Archives of Oral Biology 61 (2016) 28–35

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Overexpression of immunosuppressive cytokines is associated with poorer clinical stage of oral squamous cell carcinoma

Diego Antonio Costa Arantes[a], Nádia Lago Costa[a], Elismauro Francisco Mendonça[a], Tarcília Aparecida Silva[b], Aline Carvalho Batista[a,*]

[a] Department of Stomatology (Oral Pathology), Dental School, Federal University of Goiás, Goiânia, Brazil
[b] Department of Oral Surgery and Pathology, Dental School, Federal University of Minas Gerais, Belo Horizonte, Brazil

ARTICLE INFO

Article history:
Received 10 February 2015
Received in revised form 26 August 2015
Accepted 11 October 2015

Keywords:
Interleukin-10
Transforming growth factor β
Oral squamous cell carcinoma
Immunosuppression
Oral cancer

ABSTRACT

Objective: The aim of this study was to evaluate the expression of IL-10 and TGF-β2 in oral squamous cell carcinoma (OSCC) and its relationship with prognostic clinical and microscopic parameters.
Design: Immunohistochemistry was used to assess the expression of IL-10 and TGF-β2 in OSCC samples from 43 patients who had undergone surgical excision and neck dissection. Metastatic lymph nodes were included in the study ($n = 23$). Samples of healthy oral mucosa ($n = 20$) were used as controls. The sections were evaluated using a semi-quantitative method in conjunction with staining intensity.
Results: Our findings showed that the expression of IL-10 and TGF-β2 by neoplastic and stromal cells was high in most of the OSCC samples (>70% of samples), especially when compared to the controls (≅10% of samples) ($P < 0.05$). OSCC neoplastic cells in cervical lymph nodes were also positive for IL-10 and TGF-β2. An association between high expression of IL-10 by neoplastic cells and advanced clinical stage (T3-T4) was verified ($P = 0.02$). Although not statistically significant, the expression of TGF-β2 was also augmented in advanced stage tumours.
Conclusions: These data suggest that the ability of OSCC neoplastic cells to secrete immunosuppressive cytokines could contribute to clinical progression by maintaining a microenvironment conducive to evasion and tumour proliferation.

1. Introduction

Interleukin 10 (IL-10) and transforming growth factor β (TGF-β) are anti-inflammatory and immunosuppressive cytokines that can contribute to the immune escape of neoplastic cells (Kryczek et al., 2006; Matsuda et al., 1994; Thomas & Massague, 2005). IL-10 prevents antigen presentation by macrophages and dendritic cells, reducing the expression of the MHC class I molecules on the cell surface (Matsuda et al., 1994), regulating the differentiation of T regulatory (T reg) cells (Kryczek et al., 2006) and inducing the formation of a tumour cell phenotype resistant to the action of cytotoxic T lymphocytes (CTLs) (Kurte et al., 2004). TGF-β contributes to the loss of differentiation of tumour cells (Ma et al., 2013), angiogenesis (Pertovaara et al., 1994) and the synthesis of proteolytic enzymes (Kim, Shang, Chen, Pflugfelder, & Li, 2004). It also inhibits the production and secretion of perforin and granzyme proteins from CTLs (Thomas & Massague, 2005) and has a role in the epithelial-mesenchymal transition (Xu, Lamouille, & Derynck, 2009).

The roles of IL-10 and TGF-β in the tumoural microenvironment and their potential to influence the clinical course of the disease have been investigated in different tumours (Chandler, Rassekh, Roadman, & Ducatman, 2002; Fujieda, Sunaga, Tsuzuki, Fan, & Saito, 1999; Gholamin et al., 2009; Gonçalves et al., 2015; Hatanaka et al., 2000; Ma et al., 2013). Earlier studies showed an association between higher expression of IL-10 and poorer prognosis in melanoma (Nemunaitis, Fong, Shabe, Martineau, & Ando, 2001), oesophageal (Gholamin et al., 2009), lung (Hatanaka et al., 2000), hepatocellular (Chan et al., 2012) and ovarian carcinomas (Pisa et al., 1992).

Regarding TGF-β, the distinct expression of two isoforms (β1 and β2) of this cytokine in tumours of different types and locations has

Abbreviations: IL-10, interleukin-10; TGF-β, transforming growth factor β; OSCC, oral squamous cell carcinoma; CTLs, cytotoxic T lymphocytes; APCs, WHO, World Health Organization; UICC, Union for International Cancer Control; HE, hematoxylin and eosin; IRS, immunoreactive score.

* Corresponding author at: Disciplina de Patologia Geral e Bucal, Faculdade de Odontologia, Universidade Federal de Goiás, Praça Universitária S/N, Setor Universitário, CEP 74605-220, Brazil. Fax: +55 62 3209 6327.
E-mail addresses: diegoantonio_arantes@hotmail.com (D.A.C. Arantes), nadialago@hotmail.com (N.L. Costa), elismaur@ufg.br (E.F. Mendonça), tarcilia@odonto.ufmg.br (T.A. Silva), ali.caba@uol.com.br (A.C. Batista).

http://dx.doi.org/10.1016/j.archoralbio.2015.10.013
0003-9969/

been demonstrated (Kjellman et al., 2000; Ma et al., 2013; Tsamandas et al., 2004; Vagenas, Spyropoulos, Gavala, & Tsamandas, 2007; Belle, Rodeck, Nuamah, Halpern, & Elder, 1996). Of these isoforms, TGF-β1 is considered pleiotropic because of its ability to either promote or suppress carcinogenesis (Akhurst & Derynck, 2001). High expression of TGF-β1 has been related to a poorer prognosis for patients with oesophageal (Gholamin et al., 2009), gastric (Ma et al., 2013), breast (Walker, Dearing, & Gallacher, 1994), hepatocellular (Ito et al., 1995), lung (Hasegawa et al., 2001), prostate (Desruisseau, Ghazarossian-ragni, Chinot, & Martin, 1996), pancreatic (Javle et al., 2014) or adenocystic carcinomas (Dong et al., 2013). On the other hand, other studies found that higher expression of this same TGF isoform could be related to improved survival of patients with gastric (Vagenas et al., 2007) or colorectal carcinomas (Tsamandas et al., 2004) and bears an inverse correlation with the proliferation index of squamous cell carcinoma of the lip (Salvadori et al., 2014). Regarding isoform TGF-β2, the expression of this cytokine has been related to a worse prognosis for patients with gastric (Ma et al., 2013; Vagenas et al., 2007) or colorectal carcinomas (Tsamandas et al., 2004), gliomas (Kjellman et al., 2000) and advanced melanomas (Van Belle et al., 1996).

Studies have been undertaken to evaluate IL-10 and TGF- β expression in squamous cell carcinoma of the oral cavity (OSCC) or (Chandler et al., 2002; Chen, Wang, Lin, Lee, & Chen, 2012; Chen et al., 2013; Costa et al., 2013; Fujieda et al., 1999; Gaur, Singh, Shukla, & Das, 2014; Gonçalves et al., 2015; Hamzavi et al., 2013; Mincione et al., 2008; Salman et al., 2015), in particular, the clinicopathological significance of these cytokines for disease (Chen et al., 2012, 2013; Costa et al., 2013; Gaur et al., 2014; Gonçalves et al., 2015; Hamzavi et al., 2013). Recently, Gaur et al. (2014) observed that high expression of IL-10 and TGF-β in patients with OSCC is associated with more advanced stages of the disease (Gaur et al., 2014). Costa et al. (2013) have also suggested that elevated gene expression of IL-10 and TGF-β in OSCC could be related to immunosuppressive events that contribute to a worse prognosis for patients (Costa et al., 2013). In that same year, Hamzavi et al. (2013) and Chen et al. (2013) showed a relationship between high expression of IL-10 and a worse prognosis for patients with OSCC (Chen et al., 2013; Hamzavi et al., 2013). As regards TGF-β1, Logullo et al. (2003) and Mincione et al. (2008) showed high expression of this cytokine in well-differentiated OSCC samples (Logullo et al., 2003; Mincione et al., 2008). Subsequently, Chen et al. (2012) and Elahi, Rakhshan, Ghasemian, and Moshref (2012) observed a correlation between high expression of TGF-β1 in OSCC and an unfavourable prognosis for patients with this tumour (Chen et al., 2012; Elahi et al., 2012).

Recently, a study by our group evaluated the expression of IL-10 and TGF-β1 in tissue and saliva samples from patients with OSCC (Gonçalves et al., 2015). This study showed high expression of IL-10 in OSCC tissue and saliva samples compared to those of healthy individuals. However, TGF-β1 expression was low or absent in most samples of OSCC evaluated (Gonçalves et al., 2015). Although earlier studies have demonstrated the role of TGF-β2 in the pathogenesis of certain malignancies (Kjellman et al., 2000; Ma et al., 2013; Tsamandas et al., 2004; Vagenas et al., 2007; Van Belle et al., 1996), to date no study has evaluated the expression of this TGF isoform in OSCC. Herein, this study investigated the expression of the TGF-β2 isoform in OSCC and the relationship between this cytokine and IL-10 with prognostic clinical and microscopic factors.

2. Materials and methods

2.1. Sample selection

The study was approved by the Ethics Committee on Human Research (CEB/UFG Protocol 032/2011). In this cross-sectional study, 43 specimens were selected after the surgical excision of oral squamous cell carcinoma (OSCC) in patients who had undergone neck dissection, treated at the Head and Neck Division of the Araújo Jorge Hospital Association Against Cancer in Goiás, Goiânia, Brazil (HAJ/ACCG). The lymph nodes from all of such patients were evaluated microscopically for investigation of absence ($n = 20$ patients) or presence metastasis ($n = 23$ patients). Following this assessment, it was included in the study only one positive lymph node of each patient with metastatic OCSCC ($n = 23$ lymph nodes). Furthermore, 20 samples of clinically and histologically normal oral mucosa (obtained adjacent to oral pigmentations) were included, all of which were selected from the Oral Disease Center of Goiás State (Oral Pathology Laboratory) of the Federal University of Goiás, Brazil.

Patients who had undergone surgical treatment (removal of lesion and cervical lymph nodes) with medical follow-up were included in the study. Those patients with squamous cell carcinoma of the lip and oropharynx, those who did not return for their medical follow-up after surgical removal of the tumour and those who had received radiotherapy, chemotherapy or any other treatment before surgery were also excluded from the study. The T and N clinical staging was established according to the classification adopted by the Union for International Cancer Control (UICC) (Edge et al., 2010). Medical records provided clinico-demographic data such as age, gender, ethnicity, alcohol and tobacco consumption (yes or no), and information on follow-up, including survival time, recurrence and mortality.

2.2. Microscopic analysis

The specimens selected, previously fixed in 10% buffered formalin (pH 7.4), were embedded in paraffin and subsequently sectioned by microtome (Leica RM2165). Each block provided consecutive 5 μm sections, which were placed on slides and stained with hematoxylin and eosin (HE). These sections were used for the microscopic characterization of all samples, as all lymphonodal sections were evaluated for the presence of metastasis and those of primary OSCC were graded according to the World Health Organization (WHO) tumour classification (Barnes, Eveson, Reichart, & Sidransky, 2005) and according to the criteria described by Bryne, Koppang, Lilleng, and Kjaerheim (1992). The depth of invasion (in mm) was measured using a millimetre ruler attached to a light microscope (×100), which allowed for an investigation of the depth of tumour invasion from the surface of the epithelium to the deepest front of the invasion.

2.3. Immunohistochemistry

Immunohistochemistry was performed as previously described (Gonçalves et al., 2014, 2015). For immunohistochemical staining, serial sections of 3 μm placed on silanized slides were used. For antigenic exposure of IL-10 and TGF-β2 proteins, the sections were incubated for 25 min in citrate buffer (pH 6.0) and EDTA (pH 9.0), respectively. Then, the slides were incubated with their respective primary antibodies previously subjected to standardization tests: IL-10 monoclonal mouse anti-human antibody (Abcam Inc.—ab34843, Cambridge, MA, USA, diluted 1:300) and monoclonal rabbit anti-human TGF-β2 antibody (Santa Cruz Biotechnology—sc124019, diluted 1:50). Subsequently, the sections containing anti-IL-10 and TGF-β2 were incubated with the Advanced Kit (K4067 and 4068, Dako). Positive controls: positive expression of IL-10 in immune-inflammatory cells in OSCC and TGF-β2 in neoplastic cells of gastric carcinoma. Negative controls were obtained by omitting the primary antibody, which was replaced by phosphate-buffered saline (PBS, 0.4 mM NaCl, 10 mM $NaPO_4$)