Unusual Multinucleated Giant Cell Reaction in a Tongue Squamous Cell Carcinoma: Histopathological and Immunohistochemical Features

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Abstract

Multinucleated giant cell (MGC) reaction in oral squamous cell carcinoma (OSCC) usually represents a stromal foreign body reaction to keratin from neoplastic epithelial cells. We describe and illustrate by double immunohistochemistry a case of a tongue squamous cell carcinoma (SCC) in a 70-year-old female patient, with a copious MGC reaction not associated to keratin, showing a histopathological pattern not described before. The MGCs were directly associated with neoplastic cells, which are phagocytosed by the MGCs. Immunohistochemistry for CD68, AE1/AE3, CD163, CD11c, RANK, RANK-L, OPG were performed, as well as double staining for CD68 and AE1/AE3 to better illustrate the relationship between MGCs and neoplastic cells. The clinical and biological significance of this pattern of MGC reaction in OSCC needs to be better elucidated.

Keywords Oral squamous cell carcinoma · Multinucleated giant cells · Immunohistochemistry · Phagocytosis

Introduction

Interactions between tumoral and stromal cells are important events in cancer, and inflammation is an important reaction that can be associated with better or worse prognosis of neoplasms, depending on the type of inflammation and of the malignancy. Macrophages are an important component of the cancer-associated inflammation in several types of cancer, including oral squamous cell carcinoma (OSCC) [1–3].

There are two different cell populations in macrophages given by a mechanism called macrophage polarization by which, in response to microenvironmental signals, the macrophage expresses different functional programs, acquiring a specific phenotype such as M1 or M2. M1 macrophages (classically activated macrophages), produce pro-inflammatory cytokines: interleukin (IL)-1, IL-6, IL-12, and tumor necrosis factor alpha (TNFα), these M1 macrophages can

present a antitumoral response in cancer; while M2 macrophages (alternatively activated macrophages), may have a pro-tumoral response, by releasing IL-4, IL-10 cytokines and transforming growth factor (TGF)-β promoting tumoral growth, angiogenesis, extracellular matrix (ECM) remodeling, and even metastasis [4–7]. Macrophages fusion resulting into multinucleated giant cells (MGC) has been described as a host response to the tumoral cells in several types of carcinomas, however, the prognostic implications are not clear [8–13].

To the best of our knowledge, few cases of OSCC and lip squamous cell carcinoma (SCC) presenting MGCs have been reported, usually representing a foreign body reaction to keratin. In this study we describe and illustrate a case of tongue SCC with an unusual profuse reaction of MGC surrounding and phagocytosing neoplastic cells, but without association with extracellular keratin.

Case Report

A 70 year-old Guatemalan female presented with an ulcerated mass on the lateral border of the tongue, with an approximate duration of 4 months. An incisional biopsy was performed and the specimen was sent to the pathology

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service at Centro Clínico de Cabeza y Cuello, for histopathological analysis.

The gross specimen consisted of two fragments of soft tissue with firm-elastic consistency and brownish color. The diagnosis was invasive SCC, moderately differentiated. The superficial mucosa was covered by dysplastic stratified squamous epithelium from which originated the neoplastic proliferation, composed of sheets and islands of pleomorphic and hyperchromatic squamous epithelial cells that infiltrated deeply the subjacent connective and muscular tissues, in a background of peritumoral and intratumoral inflammatory cells. A striking and exuberant macrophagic and MGC reaction involving and infiltrating the tumoral nests, in absence of keratin pearls or extracellular keratin filaments was evident (Fig. 1a). Peritumoral mononuclear macrophages predominated in the superficial areas adjacent to surface epithelium, while the MGC were directly associated to tumoral

nests mainly in deeper areas of the tumor, adjacent to skeletal muscle fibers.

The predominant MGCs were of foreign-body type, however, also Langhans type cells were found. Most of the foreign-body type MGCs presented more than 40 nuclei, some exceeding 200 (Fig. 1b).

MGC showed cytoplasmic eosinophilic figures, some of them with a basophilic center, suggesting degenerating epithelial cells, phagocytosed by the MGCs, and in some areas, the MGCs seemed to be engulfing the neoplastic cells (Fig. 1c, d).

Immunohistochemical (IHC) reactions including double staining for CD68 and AE1/AE3 were performed to illustrate the relationship between MGCs and neoplastic cells. Additionally, to establish the polarization of M1/M2 macrophages, IHC reactions for CD163 and CD11c were also done. To determine the possible pathway of MGCs

Fig. 1 Histopathological aspects of intense multinucleated giant cell reaction in a tongue squamous cell carcinoma. **a** Inflammatory response associated to the neoplastic cells consisting in an exuberant macrophagic and MGC reaction infiltrating tumoral nests, in absence of keratin pearls and surrounded by a background of a prominent lymphoplasmacytic infiltrate. In this field are observed foreign-body type MGCs with random arrangement of nuclei (white arrowhead), and also Langhans type MGCs with a horseshoe pattern of nuclei at

the periphery of the cytoplasm (white arrows) (HE 200×). **b** A foreign-body type MGC presenting more than 200 nuclei (HE 200×). **c** Epithelial cells fagocitated in the cytoplasm of MGC (arrows) (HE 400×). **d** MGCs engulfing an eosinophilic corpuscle that could be an epithelial cell in degeneration, the cytoplasm of MGC shows a modified shape that resembles pseudopods or a phagocytic vacuole (arrowhead), also a fagocitated cell is present within the cytoplasm of the MGC (arrow) (HE 400×)

formation, IHC for RANK, RANK-L and OPG was also performed.

The IHC was performed in sections of formalin-fixed, paraffin-embedded tissues that were dewaxed with xylene and then hydrated in an ethanol series. Antigen retrieval was performed by immersing the sections for 3 min in citrate buffer solution (pH 6.0) in a pressure cooker for CK-cocktail, CD68 and, CD163 reactions, and in a microwave oven for 24 min for RANK, RANKL, OPG and CD11c. Endogenous peroxidase activity was blocked using 10% hydrogen peroxide for 15 min. Subsequently, preparations were washed in phosphate buffer solution (pH 7.4) and incubated overnight with the primary antibodies (Table 1). Slides were exposed to avidin–biotin complex and horseradish peroxidase reagents: LSAB Kit; Dako Cytomation, for CK-cocktail, CD68 and, CD163 reactions and Vectastain ABC Kit–Rabbit, Vector, for RANK, RANKL, OPG and CD11c. The reaction was finally revealed with diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich, St Louis, MO, USA), and the tissue sections were counterstained with Carazzi hematoxylin. The positive controls for each marker are cited in Table 1 and the negative controls were obtained by omitting the specific primary antibody. Information about IHC is summarized in Table 1.

Surface epithelium and islands of OSCC stained with AE1/AE3. MGCs located in the deeper stroma, contained in the cytoplasm AE1/AE3-positive figures suggestive of phagocytosed epithelial cells (Fig. 2 A, B). Most of the infiltrating mononuclear inflammatory cells and all MGCs were positive for CD68 (Fig. 2c, d). Tumor cells adjacent to the surface epithelium were surrounded by a strong reaction of peritumoral CD68-positive mononuclear macrophages and few CD68-positive MGCs were also observed on this area.

In order to better illustrate the relationship between MGCs and the neoplastic cells, IHC double staining was performed using AE1/AE3 and CD68 antibodies in the same tissue section, with the EnVision Gl2 Doublestain System, Rabbit/Mouse (DAB+/Permanent Red) (Dako/Agilent Santa Clara, CA). In the first double IHC, DAB and Permanent Red was used as chromogen for AE1/AE3 and CD68

respectively, illustrating the intense macrophagic and MGC infiltration surrounding the tumoral islands (Fig. 3a, b). Large MGCs were found at the center of the tumor islands, while smaller ones resembling Langhans-type were located peritumorally. In the second double IHC, the chromogens were inverted, for CD68 was used DAB as chromogen and for AE1/AE3 Permanent Red, confirming the same pattern between OSCC and MGCs (Fig. 3c, d). In both double IHC, we observed figures suggestive of phagocytosis of neoplastic cells by MGCs (Fig. 3b, d).

Most MGCs were positive for CD11-c and negative for CD163, the first was also expressed by the intratumoral mononuclear macrophages adjacent to MGCs, while CD163 was positive in the macrophages surrounding the tumor (Fig. 4a, b). Tumoral and stromal components were negative for OPG. Approximately half of the MGCs and most of the intratumoral mononuclear macrophages were positive for RANK, while the macrophages surrounding the tumor were negative. In focal areas neoplastic cells showed weak positivity for RANK. All MGCs were strongly positive for RANK-L, as well as the inflammatory cells surrounding the tumor nests (Fig. 4c, d).

Discussion

Cancer progression is influenced by the microenvironment conditions given by interactions between parenchymal and stromal cells. Diverse stromal reactions as fibrosis, cancer associated fibroblasts/myofibroblasts, inflammation, immune response and angiogenesis have been associated with prognosis in several types of cancer. Lymphocytes, macrophages, and dendritic cells represent the main inflammatory and immune response which can play dual roles during tumorigenesis [1, 3]. Macrophages can be abundant in oral squamous cell carcinoma and other malignancies, and according to the polarization (M1 vs. M2) are associated with a dual tumor interaction: antitumoral (M1, induced by Th1 cytokines) or pro-tumoral response (M2, induced by Th2 cytokines) [4, 6, 7]. High infiltration of M2 polarized

Table 1 Immunohistochemical reactions

Antibody	Source/clone	Dilution	Positive control	Result
CD68	Dako, PG-M1	1:400	Mucocoele (macrophages)	Positive in MM and MGC
CK-cocktail	Dako, AE1/AE3	1:500	OSCC	Strongly positive in tumoral cells
CD163	Novocastra, 10D6	1:4000	Mucocoele (macrophages)	Negative in MGC and positive in MM surrounding the tumor
CD11c	Abcam, N418	1:100	Bone marrow	Positive in MGC and in intratumoral MM
RANK	Abcam, polyclonal	1:300	T-cell lymphoma	Positive in half of the MGC and most of the intratumoral MM
RANKL	Abcam, 64C1385	1:500	Lymph node	Positive in MGC and peritumoral inflammatory infiltrate
OPG	Abcam, polyclonal	1:200	Osteoblasts	Negative in tumoral and stromal components

OSCC oral squamous cell carcinoma, MM mononuclear macrophages, MGC multinucleated giant cells

Fig. 2 Immunohistochemical features of intense multinucleated giant cell reaction in a tongue squamous cell carcinoma. **a, b** Immunohistochemistry for AE1/AE3. Positive neoplastic cell islands were infiltrated by mononuclear cells compatible with macrophages and

lymphocytes, and centrally by MGC of variable sizes, some of them containing AE1/AE3-positive figures in the cytoplasm (immunoperoxidase, 200×). **c, d** Many tumor-infiltrating mononuclear and MGCs were positive for CD68. (immunoperoxidase, **c** 50× and **d** 200×)

macrophages is associated with poor tumor outcome, and progression in OSCC [14, 15].

Th1 cytokines such as interferon-gamma and tumor necrosis factor-alpha as well as Th2 cytokines, including IL-4 and IL-13, can induce macrophage fusion, resulting into MGCs which has been described as a host response to the tumoral cells in several types of carcinomas; however, it is uncertain if the presence of MGC have prognostic implications [9, 10, 12, 13].

Cases of OSCC and lip SCC rich in MGCs have been reported in few studies, usually as a foreign body reaction to extracellular keratin. To date, presence/distribution of MGCs has not been associated with any clinical parameters [8, 11, 12, 16].

Brandwein-Gensler et al. in 2005 evaluated 292 cases of oral SCC from which 77% presented foreign body reaction to keratin, while Santos et al. reported the frequency of about 40% of MGCs in lip SCC in a series of 91 cases, showing a correlation between MGC reaction and keratinization of the tumor. In short, in these two reports and others of lip and intraoral SCC, the presence of MGCs represented a

foreign body reaction to keratin [8, 11, 16]. One exception was the report of Emanuel et al., of a cutaneous lower lip SCC, describing the presence of MGCs as an osteoclastic giant-cell-like proliferation, not related to keratin [18]. The present case shows a rather unique histopathological pattern, considering morphology, size and high number of MGCs surrounding and phagocytizing tumoral cells.

We observed a profuse reaction of mononuclear macrophages and MGCs practically in the totality of the tumor sheets and islands, with MGCs more frequent and larger in the deeper areas of the connective tissue. In addition, the MGCs were unusually large, most showing more than 40 nuclei, and some exceeding 100 or 200 nuclei, some displaying in the cytoplasm phagocytosed neoplastic cells without apparent keratinization or apoptosis.

All MGCs were positive for CD68, confirming its macrophagic origin. We also observed that most mononuclear macrophages were positive for CD163 while the majority of MGCs were negative, conversely MGCs showed predominant positivity for the M1 marker CD11-c, in accordance to the most common immunophenotype of MGCs present in

Fig. 3 Immunohistochemical double staining for AE1/AE3 and CD68. **a** In low power magnification, an intense macrophagic and MGC infiltration was observed in all the tumoral islands, while scarce positivity for CD68 was observed within the lymphoplasmacytic infiltrate in the stroma. **b** Area of phagocytosis of the neoplastic cells by MGC (yellow arrow). (AE1/AE3 immunoperoxidase and CD68 per-

manent red, **a** 100 $\times$, **b** 400 $\times$). **c** Infiltrating intratumoral macrophages and MGCs, as well as peritumoral and stromal macrophages admixed to a lymphoplasmacytic infiltrate of the stroma. **d** Areas of phagocytosis of the neoplastic cells by MGCs (yellow arrowheads). (CD68 immunoperoxidase and AE1/AE3 permanent red, **c** 200 $\times$, **d** 400 $\times$)

labial and intra-oral OSCC as previously described in the literature [12, 17, 18].

In some cancer types, activated or M1 macrophages present in the tumor, are considered to play a crucial role in preventing progression as they are potent effector cells capable of killing tumor cells by direct and indirect effects, and they also can activate and recruit cytotoxic T cells via antigen presentation. On the other hand, M2 macrophages have been associated with tumor growth and metastasis by enzymatic matrix degradation, production of immunosuppressive cytokines/chemokines and angiogenic factors [4, 19, 20]. Thus, according to previous reports, the present case also showed a predominant M1 phenotype suggesting that the MGC reaction could play an antitumor activity in labial SCC and OSCC.

Interactions between receptor activator of NF- κ B (RANK) and its ligand (RANKL) are required for osteoclastogenesis, with osteoprotegerin (OPG) acting as an inhibitor. These molecules have been studied mainly in osteoclasts

[20–22]. Khan et al. observed by Western blot, that foreign body MGCs were negative for osteoclast specific markers (RANK, NFATc1, CTSK); and in this context, our immunohistochemistry results indicate that the giant cells in the present case have an osteoclast-like phenotype [23].

Up-regulation of RANK and secretion of RANKL by OSCC cells and high expression of these markers in associated multinucleated osteoclasts, have been described previously [24, 25]. In the present case, focal areas of OSCC weakly expressed RANK, that was strongly positive in mononuclear intratumoral macrophages and about half of MGCs; RANKL was positive in all MGCs, as well as in a significant proportion of stromal and inflammatory cell components, leading us to consider that the MGC reaction was induced mainly by stromal cells instead of the tumor itself, and that the MGC differentiation was likely produced by the RANK/RANKL pathway.

In conclusion we described an unusual MGC reaction not associated to extracellular keratin in a SCC of the

Fig. 4 Immunoexpression of CD11-c, CD163, RANK and RANKL. **a** Most of MGCs were positive for CD11-c, which also was expressed by the intratumoral mononuclear macrophages adjacent to MGCs. **b** The MGCs were negative for CD163 that was positive in the macrophages surrounding the tumoral cells. **c** Approximately half of the MGCs were positive for RANK, also most of the intratumoral macrophages showed positivity, while the macrophages surrounding the tumor were negative. Also, focal areas of tumor showed weak positivity for RANK (arrows in inset). **d** All the MGCs were strongly positive for RANKL, as well as several mononuclear inflammatory cells. (immunoperoxidase, 200 $\times$, inset: 400 $\times$)

tongue. The giant cells seemed to be formed by the RANK/RANKL pathway, and they surrounded and phagocytosed neoplastic cells. The biological and clinical significance of this type of MGCs reaction in OSCC are unknown and future studies are required to better understand this phenomenon.

Compliance with Ethical Standards

Conflict of interest All of authors have indicated they have no potential conflicts of interest and no financial relationships relevant to this article to disclose.

Ethical Approval This article does not contain any studies with animals performed by any of the authors. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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