Int. J. Odontostomat.,
16(2):258-266, 2022.

Pro-and Anti-inflammatory Biomarkers as Potential Prognosis Markers in Oral Squamous Cell Carcinoma

Biomarcadores Pro y Antiinflamatorios como Posibles Marcadores de Pronóstico en el Carcinoma Oral de Células Escamosas

Karla Daniella Malta Ferreira[1]; Ruth Tramontani Ramos[2]; Camila Stofela Sodré[2]; Patrícia Nivoloni Tannure[1]; Luciana Armada[2,3]; Lúcio de Souza Gonçalves[3]; Dennis de Carvalho Ferreira[1,3] & Marcia Gonçalves Ribeiro[4]

FERREIRA, K. D. M.; RAMOS, R. T.; SODRÉ, C. S.; TANNURE, P. N.; ARMADA, L.; GONÇALVES, L. S.; FERREIRA, D. C. & RIBEIRO, M. G. Pro-and anti-inflammatory biomarkers as potential prognosis markers in oral squamous cell carcinoma. *Int. J. Odontostomat., 16(2)*:258-266, 2022.

ABSTRACT: The most widely used method to classify prognostic factors in cancers today is TNM. However, Oral Squamous Cell Carcinoma (OSCC) often demonstrates different behaviors in relation to aggressiveness and therapeutic response at the same TNM stage. So, in such cases biomarkers can be used to identify the biological diversity of these tumors more reliably, leading to better therapeutic strategies and disease management. The presence of inflammatory immune cells in the tumor microenvironment can have pro or antitumor effects and the investigation of the expression of inflammatory markers in OSSC can be usefulto design immunotherapeutic interventions. The Transforming Growth Factor alpha is a potent stimulator of cell migration that acts on cell proliferation, invasion and metastasis of cancer, as well as immune suppression and angiogenesis. Inflammatory cytokines, such as Interferon-gamma, mediate macrophage differentiation. Macrophages are an important component of the OSCC microenvironment. The greater amount of tumor-associated macrophages, especially the M2 phenotype, may be associated with a more aggressive biological behavior of the OSCC and, consequently, with reduced survival.

KEY WORDS: oral squamous cell carcinoma, transforming growth factor-alpha, epidermal growth factor receptor, cd68 antigen, cd57 antigens, interferon-gamma, biomarkers, prognosis.

INTRODUCTION

Cancer will probably be the leading cause of death as well as the most important challenge for life expectancy worldwide in the 21st century (Bray *et al*., 2018). Oral squamous cell carcinoma (OSCC), the most prevalent malignant neoplasm in the oral cavity, has a high potential for local invasion and nodal metastasis. The overall 5-year survival rate of OSCC has not changed significantly over the last 30 years (Lampri *et al*., 2015), despite the discovery of new therapies and treatment options (Cervino *et al*., 2019). In 2018, 354,864 patients worldwide were diagnosed with OSCC and 177,384 of these patients died due to this disease (Bray *et al*., 2018). Previous studies have already estimated an incidence of 550,000 cases of OSCC in 2040 with 275,000 deaths (Bray *et al*., 2018). The main etiological and predisposing factors for OSCC include the use of tobacco products, drinking habits, ultraviolet radiation (specifically for lip cancer), human papillomavirus (HPV) infection, poor oral health, nutritional deficiencies, and genetic polymorphisms (McDermott & Bowles, 2019).

[1] Postgraduate Program in Odontology, Veiga de Almeida University, Rio de Janeiro, Brazil.
[2] Faculty of Dentistry, UNIVERITAS, Rio de Janeiro, Brazil.
[3] Faculty of Dentistry, Estácio de Sá University, Rio de Janeiro, Brazil.
[4] Service of Medical Genetics, IPPMG - Martagão Gesteira Pediatric Institute, Federal University de Rio de Janeiro, Rio de Janeiro, Brazil.

Received: 2021-07-09 Accepted: 2022-05-13

Nowadays, the TNM staging system seems to be the most reliable prognostic factor for OSCC; however, OSCCs often have a diverse behavior especially regarding their aggressiveness and therapy responses at similar TNM stages. Therefore, TNM staging might benefit from the use of biomarkers that reflect the biological diversity of these tumors more reliably, leading to better therapeutic strategies and disease management (Hadler-Olsen & Wirsing, 2019). Biomarkers can be defined as biological molecules such as nucleic acids, proteins, peptides, enzymes, antibodies, metabolites, lipids, carbohydrates and growth factors able to mediate the interaction between neoplastic cells and their microenvironment (Santosh *et al*., 2016), which together form a self-sufficient biological structure (Peltanova *et al*., 2019).

Currently, a large number of biomarkers have been identified and are under investigation (Lampri *et al*., 2015). They can be used to estimate disease risk, track unknown primary cancer, distinguish benign neoplasms from malignant tumors, determine prognosis, monitor the disease status, detect recurrence as well as therapy effectiveness or failure (Santosh *et al*., 2016). Fortunately, considerable progress in the identification and validation of biomarkers with a high potential to improve cancer diagnosis and treatment has been made (Lampri *et al*., 2015).

The presence of inflammatory immune cells in the tumor microenvironment with functional plasticity and that can exert either pro- or anti-tumor effects (Takahashi *et al*., 2017) along with investigations of inflammatory marker expressions in OSCC might be helpful to design immunotherapeutic interventions. Since several tumor biomarkers have already been suggested to predict the prognosis of OSCC in patients, we decided to conduct a review to assess whether the expression of pro- (TGF-α, CD-68) and anti-inflammatory (IFN-γ, CD57) biomarkers can be used as a prognosis in OSCC.

Pro-inflammatory Biomarkers

Transforming Growth Factor α (TGF-α). TGF- a was described by Roberts *et al*. (1981), and was initially called the epidermal growth factor-potentiated TGFs. TGF-α is a potent cell migration stimulator that also acts on cell proliferation, cancer invasion and metastasis, as well as inducing immune suppression, and angiogenesis (Sherbet *et al*., 2011; Le *et al*., 2019). The biologic importance of this growth factor in head and neck cancer progression is supported by the demonstration that the survival rate of patients correlates significantly with the expression levels of TGF-α in the primary tumor, independent of other clinical and pathological parameters. This led us to believe that this protein is probably involved in the development and progression of cancer.

TGF-α is a small mitogenic peptide with 50 amino acid residues and three disulfide bridges (Sherbet *et al*., 2011). TGF-α belongs to the endothelial growth factor family and is encoded by the TGFA gene, which is expressed during craniofacial development (Le *et al*., 2019). The secondary structure is very similar to the epidermal growth factor (EGF) and has a sequence homology of 40 % with this (Sherbet *et al*., 2011). Because of its structure, TGF-α can bind by competition to EGFR through Phe15 and Leu48, which resemble Tyr13 and Leu47 of EGF giving comparable binding interactions (Sabbah *et al*., 2020).

TGF-α is produced by transformed cells, embryonic cells, and tumor cells, and is closely involved in both normal and aberrant cell development and differentiation (Wong, 1993; Sherbet *et al*., 2011). Under normal conditions, epithelial cells are the major producers of this peptide (Wong, 1993), so that they can act physiologically in tissue regeneration and bone homeostasis (Sun *et al*., 2018). Levels of TGF-α, EGFR and EGFR mRNA in tissues can be measured by several molecular techniques, including radiolabeled ligand binding, protein blotting, RNA blotting, in situ hybridization, quantitative reverse transcription-polymerase chain reaction (rt-PCR) and immunohistochemical analysis (Grandis *et al*., 1998).

The overexpression of TGF-α deregulates cell proliferation and promotes the development and progression of the tumor (Endo *et al*., 2000; Sherbet *et al*., 2011; Sabbah *et al*., 2020). This overexpression has been observed in a wide range of human tumors, such as gastric, bladder, lung, brain, breast, colon, prostate, ovary, renal, liver, pancreas, bone metastasis, and head and neck (Sun *et al*., 2018; Le *et al*., 2019).

TGF-α also facilitates the epithelial-mesenchymal transition (EMT), which is crucial for the metastasis process and consequently a poor prognosis (Le *et al*., 2019; Yokokawa *et al*., 2020). The epithelial tumor cells leave a primary tumor from the disruption of tight junctions and adherent junction complexes, delocalization of tight junction proteins (zonula occludens-1, claudin-1, and occluding), and the

occurrence of reorganization of the actin cytoskeleton anchored to the focal adhesion complexes (Furue, 2011). Thus, the silencing of the TGF-α expression may be a potential therapeutic approach in cancer treatment (Le *et al*., 2019).

Currently, no alternative receptor protein able to bind TGF-α has been described in the literature other than EGFR (Endo *et al*., 2000; Ekblad *et al*., 2015). EGFR is a member of the tyrosine kinase receptor family, which generates an intracellular signaling cascade that results in cell proliferation (Lampri *et al*., 2011; Shahsavari *et al*., 2020). Cells that acquire the ability to overexpress this ligand increasing the number of EGFR on their surface can create an autocrine growth pathway, resulting in uncontrolled growth with cellular proliferation and inhibition of apoptosis (Lampri *et al*., 2015). Cells showing EGFR overexpression acquire growth advantages, especially when this is accompanied by increased expression of TGF-α (Diniz-Freitas *et al*., 2007).

Both EGFR and TGF-α are frequently overexpressed in tumor specimens of patients with head and neck squamous cell carcinoma (HNSCC) (Endo *et al*., 2000; Byeon *et al*., 2019). The binding of TGF-α to EGFR activates the Signal Transducer and Activator of Transcription (STAT) 1, and STAT 3, phosphatidylinositol 3-kinase (PI3k)/AKT/mTOR, PLCg/PKC, and the Ras-MAPk/ERk pathways to cell survival and proliferation in HNSCC (Lampri *et al*., 2015; Byeon *et al*., 2019). This suggests that the maintenance of an autocrine loop in HNSCC is dependent on the elevated levels of EGFR and the presence of TNF-α (Endo *et al*., 2000).

Tobacco smoke, a classic etiological factor for HNSCC, can increase TGF-α production, and consequently resulting in direct EGFR activation (Byeon *et al*., 2019). The high expression of EGFR is a negative prognostic factor often associated with nodal or distant metastasis, poor survival (Grandis *et al*., 1998; Lampri *et al*., 2015), local treatment failure (Byeon *et al*., 2019) and increased resistance to radiotherapy and chemotherapy in patients with HNSCC (Yokokawa *et al*., 2020). However, Shahsavari *et al*. (2020) did not find any significant correlation between EGFR expression and the TNM stage of OSCC.

TGF-α seems to be overexpressed in areas of mild dysplasia compared to normal mucosa, but no additional expression of this factor can be seen in more aggressive stages of dysplasia or even in carcinoma. However, EGFR levels increase progressively accompanying aggressiveness of dysplasia until they reach the maximum expression in HNSCC (Grandis *et al*., 1998). Similar to what happens in other cancers, the levels of EGFR expression seem to be upregulated in the normal mucosa of HNSCC patients, suggesting that increased EGFR expression is an early event in the development of HNSCC (Lampri *et al*., 2015). In fact, previous studies have already determined that about 80 % of OSCC specimens overexpress EGFR, with rare events of EGFR mutations or EGFR amplifications (Byeon *et al*., 2019; Shahsavari *et al*., 2020; Yokokawa *et al*., 2020).

Several agents that are known to trigger mechanisms of EGFR nuclear translocation in HNSCC are: EGFR ligands, Cetuximab, Epstein Barr Virus, radiation, and Src family kinase (SFK). The therapeutic resistance and cancer progression may be caused by increased activity of EGFR, which induces cell proliferation and repair of DNA damage caused by chemoradiotherapy (Byeon *et al*., 2019).

The efficacy of EGFR in HNSCC is at an advanced research stage, currently in the Phase III clinical trial investigation (Santos *et al*., 2016), and the aim of these researchers is to find specific inhibitors of EGFR. Two classes of EGFR inhibitors have already been developed and approved for various cancer therapies; they are the reversible tyrosine kinase inhibitors (TKI) (gefitinib, erlotinib), and the EGFR blocking antibodies (cetuximab, panitumumab) (Ekblad *et al*., 2015). Cetuximab is a monoclonal antibody that acts as a competitive inhibitor at the ligand-binding site of EGFR in HNSCC (Ekblad *et al*., 2015), which consequently results in the abrogation of EGFR dimerization (Byeon *et al*., 2019). The administration of Cetuximab in radiotherapy or chemotherapy has enhanced tumor control (Ekblad *et al*., 2015), and its association with platinum-based and 5-fluorouracil chemotherapy are the first-line in recurrent, unresectable or metastatic HNSCC treatment (National Comprehensive Cancer Network, 2020). Meanwhile, the cetuximab monotherapy as second-line therapy for platinum resistant recurrent/metastatic HNSCC had a poor response rate. The reversible action of TKI on EGFR has not shown any clinical benefit in HNSCC, but multi target TKIs (lapatinib, afatinib, dacomitinib) have shown promising results in recent clinical trials (Byeon *et al*., 2019).

Cancer tissues form a complex structure composed by malignant cells, nonmalignant cells, and inflammatory cells in a tumor microenvironment (Santosh *et al*., 2016). Ekblad *et al*. (2015) investigated the effect of the EGFR ligand TGF-α and cetuximab, alone and in combination, on HNSCC cell lines. They showed that TGF-α can have both growth-stimulating and growth-inhibiting effects in the same cell line just like cetuximab that can have a similar behavior, and which can be explained by the influence of tumor microenvironment.

The initial response rates to cetuximab monotherapy are far from therapeutically encouraging, mainly because responses seem to decline rapidly after a short period of effect. The major mechanisms of resistance of EGFR-targeted monoclonal antibodies has been identified as: EGFR and TGF-α overexpression, deregulation of EGFR internalization and its degradation by ubiquitination, the intracellular binding of MDG1 to EGFR that avoids extracellular targeting, EGFR nuclear translocation, enhanced SFK-mediated signaling, constitutively activated EGFR in a ligand-independent manner, KRAS mutation with constant activation of EGFR downstream signals, PI3K/AKT signal activation, increased heterodimerization of EGFR or HER2 with HER3, crosstalk with HGF-MET or VEGF-VEGFR1, and EMT (Byeon *et al*., 2019).

The proto-oncogene Src is currently being investigated for apparently promoting resistance to EGFR inhibitors. This proto-oncogene is a non-receptor tyrosine kinase, expressed in several cancers and correlates with tumor progression and metastasis development (Simatou *et al*., 2020). Once activated, Src phosphorylates the intracellular domain of EGFR facilitating the interaction between STAT 3 and 5 and EGFR forming a multiple molecular kinase complex able to transduce EGFR signals to STATs (Xi *et al*., 2003). Phase I/II clinical trials with Dasatinib, a src inhibitor including several members of the SFK family, are underway for EGFR-resistant HNSCC (Stabile *et al*., 2017).

Cluster of Differentiation 68 (CD68)

CD68 is a type I transmembrane glycoprotein with advanced glycation, composed of 354 amino acids, associated with an endosomal/lysosomal compartment. CD68 can be found in several types of non-hematopoietic cells, umbilical cord mesenchymal stem cells, fibroblasts, endothelial cells, various tumor cell lines and in smooth muscle cells of human arteries. This glycoprotein is highly expressed in the lineage of mononuclear phagocytes, such as macrophages, microglia, osteoclasts and myeloid dendritic cells (Chistiakov *et al*., 2017; Zhao *et al*., 2020). In studies using immunohistochemistry, CD68 is a marker for macrophages as well as for subsets of lymphocytes, fibroblasts and endothelial cells (Wirsing *et al*., 2018).

Tumor cells express immune markers to escape macrophage-mediated phagocytosis and effects of CD8 + cytotoxic T cell damage during invasion of a normal non-tumor tissue environment (Chistiakov *et al*., 2017). Macrophages, derived from circulating monocytes, play an important role in both innate and adaptive immunity (Ni *et al*., 2015). These tumor-associated macrophages (TAMs) become activated after infiltrating the tumor microenvironment due to their phenotypic plasticity, and can exhibit two states of functional polarization; either the pro-inflammatory M1 phenotype (anti-tumorigenic) or the anti-inflammatory M2 (protumorigenic) phenotype. TAMs acquire immunosuppressive properties and inhibit the cytotoxic activity of CD8 + T cells in tumors. CD68 is a pan macrophage marker that can express both phenotypes (M1 and M2) (Ni *et al*., 2015; Fang *et al*., 2017; Kikuchi *et al*., 2021).

The CD68 expression has been related to a higher expression of vimentin and lower expression of E-cadherin, which are important markers of EMT (Zhang *et al*., 2016). Based on this, TAMs can induce tumor cell EMT, resulting in the progression of OSCC and participating in the carcinogenesis process by activating the Gas6/Axl-NF-kB signaling pathway (Wei & Hujie, 2018).

A high density of CD68 TAMs is accompanied by high levels of stromal and high serum levels of the vascular endothelial growth factor (VEGF), which favor angiogenesis. Possibly, there is a positive regulation of VEGF in macrophages exposed to ionizing radiation that decreases the antitumor efficacy of radiotherapy. Therefore, CD68 could be used as an indicator to monitor radiotherapy treatment (Yu *et al*., 2015).

Overexpression of CD68 in tumor tissue may indicate a prometastatic state, be correlated with the degree of tumor differentiation, the chance of lymph node metastasis and a poor prognosis (Hu *et al*., 2016; Zhao *et al*., 2020). In the study by Dantas *et al*. (2019), the CD68 expression in patients with OSCC was higher in the primary tumor, compared to the surgical margin and lymph nodes, however they found no association

between the CD68 expression and prognosis. In contrast to the previous study, Wirsing *et al*. (2018) found an association between CD68 and survival in OSCC patients.

The conflicting results of survival may be due to differences in the immunohistochemical procedures, in the scoring system, in the marker used (Wirsing *et al*., 2018), in the location of the TAM infiltration (Zhao *et al*., 2020) and by the decrease of the patient's immune response (Wei & Hujie, 2018). TAMs at different tumor sites can have different effects on cancer prognosis (Kikuchi *et al*., 2021) but the impacts of CD68 micro locations on the clinical outcomes of patients with OSCC are limited (Ni *et al*., 2015). CD68 staining does not differentiate between M1 and M2 macrophages when using immunohistochemistry; therefore, some researchers have suggest combining CD68 and CD163 to generate more specificity for the M2 macrophages (Hu *et al*., 2016; Wei & Hujie, 2018).

Kikuchi *et al*. (2021), found a high density of CD68 in the intratumor region related to poor survival rates; however, in the stroma region this relationship was not true. On the other hand, in the study by Ni *et al*. (2015), the greatest expression of CD68 appeared in the tumor stroma, a place that also has a relationship with low survival rates of patients with high tumor staging and with lymph node metastasis. Hu *et al*. (2016), found this association between the expression of CD68 in the "tumor nest" and the prognosis, but did not show any relationship in the tumor stroma and the tumor cells.

In contrast to previous research, Zhao *et al*. (2020) identified that patients with high CD68 expression in cancer-associated fibroblasts (CAF) revealed low postoperative recurrence and better prognosis, with greater overall survival (OS), disease-free survival (DFS) and recurrence-free survival (RFS). However, after multivariate analyzes, CD68 expression was not considered an independent risk factor for OS, RFS and DFS in the tumor center or on the invasive front of patients with OSCC. Thus, TAMs and CAFs may show a distinct expression pattern of CD68 in the OSCC tumor microenvironment.

Anti-Inflammatory Markers

Interferon-gamma (IFN-γ). IFN-γ, which is the only representative of the type II interferon family, is a cytokine that plays a key role in mediating the immune response to different stimuli, such as microbial agents, viruses and tumors. Furthermore, as a pleiotropic agent, it is involved in the immunomodulation of innate and adaptive responses (Mendoza *et al*., 2019). Its production occurs mainly in natural killer cells (NK) and in CD4+ and CD8+ T-lymphocytes (Jorgovanovic *et al*. 2020).

Although the role of IFN-γ in the antitumor immune response remains controversial, it has demonstrated high physiological importance in immunomodulation against pathogens. A significant amount of evidence suggests that IFN-γ mediates several tumor suppressive effects, such as: induction of apoptosis by upregulation of the JAK-STAT/caspases pathway; inhibition of metastases by the FN-1 expression; stimulation of tumor senescence via the p16INK4a-Rb pathway and tumor dormancy by regulating the IDO1-Kyn-Ahr-p27 pathway (Jorgovanovic *et al*. 2020). On the other hand, there is evidence to indicate that IFN-γ also has a pro-tumorigenic role that is directly related to its concentration in the tumor microenvironment (Jorgovanovic *et al*., 2020). Low doses of IFN-γ tend to increase the metastatic potential, while the reverse effect is observed at high doses (Chen *et al*., 2011). However, the accumulation of IFN-γ in the peritumoral lymphatic vessels is also able to increase the expression of PD-L1, which reduces the migration of T-lymphocytes to the tumor microenvironment and severely affects the immune response (Lane *et al*., 2018).

A low expression of IFN-γ in OSCC associated with high levels of IL-10 and low levels of CD163 are linked to lower overall survival and tumor progression (Wang *et al*., 2014). Lower levels of IFN-γ were found in metastatic OSCC than in non-metastatic OSCC and in tissues used as controls (Costa *et al*., 2013). Interestingly, the lowest levels of IFN-γ were not associated with macrophages, which suggests that they are associated with other cell types such as CD4+ Th1 T-lymphocytes (Costa *et al*., 2013). Consistent with these findings, Boxberg *et al*. (2019) used in situ hybridization to confirm that tumor-infiltrating lymphocytes in OSCC had high levels of IFN-γ, which would correspond to a T-cell inflamed tumor microenvironment. These phenotypes demonstrate great responsiveness, including to immunotherapy (Trujillo *et al*., 2018).

Cluster of differentiation 57 (CD57). CD57 is an epitope that contains a sulfated carbohydrate (glycoepitope). It was first identified in 1981 as a marker

of NK cells (Nielsen *et al*., 2013). This marker is recognized by murine monoclonal antibodies, Human Natural Killer 1 (HNK-1) and Leu-7. Initially CD57 was considered an exclusive marker of NK cells, however, it was also found in cells derived from the neural crest (Lipinski *et al*., 1983) and in CD8+ T-lymphocytes (Markey & McDonald, 1989). In this latter cell population, persistent immune stimulation has already been shown to induce a change in the expression pattern of CD28+CD57- to CD28-CD57+ (Vallejo *et al*., 2005). CD57+ T lymphocytes have shorter telomeres, low telomerase activity and reduced capacity for proliferation, in addition to being highly cytotoxic (Nielsen *et al*., 2013; Vallejo *et al*., 2005). Therefore, CD8+CD57+ T-lymphocytes are considered terminally differentiated oligoclonal populations (Nielsen *et al*., 2013). A similar maturation process involving CD57 was also observed in NK cells, which show the CD56dimCD57+ phenotype after stimulation with IL-2 or co-culture with target cells (Lopez-Vergès *et al*., 2010).

The role of NK cells in immune recognition of cancer has been well established by a significant amount of evidence (Narendra *et al*., 2013). Higher frequencies of peripheral or tumor-associated NK cells in cancer patients have been associated with better results (Nielsen *et al*., 2013).

Research found a greater expression of CD57 in OSCC tissues was correlated with a better overall survival of patients (Taghavi *et al*., 2016), and was associated with greater differentiation of tumor squamous cells (Agarwal *et al*., 2016) and early clinical stages (Fang *et al*., 2017). The study by Fang *et al*. (2017) also found that the higher expression of CD57 was correlated with the absence of metastasis in the lymph nodes.

Conversely, a recent retrospective, case-control study by Elahi & Rakshan (2020) found that the upregulation of CD57, CD16 and MED15 were associated with poor prognoses. This worsening of prognosis was accentuated mainly in older patients with a high TNM stage (Elahi & Rakshan, 2020). Furthermore, this recent study differs from its predecessors due to the use of quantitative PCR (qPCR) to analyze the expression of the tumor markers, whereas those previously mentioned used less accurate methods, such as immunohistochemistry (IHC). However, other previous IHC-based studies have also found a negative association between survival and CD57 expression. Zancope *et al*. (2010) found no prognostic value in the expression of CD57 in NK cells infiltrated in tumors; however, this study used a small sample size. Fraga *et al*. (2012) found that CD57 could not be considered an independent prognostic variable, even though they found an association between the frequency of locoregional metastases and the density of CD57+ cells.

Nevertheless, there are many methodological differences between studies that have proven to be paradoxical. In addition, the immune-tumor interaction appears to be more complex than is currently known. The accumulation of CD8+CD57+ T-lymphocytes, for example, is associated with reduced survival of patients with different tumors, such as lymphomas, myelomas and melanomas (Nielsen, 2013). This seems to have a relation with the prolonged stimulation of this population, in a scenario in which the antitumor response is ineffective. Therefore, further studies are still needed to clarify the role of CD57 as a tumor marker in OSCC, especially with techniques that provide more accurate results.

CONCLUSION AND FUTURE PROSPECT

OSSC is the most prevalent malignant neoplasm in the oral cavity with a 5-year survival ratio ranging from 5 to 80 %, depending on the stage of the disease. The TNM classification system alone or in combination with histological grading is not sufficient to predict the prognosis of OSCC. These facts highlight the urgent need to identify novel prognostic parameters, such as biomarkers for improving patient survival, life expectancy and quality-of-life. The application of new therapeutic agents such as anti-EGFR monoclonal antibodies or multi-target tyrosine kinase inhibitors is promising as an alternative strategy for OSCC therapy. Nevertheless, the inclusion of EGFR inhibitors in the treatment protocols has already come up against tumors that are unresponsive tumors to this therapy. However, the latest strategies for the treatment of OSCC are focused on alternative drugs which are able to downstream signals (such as src) to improve the prognosis of these tumors. Such research is already undergoing clinical trials.

There is suggestive evidence that TGF-α overexpression may occur in mild dysplastic lesions. Abnormal expression of this protein can be considered potential endpoints and molecular prognostic factors for chemoprevention studies mainly in the presence of

premalignant oral lesions. Randomized studies with EGFR inhibitors should be performed to assess their potential as chemo-preventive agents, followed by the observation of cancer development in patients previously diagnosed with cancerous lesions. The fact that folate deficiency has been associated with colon cancer may provide a pathway to discovery of natural chemo-preventive compounds for high-risk malignancies.

There are still many divergent results concerning the CD68, CD57 and IFN-γ markers. While some studies have linked them to a poor prognosis, others have found that they are associated with a better antitumor immune response. Recent studies have found that different locations of these markers are linked to different prognoses. Many TAMs are correlated with low survival, perhaps through the EMT of the tumor cells induced by these macrophages, which would facilitate invasive growth and increase metastatic potential, but they are not independent predictive factors.

Methodological differences between the studies and the very complexity of the interaction between the immune system and the tumor microenvironment may be the cause of these discrepancies. Future research should consider the heterogeneity of the cell populations involved in the tumorigenic process, as well as in the acquisition of tumor phenotypes.

The current availability of prognostic biomarkers for OSCC remains far from ideal and more comprehensive studies on the role of such biomarkers will be essential for better results in this type of cancer treatment and management.

FERREIRA, K. D. M.; RAMOS, R. T.; SODRÉ, C. S.; TANNURE, P. N.; ARMADA, L.; GONÇALVES, L. S.; FERREIRA, D. C. & RIBEIRO, M. G. Biomarcadores pro y antiinflamatorios como posibles marcadores de pronóstico en el carcinoma oral de células escamosas. *Int. J. Odontostomat., 16(2)*:258-266, 2022.

RESUMEN: El método más utilizado para clasificar los factores de pronóstico en los cánceres en la actualidad es TNM. Sin embargo, el carcinoma oral de células escamosas (COCE) a menudo muestra diferentes comportamientos en relación con la agresividad y la respuesta terapéutica en la misma etapa TNM. Entonces, en tales casos, los biomarcadores pueden usarse para identificar la diversidad biológica de estos tumores de manera más confiable, lo que lleva a mejores estrategias terapéuticas y manejo de la enfermedad. La presencia de células inmunes inflamatorias en el microambiente tumoral puede tener efectos pro o antitumorales y la investigación de la expresión de marcadores inflamatorios en COCE puede ser útil para diseñar intervenciones inmunoterapéuticas. El factor de crecimiento transformante α es un potente estimulador de la migración celular que actúa sobre la proliferación celular, la invasión y metástasis del cáncer, así como la inmunosupresión y la angiogénesis. Las citocinas inflamatorias, como el IFN-γ, median en la diferenciación de macrófagos. Los macrófagos son un componente importante del microambiente COCE. La mayor cantidad de macrófagos asociados a tumores, especialmente el fenotipo M2, puede estar asociada a un comportamiento biológico más agresivo del COCE y, en consecuencia, a una menor supervivencia.

PALABRAS CLAVE: carcinoma oral de células escamosas, factor de crecimiento transformante alfa, receptor del factor de crecimiento epidérmico, antígeno 68; antígeno 57, interferón-gamma, biomarcadores, pronóstico.

REFERENCES

Agarwal, R.; Chaudhary, M.; Bohra, S. & Bajaj, S. Evaluation of natural killer cell (CD57) as a prognostic marker in oral squamous cell carcinoma: An immunohistochemistry study. *J. Oral Maxillofac. Pathol., 20(2)*:173-7, 2016

Boxberg, M.; Leising, L.; Steiger, K.; Jesinghaus, M.; Alkhamas, A.; Mielke, M.; Pfarr, N.; Götz, C.; Wolff, K. D.; Weichert, W.; *et al*. Composition and clinical impact of the immunologic tumor microenvironment in oral squamous cell carcinoma. *J. Immunol., 202(1)*:278-91, 2019.

Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R. L.; Torre, L. A. & Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin., 68(6)*:394-424, 2018.

Byeon, H. K.; Ku, M. & Yang, J. Beyond EGFR inhibition: multilateral combat strategies to stop the progression of head and neck cancer. *Exp. Mol. Med., 51*:1-14, 2019.

Cervino, G.; Fiorillo, L.; Herford, A. S.; Romeo, U.; Bianchi, A.; Crimi, S.; D'Amico, C.; De Stefano, R.; Troiano, G.; Santoro, R.; *et al*. Molecular biomarkers related to oral carcinoma: clinical trial outcome evaluation in a literature review. *Dis. Markers, 2019*:8040361, 2019.

Chen, H. C.; Chou, A. S.; Liu, Y. C.; Hsieh, C. H.; Kang, C. C.; Pang, S. T.; Yeh, C. T.; Liu, H. P. & Liao, S. K. Induction of metastatic cancer stem cells from the NK/LAK-resistant floating, but not adherent, subset of the UP-LN1 carcinoma cell line by IFN-γ. *Lab. Invest., 91(10)*:1502-13, 2011.

Chistiakov, D.; Killingsworth, M. C.; Myasoedova, V. A.; Orekhov, A. N. & Bobryshev, Y. V. CD68/macrosialin: not just a histochemical marker. *Lab. Invest., 97(1)*:4-13, 2017.

Costa, N. L.; Valadares, M. C.; Souza, P. P.; Mendonça, E. F.; Oliveira, J. C.; Silva, T. A. & Batista, A. C. Tumor-associated macrophages and the profile of inflammatory cytokines in oral squamous cell carcinoma. *Oral Oncol., 49(3)*:216-23, 2013.

Dantas, T. S.; Silva, P. G. B.; Verde, M. E. Q. L.; Ribeiro Junior, A. L.; Cunha, M. P. S. S.; Mota, M. R. L.; Alves, A. P. N. N.; Leitão, R. F. C. & Sousa, F. B. Role of inflammatory markers in prognosis of oral squamous cell carcinoma. *Asian Pac. J. Cancer Prev., 20(12)*:3636-42, 2019.

Diniz-Freitas, M.; Otero-Rey, E.; Blanco-Carrión, A.; Garcia-Caballero, T.; Rey, J. M. & Garcia-Garcia, A. *Prognostic Indicators in Oral Squamous Cell Carcinoma. Oral Cancer Research Advances*. Nova Science Publishers, 2007. pp.51-93.

Ekblad, L.; Welinder, C.; Kjellén, E.; Brun, E. & Wennerberg, J. Anti- or pro-proliferation - Conditional options for TGF-α and cetuximab in head and neck squamous cell carcinoma. *Oral Oncol., 51(1)*:46-52, 2015.

Elahi, M. & Rakhshan, V. MED15, transforming growth factor beta 1 (TGF-β1), FcgRIII (CD16), and HNK-1 (CD57) are prognostic biomarkers of oral squamous cell carcinoma. *Sci. Rep., 10(1)*:8475, 2020.

Endo, S.; Zeng, Q.; Burke, N. A.; He, Y.; Melhem, M. F.; Watkins, S. F.; Lango, M. N.; Drenning, S. D.; Huang, L. & Rubin Grandis, J. TGF-alpha antisense gene therapy inhibits head and neck squamous cell carcinoma growth in vivo. *Gene Ther., 7(22)*:1906-14, 2000.

Fang, J.; Li, X.; Ma, D.; Liu, X.; Chen, Y.; Wang, Y.; Lui, V. W. Y.; Xia, J.; Cheng, B. & Wang, Z. Prognostic significance of tumor infiltrating immune cells in oral squamous cell carcinoma. *BMC Cancer, 17(1)*:375, 2017.

Fraga, C. A.; de Oliveira, M. V.; Domingos, P. L.; Botelho, A. C.; Guimarães, A. L.; Teixeira-Carvalho, A.; Correa-Oliveira, R. & De Paula, A. M. Infiltrating CD57+ inflammatory cells in head and neck squamous cell carcinoma: clinicopathological analysis and prognostic significance. *Appl. Immunohistochem. Mol. Morphol., 20(3)*:285-90, 2012.

Furue, M. Epithelial tumor, invasion and stroma. *Ann. Dermatol., 23(2)*:125-31, 2011.

Grandis, J. R.; Melhem, M. F.; Gooding, W. E.; Day, R.; Holst, V. A.; Wagener, M. M.; Drenning, S. D. & Tweardy, D. J. Levels of TGF-a and EGFR protein in head and neck squamous cell carcinoma and patient survival. *J. Natl. Cancer Inst., 90(11)*:824-32, 1988.

Hadler-Olsen, E. & Wirsing, A. M. Tissue-infiltrating immune cells as prognostic markers in oral squamous cell carcinoma: a systematic review and meta-analysis. *Br. J. Cancer, 120(7)*:714-27, 2019.

Hu, U.; He, M. Y.; Zhu, L. F.; Yang, C. C.; Zhou, M. L.; Wang, Q..; Zhang, W.; Zheng, Y. Y.; Wang, D. M.; Xu, Z. Q.; *et al*. Tumor-associated macrophages correlate with the clinicopathological features and poor outcomes via inducing epithelial to mesenchymal transition in oral squamous cell carcinoma. *J. Exp. Clin. Cancer Res., 35*:12, 2016.

Jorgovanovic, D.; Song, M.; Wang, L. & Zhang, Y. Roles of IFN-γ in tumor progression and regression: a review. *Biomark. Res., 8*:49, 2020.

Lampri, E. S.; Chondrogiannis, G.; Ioachim, E.; Varouktsi, A.; Mitselou, A.; Galani, A.; Briassoulis, E.; Kanavaros, P. & Galani, V. Biomarkers of head and neck cancer, tools or a gordian knot? *Int. J. Clin. Exp. Med., 8(7)*:10340-57, 2015.

Lane, R. S.; Femel, J.; Breazeale, A. P.; Loo, C. P.; Thibault, G.; Kaempf, A.; Mori, M.; Tsujikawa, T.; Chang, Y. H. & Lund, A. W. IFNγ-activated dermal lymphatic vessels inhibit cytotoxic T cells in melanoma and inflamed skin. *J. Exp. Med., 215(12)*:3057-74, 2018.

Le, B. T.; Raguraman, P.; Kosbar, T. R.; Fletcher, S.; Wilton, S. D. & Veedu, R. N. Antisense oligonucleotides targeting angiogenic factors as potential cancer therapeutics. *Mol. Ther. Nucleic Acids, 14*:142-57, 2019.

Lipinski, M.; Braham, K.; Caillaud, J. M.; Carlu, C. & Tursz, T. HNK-1 antibody detects an antigen expressed on neuroectodermal cells. *J. Exp. Med., 158(5)*:1775-80, 1983.

Lopez-Vergès, S.; Milush, J. M.; Pandey, S.; York, V. A.; Arakawa-Hoyt, J.; Pircher, H.; Norris, P. J.; Nixon, D. F. & Lanier, L. L. CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset. *Blood, 116(19)*:3865-74, 2010.

Markey, A. C. & MacDonald, D. M. HNK-1 antigen is not specific for natural killer cells. *J. Invest. Dermatol., 92(5)*:774-5, 1989.

McDermott, J. D. & Bowles, D. W. Epidemiology of head and neck squamous cell carcinomas: impact on staging and prevention strategies. *Curr. Treat Options. Oncol., 20(5)*:1-13, 2019.

Mendoza, J. L.; Escalante, N. K.; Jude, K. M.; Sotolongo Bellon, J.; Su, L.; Horton, T. M.; Tsutsumi, N.; Berardinelli, S. J.; Haltiwanger, R. S.; Piehler, J., *et al*. Structure of the IFNγ receptor complex guides design of biased agonists. *Nature, 567(7746)*:56-60, 2019.

Narendra, B. L.; Reddy, K. E.; Shantikumar, S. & Ramakrishna, S. Immune system: a double-edged sword in cancer. *Inflamm. Res., 62(9)*:823-34, 2013.

National Comprehensive Cancer Network (NCCN). *Head and Neck Cancer - NCCN Clinical Practice Guidelines in Oncology (Guideline Version 2.2020), 2020*. Available from: https://www.nccn.org/professionals/physician_gls/pdf/head-and-neck.pdf

Ni, Y. H.; Ding, L.; Huang, X. F.; Dong, Y. C.; Hu, Q. G. & Hou, Y. Y. Microlocalization of CD68+ Tumor-Associated Macrophages in Tumor Stroma Correlated With Poor Clinical Outcomes in Oral Squamous Cell Carcinoma Patients. *Tumour Biol., 36(7)*:5291-8, 2015.

Nielsen, C. M.; White, M. J.; Goodier, M. R. & Riley, E. M. Functional Significance of CD57 Expression on Human NK Cells and Relevance to Disease. *Front. Immunol., 4*:422, 2013.

Peltanova, B.; Raudenska, M. & Masarik M. Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: A systematic review. *Mol. Cancer, 18(1)*:1-4, 2019.

Roberts, A. B.; Anzano, M. A.; Lamb, L. C.; Smith, J. M. & Sporn, M. B. New class of transforming growth factors potentiated by epidermal growth factor: Isolation from non-neoplastic tissues. *Proc. Natl. Acad. Sci. U. S. A., (9 II)*:5339-43, 1981.

Sabbah, D. A.; Hajjo, R. & Sweidan, K. Review on Epidermal Growth Factor Receptor (EGFR) Structure, Signaling Pathways, Interactions, and Recent Updates of EGFR Inhibitors. *Curr. Top. Med. Chem., 20(10)*:815–34, 2020.

Santosh, A.; Jones, T. & Harvey, J. A review on oral cancer biomarkers: Understanding the past and learning from the present. *J. Cancer Res. Ther., 12(2)*:486-92, 2016.

Shahsavari, F.; Miri, R. & Ghorbanpour, M. Expression of epidermal growth factor receptor in oral and esophageal squamous-cell carcinoma. *Dent. Res. J. (Isfahan), 17(2)*:85, 2020.

Sherbet, G. V. *The Epidermal Growth Factor (EGF) Family. Growth Factors and Their Receptors in Cell Differentiation, Cancer and Cancer Therapy*. Amsterdam, Elsevier, 2011. pp.173-98.

Simatou, A.; Simatos, G.; Goulielmaki, M.; Spandidos, D.; Baliou, S. & Zoumpourlis, V. Historical retrospective of the SRC oncogene and new perspectives (Review). *Mol. Clin. Oncol., 13(4)*:21, 2020.

Stabile, L. P.; Egloff, A. M.; Gibson, M. K.; Gooding, W. E.; Ohr, J.; Zhou, P.; Rothenberger, N. J.; Wang, L.; Geiger, J. L.; Flaherty, J. T.; *et al*. IL6 is associated with response to dasatinib and cetuximab: Phase II clinical trial with mechanistic correlatives in cetuximab-resistant head and neck cancer. *Oral Oncol., 69*:38-45, 2017.

Sun, J.; Cui, H.; Gao, Y.; Pan, Y.; Zhou, K.; Huang, J.; Lan, J.; Wei, Q.; Liu, X.; Liu, L.; *et al*. TGF- a overexpression in breast cancer bone metastasis and primary lesions and tgf-α enhancement of expression of procancer metastasis cytokines in bone marrow mesenchymal stem cells. *Biomed. Res. Int., 2018*:6565393, 2018.

Taghavi, N.; Bagheri, S. & Akbarzadeh, A. Prognostic implication of CD57, CD16, and TGF-β expression in oral squamous cell carcinoma. *J. Oral Pathol. Med., 45(1)*:58-62, 2016.

Takahashi, H.; Sakakura, K.; Kudo, T.; Toyoda, M.; Kaira, K. & Oyama, T, Chikamatsu K. Cancer-associated fibroblasts promote an immunosuppressive microenvironment through the induction and accumulation of protumoral macrophages. *Oncotarget, 8(5)*:8633-47, 2017.

Trujillo JA, Sweis RF, Bao R, Luke JJ. T Cell-inflamed versus non-T cell-inflamed tumors: a conceptual framework for cancer immunotherapy drug development and combination therapy selection. *Cancer Immunol. Res., 6(9)*:990-1000, 2018.

Vallejo, A. N. CD28 extinction in human T cells: altered functions and the program of T-cell senescence. *Immunol. Rev., 205*:158-69, 2005.

Wang, S.; Sun, M.; Gu, C.; Wang, X.; Chen, D.; Zhao, E.; Jiao, X. & Zheng J. Expression of CD163, interleukin-10, and interferon-gamma in oral squamous cell carcinoma: mutual relationships and prognostic implications. *Eur. J. Oral Sci., 122(3)*:202-9, 2014.

Wei, W. & Hu-Jie, C. Association of the infiltration of tumor-associated macrophages, expression of Smad7 protein and prognosis in oral squamous cell carcinoma. *Arch. Oral Biol., 95*:22-9, 2018.

Wirsing, A. M.; Ervik, I. K.; Seppola, M.; Uhlin-Hansen, L.; Steigen, S. E. & Hadler-Olsen, E. Presence of high-endothelial venules correlates with a favorable immune microenvironment in oral squamous cell carcinoma. *Mod. Pathol., 31(6)*:910-22, 2018.

Wong, D. T. W. TGF-a and oral carcinogenesis. *Eur. J. Cancer Part B Oral Oncol., 29(1)*:3-7, 1993.

Xi, S.; Zhang, Q.; Dyer, K. F.; Lerner, E. C.; Smithgall, T. E.; Gooding, W. E.; Kamens, J. & Grandis, J. R. Src kinases mediate STAT growth pathways in squamous cell carcinoma of the head and neck. *J. Biol. Chem., 278(34)*:31574-83, 2003.

Yokokawa, M.; Morita, K. I.; Oikawa, Y.; Kayamori, K.; Sakamoto, K.; Ikeda, T. & Harada, H. Co-expression of EGFR and MET has a synergistic effect on the prognosis of patients with oral squamous cell carcinoma. *J. Oral Pathol. Med., 49(3)*:235-42, 2020.

Yu, X.; Guo, C.; Fisher, P. B.; Subjeck, J. R. & Wang, X. Y. Scavenger receptors: emerging roles in cancer biology and immunology. *Adv. Cancer Res., 128*:309-64, 2015.

Zancope, E.; Costa, N. L.; Junqueira-Kipnis, A. P.; Valadares, M. C.; Silva, T. A.; Leles, C. R.; Mendonça, E. F. & Batista, A. C. Differential infiltration of CD8+ and NK cells in lip and oral cavity squamous cell carcinoma. *J. Oral Pathol. Med., 39(2)*:162-7, 2010.

Zhang, J.; Yan, Y.; Yang, Y.; Wang, L.; Li, M.; Wang, J.; Liu, X.; Duan, X. & Wang, J. High infiltration of tumor-associated macrophages influences poor prognosis in human gastric cancer patients, associates with the phenomenon of EMT. *Medicine (Baltimore), 95(6)*:e2636, 2016.

Zhao, X.; Ding, L.; Lu, Z.; Huang, X.; Jing, Y.; Yang, Y.; Chen, S.; Hu, Q. & Ni, Y. Diminished CD68 + cancer-associated fibroblast subset induces regulatory T-cell (Treg) infiltration and predicts poor prognosis of oral squamous cell carcinoma patients. *Am. J. Pathol., 190(4)*:886-9, 2020.

Corresponding author:
Karla Daniella Malta Ferreira
Evangelical Hospital of Cachoeiro de Itapemirim
Manoel Braga Machado Street, 02. Ferroviários
Cachoeiro de Itapemirim
Espírito Santo
CEP 29308-065
BRAZIL

E-mail: karlamaltaferreira@gmail.com