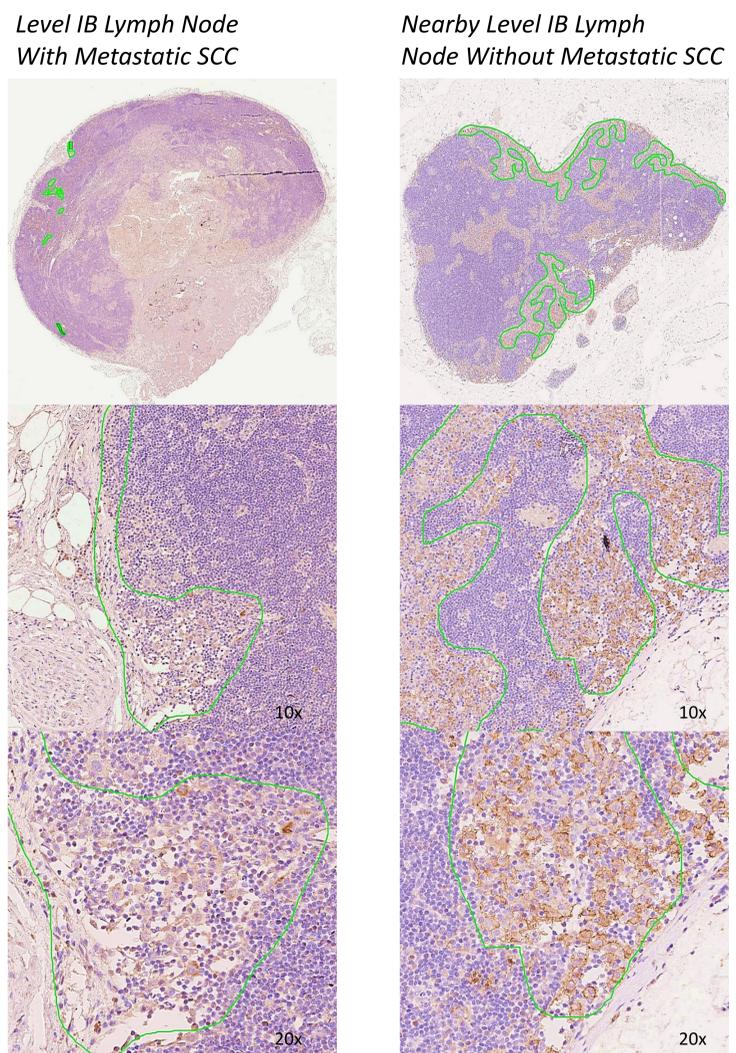
Table 1. Concentration of CD169+ Lymph Node Macrophages

Patient	Positive LN (CD169+ cells/mm ²)	Negative LN (CD169+ cells/mm ²)
68M T1N1 FOM	87.8	154.3
55F T4aN2c Tongue	246.6	534.4
81M T4aN2b Buccal	322.9	449.9
60M T3N2b Tongue	237.2	350
44M T4aN2c FOM	98.9	231
52F T4aN2a Tongue	107.7	555.1
68F T4aN2c Hard Palate	51.3	646.2
Mean	164.6	417.3
Mean Difference	252.6	
p value	.007	

Methods and Materials

CD169 staining was performed on formalin fixed paraffin embedded tissue from seven oral cavity SCC patients with nodal disease. CD169 staining of the positive lymph nodes was compared to the same patient's nearby negative dLN. Quantitative analysis of CD169 staining was performed using Aperio Imagescope by counting number of positive cells per millimeter squared (mm²) within the subcapsular sinus. Each slide was counted by two independent counters blinded to the identity of the sample and averaged. To calculate the number of positive cells per unit area, the annotated subcapsular sinus area was measured using Aperio Imagescope software program.

Figure 1. CD169 staining performed on two nearby lymph nodes from patient with T4aN2b buccal SCC



Discussion

For nodal metastasis to occur, it is imperative that tumor cells effectively disarm the immune defenses in the new nodal environment. With this preliminary research on CD169 sinus macrophages, we offer a possible explanation to the mechanism of nodal metastases in HNSCC. All seven patients contained significantly fewer CD169+ cells/mm² in the subcapsular sinus of lymph nodes with metastatic SCC when compared to nearby negative draining lymph nodes from the same patient. This suggests that CD169+ subcapsular sinus macrophages may play an important role as sentinel macrophages in the protection of lymph nodes from metastatic SCC. We further hypothesize that soluble mediators secreted by HNSCC cells are capable of altering the local immune microenvironment and CD169+ concentration of draining lymph nodes.

Conclusions

1. Lymph nodes with metastatic SCC contain lower levels of sentinel CD169+ macrophages in the subcapsular sinus when compared to nearby lymph nodes without metastatic SCC.
2. Further research is necessary to understand the role that CD169+ macrophages play in SCC lymph node metastases and its clinical significance related to prognosis and response to treatment.

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References

1. Coskun HH, Medina JE, Robbins KT, et al. Current philosophy in the surgical management of neck metastases for head and neck squamous cell carcinoma. *Head & neck*. Jun 2015;37(6):915-926.
2. Ohnishi K, Yamaguchi M, Erdenebaatar C, et al. Prognostic significance of CD169-positive lymph node sinus macrophages in patients with endometrial carcinoma. *Cancer science*. Jun 2016;107(6):846-852.
3. Shiota T, Miyasato Y, Ohnishi K, et al. The Clinical Significance of CD169-Positive Lymph Node Macrophage in Patients with Breast Cancer. *PLoS one*. 2016;11(11):e0166680.
4. Saito Y, Ohnishi K, Miyashita A, et al. Prognostic Significance of CD169+ Lymph Node Sinus Macrophages in Patients with Malignant Melanoma. *Cancer immunology research*. Dec 2015;3(12):1356-1363.
5. Swartz MA. Immunomodulatory roles of lymphatic vessels in cancer progression. *Cancer immunology research*. Aug 2014;2(8):701-707.
6. Schaale K, Brandenburg J, Kispert A, Leitges M, Ehlers S, Reiling N. Wnt6 is expressed in granulomatous lesions of Mycobacterium tuberculosis-infected mice and is involved in macrophage differentiation and proliferation. *Journal of immunology*. Nov 15 2013;191(10):5182-5195.