Analysis of M1 and M2 tumor associated macrophages in tongue squamous cell carcinomas

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AIM

Tumor associated macrophages (TAMs) are the dominant leukocyte population found in the tumor microenvironment and their mobilization into tumor tissues is a critical event in tumor initiation, growth, and development. TAMs are a heterogeneous population of innate myeloid cells and take on various phenotypes depending on the context of the molecular stimuli from the microenvironment. Basically, there are two main recognized categories: classically-activated pro-inflammatory M1 macrophages (expressing the CD11c antigen) and alternatively-activated anti-inflammatory M2 macrophages (expressing the CD163 antigen). Although TAMs have been detected in head and neck cancers, little is known about their phenotype in the context of the tongue squamous cell carcinoma (TSCC), which have the highest incidence in maxillofacial malignant tumors. The aim of the present retrospective study was to characterize the macrophage polarization in low grade (G1) and high grade (G3) TSCC, and to examine the importance of their relative localization (tumor stroma, inflammation area or tumor nest) on tumor-promoting capabilities and tumor-prognostic relevance.

MATERIALS AND METHODS

This study included surgical specimens obtained from 71 patients with TSCC (36 graded as G1 and 35 graded as G3). 4-µm serial sections from formalin-fixed, paraffin embedded blocks were cut for each case and mounted on poly-L-lysine-coated glass slides. The sections were incubated for one hour at room temperature, with anti-CD163 monoclonal antibody (MA5-11458 clone

10D6 Thermo Scientific, dilution 1:100) and anti-CD11c monoclonal antibody (ab52632 clone EP1347Y Abcam, dilution 1:200). Immunohistochemical staining of TAMs was evaluated both in inflammatory infiltrate and tumor stroma. To evaluate the expression of CD11c and CD163, the percentage of positive cells was scored as 0 (< 10%), 1 (10-25%), 2 (26-50%), and 3 (> 50%). The staining intensity was scored as 0 for negative, 1 for weak, 2 for moderate, and 3 for intense expression.

RESULTS

Infiltration of CD11c+ (M1) or CD163+ (M2) TAMs in the tumor nest was not associated with any clinicopathological features. On the contrary, CD11c+ and CD163+ samples were significantly more numerous in G3 group, both in terms of extension and intensity of staining. In addition, a positive correlation was found between inflammatory-CD11c and stromal-CD163 expression, both in extension and intensity of staining. Moreover, Kaplan-Meier analysis showed better disease-free survival (DFS) in G3 patients with inflammatory-CD11c expression, while inflammatory-CD163 expression was associated with a worse DFS. Finally, patients with stromal-CD163 expression showed better DFS in both G1 and G3 patients.

DISCUSSION

These findings highlight the importance of analyzing the phenotype of TAMs (CD11c+ and CD163+) both in inflammatory infiltrate and tumor stroma as a new promising prognostic marker in TSCC.

Keywords: tumor associated macrophage, CD11c, CD163, OSCC, immunoistochemistry