

Density and migration of mast cells in lip squamous cell carcinoma and actinic cheilitis

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Summary. Mast cells (MCs) display a diversity of roles that may contribute to the stromal microenvironment alterations during tumor progression. The aim of this study was to investigate MC populations expressing tryptase and c-kit in lip squamous cell carcinoma (lip SCC) (n=37), actinic cheilitis (AC) (n=15) and normal lip mucosa (control) (n=6), as well as their relationship with microscopic parameters (collagen degeneration, elastin changes, angiogenesis and proliferative index). Tryptase, c-kit, CD31 and Ki-67 expressions were analyzed by means of immunohistochemistry and collagen and elastic fibers were visualized with Picrosirius and Verhoeff's stain, respectively. The numbers of tryptase+ MC were significantly higher in lip SCC when compared with control (P=0.01), while a similar density of these cells was observed in AC and lip SCC (P=0.09). The density of c-kit+ MC was similar in all groups examined (P=0.65). MC migration (c-kit+/Tryptase+ relationship) was 69% in lip SCC, 60% in AC and 100% in control. The number of CD31+ blood vessels was significantly higher in the lip SCC when compared with control and AC (P<0.01). The increase of MCs and angiogenesis in lip SCC may reflect an important modification in the tumor microenvironment during squamous photo-carcinogenesis.

Key words: Mast cell, Lip squamous cell carcinoma, Actinic cheilitis, Tryptase, C-kit

Introduction

Chronic sun exposure, particularly to ultraviolet B (UV radiation of 280-320 nm), has been described as a significant risk factor for the development of actinic cheilitis (AC), which is a pre-malignant lesion, and of lip squamous cell carcinoma (SCC) (English et al., 1998; Rojas et al., 2004, 2005). SCC of the lip is a common malignancy in Brazil (latitude 10.00 S) (Antunes et al., 2001; Vartanian et al., 2004; Durazzo et al., 2005) and, in particular, in the state of Goiás (latitude 15.55 S), whose economy is based on farming and cattle raising (Curado, 2003). Lip SCC patients usually have a better prognosis and a lower rate of regional lymph node metastasis and mortality when compared with oral cavity SCC (Leite and Koifam, 1998; Antunes et al., 2001; Vartanian et al., 2004); however the local invasion of this tumor contributes to extensive surgical procedures with loss of aesthetics and quality of life (Ortiz-Luna et al., 2004).

Although many genetic and environmental factors are involved in photocarcinogenesis (Nishigori, 2006), evidence suggests that the tumor microenvironment is able to influence several cell processes, such as growth, death, differentiation, gene expression, migration and invasion (Balkwill and Mantovani, 2001; Coussens and Werb, 2002; Pupa et al., 2002; Mignogna et al., 2004; Balkwill, 2004). In fact, the tumor-associated inflammatory infiltrate may contribute to squamous tumorigenesis (Balkwill and Mantovani, 2001; O'Byrne and Dalglish, 2001; Coussens and Werb, 2002; Balkwill, 2004; Mignogna et al., 2004).

In this context, a recent study has shown that mast cells (MCs) are significantly increased in chronically sun-exposed human skin (Grimbaldeston et al., 2006). In

addition, UV light induces MC synthesis and release of several mediators, such as metalloproteinases, chymase, basic fibroblast growth factor (bFGF), tryptase, heparin, histamine, tumor necrosis factor alpha (TNF- α), several interleukins (IL-3, -4, -5, -6, -8, -10, -13, and -16), chemokines (MCP-1/CCL2, MIP-1 β /CCL3, MIP-1 β /CCL4 and RANTES/CCL5) and lipidic mediators (Metcalfe et al., 1997; Zhao et al., 2002; Walsh, 2003; Grimbaldston et al., 2006), capable of stimulating extracellular matrix degradation, angiogenesis and innate and acquired immune responses (Metcalfe et al., 1997; Walsh, 2003; Grimbaldston et al., 2006). Furthermore, MCs present a long half-life and are capable of undergoing multiple cycles of degranulation followed by regranulation (Grimbaldston et al., 2006).

MCs are derivatives of hematopoietic progenitor cells in the bone marrow. These progenitor cells enter the circulation and migrate to peripheral tissues where, under the influence of local microenvironmental factors and Stem cell factor (SCF) (Galli et al., 1995; Gurish and Boyce, 2002; Grimbaldston et al., 2006), they complete differentiation into mature MCs (Galli et al., 1995; Gurish and Boyce, 2002; Grimbaldston et al., 2006). SCF stimulates directional motility of both mucosal and connective tissue-type MC (Meininger et al., 1992), and also plays a role in survival (Valent et al., 1992; Galli et al., 1995; Gurish and Boyce, 2002) and activation via the up-regulation of TNF- α and chemokine production and the induction of histamine release (Oliveira and Lukacs, 2001). SCF mediates its actions by interacting with SCF-receptor or c-kit (encoded by the c-kit proto-oncogene) and is up-regulated by chronic UV irradiation (Kligman and Murphy, 1996). The SCF/c-kit activation pathway is crucial for MC infiltration, as shown by mice carrying mutations in the c-kit locus, which exhibit reduced c-kit tyrosine kinase-dependent signaling, resulting in mast cell deficiency (Grimbaldston et al., 2005).

Recent studies have shown that MCs are significantly increased in human AC (Rojas et al., 2004) and lip SCC (Rojas et al., 2005) and are associated with tumor-favoring effects (Iamaroon et al., 2003; Rojas et al., 2005). Moreover, in an experimental model of carcinoma development through the up-regulation of angiogenesis during pre-malignant and malignant stages of squamous epithelial carcinogenesis (Flynn et al., 1991; Coussens et al., 1999). On the other hand, some studies suggest a tumor antagonistic effect of MCs, given that mast cell-deficient mice have an increased tumor incidence after treatment with a carcinogenic agent (Tanooka et al., 1982).

In this study, we investigated MC populations expressing tryptase and c-kit in lip SCC, AC and normal lip mucosa and their relationship with microscopic parameters, such as angiogenesis, extracellular matrix degradation, degree of elastin changes and tumor proliferative index.

Materials and methods

Samples

The thirty-seven surgically excised specimens of primary lip squamous cell carcinoma (lip SCC) were obtained from the archives of the Anatomopathology and Cytopathology Division of Araújo Jorge Hospital, Association of Cancer Combat of Goiás, Goiânia, Brazil. All patients were submitted to surgical treatment and none received radiotherapy, chemotherapy or other treatment prior to surgery. Clinical data (gender, age, ethnic group, sun-exposition, tobacco and alcohol consumption, tumor location, extension, T and N stages) and follow up information (recurrence and death) were obtained from medical records.

The fifteen cases of actinic cheilitis (AC) and six cases of lip mucosa without microscopic inflammation and without changes in epithelium and connective tissues (control) were obtained from the archives of the Oral Pathology Laboratories of the Dental Schools of the Federal University of Goiás and the Federal University of Minas Gerais, Brazil.

Furthermore, to investigate the influence of aging on mast cell density and migration, five lip mucosal samples without epithelial dysplasia and eight lip SCC of young patients were analyzed. This study was approved by the institutional ethics committee for human subjects.

Light microscopy

All specimens were fixed in 10% buffered formalin (pH 7.4) and paraffin-embedded. The microscopic features were evaluated by the analysis of one 5 μ m-section of each sample, stained routinely with hematoxylin and eosin. The microscopic features were analyzed according to the World Health Organization (WHO) classification of tumors, 1997 (Pindborg et al., 1997), by 2 independent examiners (T.A.S. and A.C.B.). In addition, the intensity of the associated inflammatory infiltrate (mild, moderate or intense) was determined in lip SCC specimens. We analyzed 10 representative microscopic high-power fields (x400) using an integration graticule and considered specimens to demonstrate: mild inflammation when the majority of the fields (> 7 fields) demonstrated less than 35% of integration graticule space filled by inflammatory cells; moderate inflammation when the majority of the fields (> 7 fields) demonstrated 35-70% of integration graticule space filled by inflammatory cells and intense when the majority of fields (> 7 fields) demonstrated more than 70% of integration graticule space filled by inflammatory cells.

Histochemistry

Collagen degeneration was evaluated in the

Mast cells in lip squamous cell carcinoma

subepithelial area and the tumor front of the samples of AC and lip SCC, respectively, using Picrosirius red staining. These samples were divided into four grades: grade I, up to 25% of collagen degeneration; grade II, 25-50%; grade III, 50-75% and grade IV more than 75%, as adapted from Silva (2007) (Silva et al., 2007). As a microscopical marker of photodamage, the accumulation in number and diameter of elastic fibres was measured with Verhoeff's stain. The elastin content was also assessed in the subepithelial area/tumor front of the AC and lip SCC samples using a scoring index of 1+ to 4+, where 1+ represents a fine meshwork of delicate elastin fibers with a normal appearance, 2+ represents a mild increase in fiber densities, 3+ represents a marked increase in fiber densities and thickness, and 4+ represents an extremely marked increase in fiber densities and thickness (Grimbaldeston et al., 2006).

Immunohistochemistry

Mouse anti-human MC tryptase monoclonal antibody (M7052, DAKO, Glostrup, Denmark) was utilized at 1:2000 and rabbit anti-human c-kit (CD 117) polyclonal antibody (A4502, DAKO) was utilized at 1:200. To evaluate angiogenesis, the identification of blood vessels was made using mouse anti-human CD31 monoclonal antibody (clone 1A10, Novocastra, Newcastle, UK), diluted at 1:200. Additionally, for the assessment of growth fractions of the lip SCC cases, an immunohistochemical study of the expression of Ki-67 (clone MM1, Novocastra), diluted 1:100, was also carried out. Briefly, paraffin-embedded tissues were sectioned (3 μ m) and collected in serial sections on glass slides coated with 2% 3-aminopropyltriethylsilane (Sigma Chemicals, St. Louis, MO). The sections were deparaffinized by immersion in xylene, and this was followed by immersion in alcohol and then incubation with 3% hydrogen peroxide diluted in Tris-buffered saline (TBS) (pH 7.4) for 40 minutes. Next, the sections were immersed in citrate buffer, pH 6.0, for 20 minutes at 95°C for antigen retrieval. Soon afterwards, the sections were blocked by incubation with 3% normal goat serum diluted in distilled water, at room temperature, for 20 minutes. The slides were then incubated with the primary antibodies at 4°C overnight in a humidified chamber.

After washing in TBS, the sections were treated with labeled streptavidin-biotin (LSAB) kits (K0492, DAKO). The sections were then incubated with 3,3'-Diaminobenzidine (DAB) in a chromogen solution (K3468, DAKO) for 2 to 5 minutes at room temperature. Finally, the sections were stained with Mayer's hematoxylin and were covered. Negative controls were obtained by the omission of primary antibodies, which were substituted by 1% PBS-BSA and by non-immune rabbit (X0902, DAKO) or mouse serum (X0910, DAKO).

Quantitative and qualitative analysis

The number of tryptase⁺ MCs and c-kit⁺ MCs and blood vessels were determined separately, in the region adjacent to the subepithelial area of the controls and AC areas and at the tumor front of lip SCC, using an integration graticule (CARL ZEISS-474068000000-Netzmikrometer, 12.5x). The blood vessels (macro and especially microvessels) were identified by the immunostaining of CD31 protein present in the endothelial membrane. Only blood vessels (CD31⁺ blood vessels) that demonstrated vascular lumens were considered, even when vascular lumens were very small (microvessels). All cells and blood vessels were enumerated in 10 representative and consecutive microscopic high-power fields (x400) and, at this magnification, each field (integration graticule) had an area of 0.0961 mm². The locations and distributions of tryptase⁺ MC, c-kit⁺ MC and CD31⁺ blood vessels were also analyzed. Descriptive analyses were expressed as mean \pm standard deviation (SD) of n observations, per mm². Comparative analyses were performed using the non-parametric Kruskal Wallis, followed by the Dunn test and/or Mann-Whitney test. The number of cells demonstrating staining for Ki-67 was calculated as the proportion of the total neoplastic population in lip SCC. The correlation between tryptase⁺ and c-kit⁺ MC numbers was calculated, as was the correlation between microscopical characteristics (grade of collagen degeneration, grade of elastin changes, density of CD31⁺ blood vessels and proportions of Ki-67⁺ neoplastic cells), using Spearman correlation coefficients. Significance was set at 0.05.

Results

During the survey of the archives of the Anatomopathology Division of the Araújo Jorge Hospital over two years and during the median of follow up of 48 months (38-172 months), only one patient with lip SCC presented cervical lymph node metastasis and died. Other clinical features of our series of 37 patients with lip SCC are summarized below (Table 1). The microscopic analysis revealed, in the majority of the samples of lip SCC, an intense inflammatory infiltrate located adjacent to the tumor front, and solar elastosis in proximity with tumor islands was observed in all specimens. Details concerning microscopic findings are summarized in Table 2. The analysis of 8 additional younger patients with lip SCC demonstrated that all were Caucasian, with a male predominance (80%), an age that varied from 33-40 years (mean= 35 years old) and none presented cervical lymph node metastasis or died.

The analysis of 15 patients with pre-malignant lesions (Actinic Cheilitis) revealed that all were Caucasian, with a male predominance (60%) and an age that varied from 45-90 years (mean= 53.6 \pm 16.99). All

specimens were obtained from the inferior lip and evolution varied from 1 to 15 years. All lesions were associated with sun exposure. Microscopically-mild epithelial dysplasia and mild inflammatory infiltrate were found in all AC samples. In addition, these samples demonstrated intense collagen degeneration (grade IV) in 64% of the samples and collagen degeneration of up to 25% (grade I) was observed in the remainder of the samples. With regard to the changes in elastin fibers, 80% of the AC samples presented a marked increase in fiber density and thickness (grade +3) and 20% showed an extremely marked increase in fiber density and thickness (grade +4).

Resident MCs were clearly identified by the use of anti-tryptase antibody and MC migration was evaluated with an anti-c-kit antibody. Tryptase- or c-kit-expressing cells infiltrating the pre-malignant and malignant lesions and controls corresponded, as expected, to MC, according to morphological appearance. In lip SCC, AC and controls, both tryptase⁺ and c-kit⁺ MCs were scattered relatively evenly throughout the connective tissue (Fig. 1A-F) and, in lip SCC, MC were also observed in morphological contact with tumoral cells (Fig. 1C, F).

The numbers of tryptase⁺ MC were significantly higher in lip SCC when compared with control (P=0.013). A similar population of these cells was

observed in AC and lip SCC (P=0.09) (Fig. 2). The density of c-kit⁺ MC was similar in lip SCC, AC and control (P=0.65) (Fig. 2). However, MC migration (c-kit⁺/Tryptase⁺ relationship) was 69% in lip SCC, 60% in AC and 100% in normal lip mucosa. When the two MC populations were compared, significantly higher numbers of tryptase⁺ in relation to c-kit⁺ cells were observed in lip SCC (P=0.01), but not in the AC and control (P=0.08) (Fig. 2).

Our results also demonstrated that the densities of tryptase⁺ and c-kit⁺ MC were similar in lip SCC when comparing old (125±21 and 86±14) and young patients (131±30 and 87±23) (P=0.07). In addition, a significant increase in MCs numbers was observed in lip SCC of young patients when compared with lip mucosal samples without epithelial dysplasia of young subjects (P<0.01).

The density of CD31⁺ blood vessels was significantly higher in the lip SCC and AC when compared with control (P<0.01) (Figs. 3, 4). Our results also demonstrate a significant increase in the number of blood vessels in lip SCC when compared with AC (P<0.01). In addition, when the lip SCC samples were dichotomized by the median density values of the CD31⁺ blood vessels (high and low groups), the group with the highest density of vessels showed a tendency to have a higher density of tryptase⁺ (143±75) and c-kit⁺ MCs (170±153), when compared with the groups with lower densities of vessels (109±48 and 80±27, respectively). In a similar manner, when the lip SCC samples were dichotomized to form two groups: low (grade 1 and 2) and high (grade 3 and 4) degradation of collagen, the group with the highest degree of collagen degradation presented a higher density of tryptase⁺ (129±52) and c-kit⁺ MCs (85±50), when compared with the group with the lower degree of collagen degradation (88±52 and 80±36, respectively); however these associations were not confirmed statically (P=0.10).

Furthermore, our results showed no association or correlation between tryptase⁺ or c-kit⁺ MC with other

Table 1. Main clinical findings of patients with lip SCC (n= 37).

Clinical features	Number of cases	%
Age		
≤ 61 years	18	48.6
> 61 years	19	51.4
Gender		
Male	26	70.3
Female	11	29.7
Ethnic group		
Caucasian	35	94.6
Non-Caucasian	02	5.4
Location		
Lower lip	33	89.2
Upper lip	04	10.8
Chronic sun-exposure		
Yes	30	81.1
No	07	18.9
Tobacco		
Yes	17	46
No	20	54
Alcohol		
Yes	08	21.6
No	29	78.4
T stage		
T1-T2	30	81.1
T3-T4	07	18.9
N stage		
N0	36	97.3
N1-N2	01	2.7
Clinical outcome		
Dead	01	2.7
Alive	36	97.3

Table 2. Main microscopic features of specimens of lip SCC (n= 37).

Microscopic features	Number of cases	%
Percentage of Ki-67 positive cells		
≤ 5.33%	15	40.5
>5.33%	11	59.5
Collagen degeneration (Picosirus stain)		
Grade I-II	11	29.7
Grade III-IV	26	70.3
Elastin changes (Verhoeff stain)		
+1 - +2	24	75
+3 - +4	8	25
Intensity of inflammatory infiltrate		
Mild	0	0
Moderate	08	21.7
Intense	29	78.3
Histological grading (WHO grade)		
Grade I (well differentiated)	37	100

Mast cells in lip squamous cell carcinoma

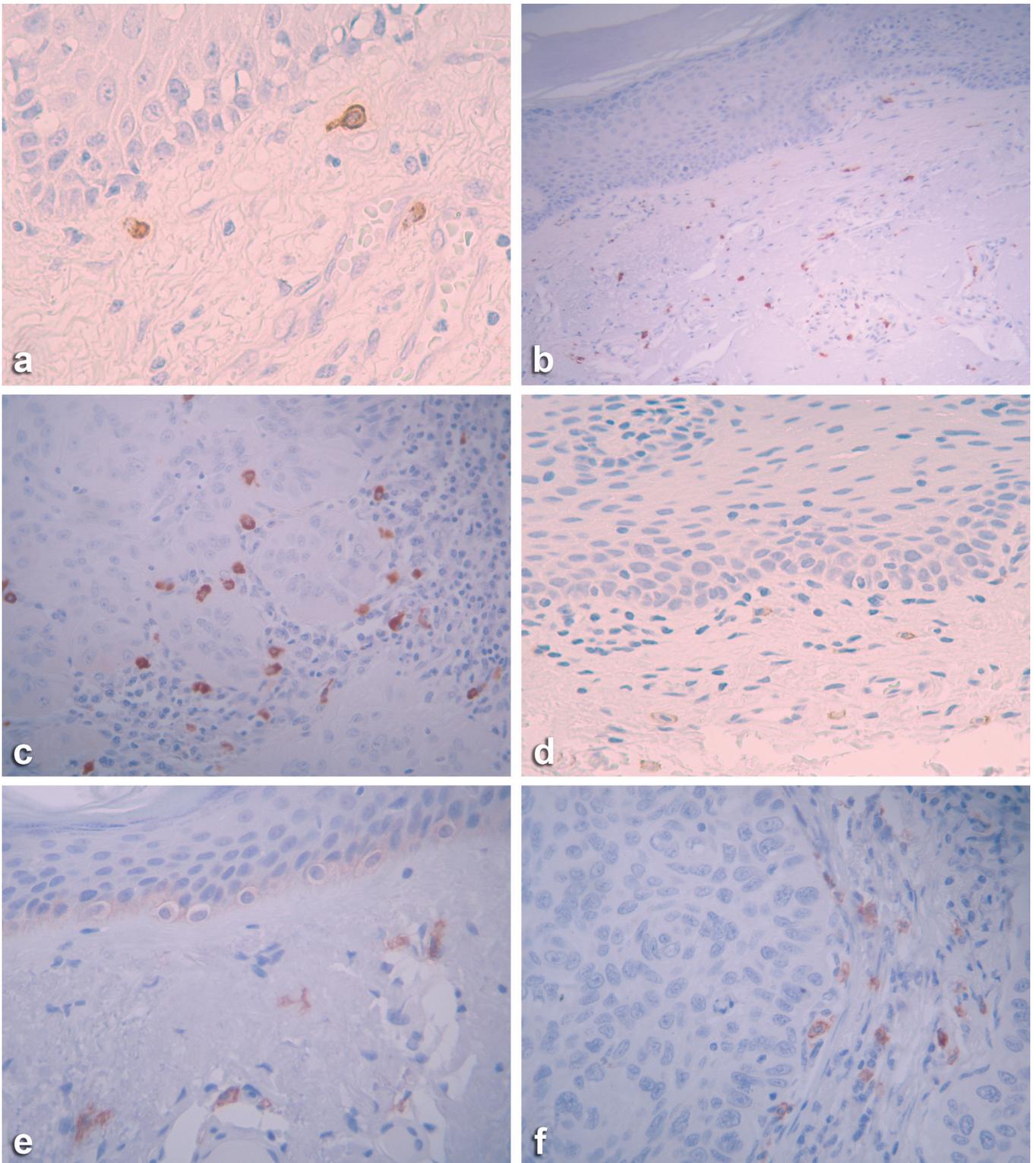


Fig. 1. Tryptase⁺ (A, B and C) and c-kit⁺ (D, E and F) mast cells (MC) distributed throughout the connective tissue of normal lip mucosa (control) (A and D), actinic cheilitis (AC) (B and E) and in the peritumoral area of lip squamous cell carcinoma (lip SCC) (C and F). Photomicroscopies (C) and (F) show a high number of MCs located in the front of the tumoral invasion of lip SCC and in close contact with neoplastic cells. Immunohistochemical staining. A, C-F, x 250; B, x 100.

Mast cells in lip squamous cell carcinoma

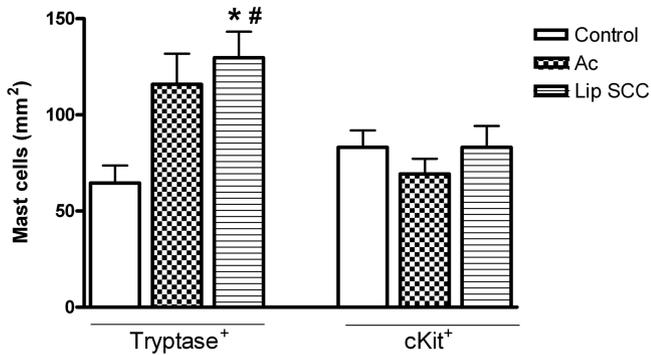


Fig. 2. Densities of tryptase⁺ and c-kit⁺ mast cells (MC) in microscopically normal lip mucosa (control), actinic cheilitis (AC) and lip squamous cell carcinoma (Lip SCC). Results are expressed as mean MC/mm² ± SD (n=6 for control samples, n=15 for AC samples and n=37 for Lip SCC samples). (*) P<0.05 when compared with control and (#) P=0.01 when compared tryptase⁺ and c-kit⁺ cells in Lip SCC (Kuskall Wallis and Dunn test).

microscopic features, such as the proliferative index, which can be measured by staining neoplastic cells with Ki-67 antibodies and the degree of elastin changes.

Discussion

The inflammatory and immunological responses that occur in the tumor microenvironment have been a matter of debate and of several studies (Balkwill and Mantovani, 2001; O'Byrne and Dalglish, 2001; Coussens and Werb, 2002; Grimbaldston et al., 2006). Whilst the immune-inflammatory cells may participate in local tumoral immunity, reducing tumoral invasion, these cells may also contribute to cancer progression and metastasis (Balkwill and Mantovani, 2001; O'Byrne and Dalglish, 2001; Coussens and Werb, 2002; Balkwill, 2004; Grimbaldston et al., 2006). In this context, MCs play an important role in host defense, due to their location at the host-environment interface and their ability to store preformed mediators that could

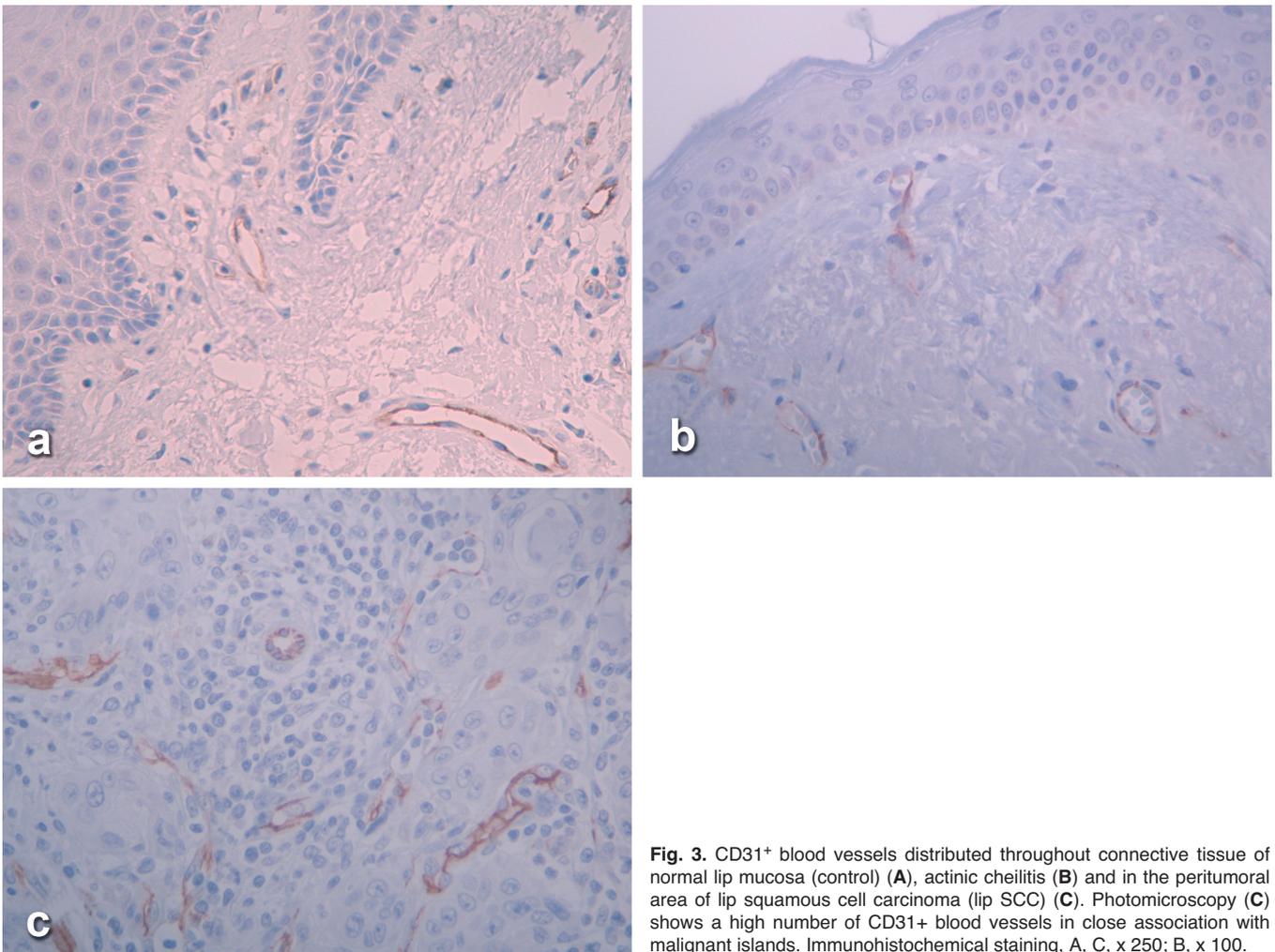


Fig. 3. CD31⁺ blood vessels distributed throughout connective tissue of normal lip mucosa (control) (A), actinic cheilitis (B) and in the peritumoral area of lip squamous cell carcinoma (lip SCC) (C). Photomicroscopy (C) shows a high number of CD31⁺ blood vessels in close association with malignant islands. Immunohistochemical staining, A, C, x 250; B, x 100.

Mast cells in lip squamous cell carcinoma

contribute to immunoregulation, matrix degradation, elastose change and angiogenesis (Tanooka et al., 1982; Flynn et al., 1991; Metcalfe et al., 1997; Coussens et al., 1999; Hart et al., 2000, 2002; Ranieri et al., 2002; Grimaldeston et al., 2006).

Recently, we investigated the MCs in oral cavity SCC (non-sun-exposed oral tissues) and demonstrated that the densities and migration of these cells are significantly reduced in this tumor, when compared with control and pre-malignant lesions (Oliveira-Neto et al., 2007). In this same study, the survival analysis demonstrated that the patients with highest numbers of tryptase⁺ and c-kit⁺ MCs demonstrated a tendency for a better prognosis than patients with low counts (Oliveira-Neto et al., 2007). In the present study, the MCs in lip SCC were significantly increased when compared with control. In addition, a significant decrease in MC migration was not observed, as determined by the ratio between c-kit⁺/tryptase⁺ cells, in lip SCC (69%) and AC (60%) when compared with our previous data for oral cavity SCC (19%). These results suggested that the MCs may be involved, direct or indirectly, with tumoral immunity, contributing to a better prognosis of the lip SCC patients, when compared with oral cavity SCC patients (Leite and Koifman, 1998; Antunes et al., 2001; Vartanian et al., 2004). On the other hand, some studies have shown a direct correlation between the number of dermal MCs and the degree of immunosuppression, suggesting that MCs are related to a worse prognosis (Hart et al., 1998, 2001, 2002; Grimaldeston et al., 2006).

Some studies have demonstrated that MC density and activity could be influenced by chemical and physical carcinogens, such as tobacco and sun exposure (Sand et al., 2002; Grimaldeston et al., 2006). In a

series of studies, Grimaldeston et al. (2002) demonstrated an increase in MC in human photo-damaged skin and suggested that this cell could represent a significant predisposing factor for the development of basal cell carcinomas (BCC) (Grimaldeston et al., 2000, 2002, 2006); however, these authors have not yet established a link between dermal MC prevalence and the presence of BCC (Grimaldeston et al., 2003, 2006). Nevertheless, evidence suggests that the pattern of sun exposure and the dose of solar ultraviolet radiation (UVR), associated with the development of BCC, differs from that of squamous cell carcinoma (SCC) (Kricger et al., 1995; Strom and Yamamura, 1997; English et al., 1998; Armstrong and Kricger, 2001; Grimaldeston et al., 2002). Intermittent intense sun exposure has been described as a significant risk factor for BCC (Kricger et al., 1995; Armstrong and Kricger, 2001; Grimaldeston et al., 2002), whereas the incidence of SCC increases with age and cumulative sun exposure over a lifetime (Kricger et al., 1995; Armstrong and Kricger, 2001; Grimaldeston et al., 2002).

We found an increase in the MC subpopulation in lip SCC, compared with normal lip vermilion and AC. Besides, we demonstrated that this increase in MC density was independent of the patients' ages. Our results are in line with previous studies suggesting a role for MC in tumor progression at the invasion front (Rojas et al., 2005). However, it has been shown that the SCC of skin (especially facial or scalp) is not associated with high dermal MC prevalence in humans (Grimaldeston et al., 2002).

The location of MCs near blood vessels supports a role for MC products, such as bFGF, TGF, TNF- α , tryptase and heparin, in promoting the angiogenesis of remodeled tissues (Coussens et al., 1999; Iamaroon et al., 2003; Grimaldeston et al., 2006). In this study, we investigated MC populations expressing tryptase and c-kit in lip SCC and AC and their relationship with the increase in CD31⁺ blood vessels. Our results demonstrate that, in spite of a significant increase in angiogenesis in lip SCC when compared with control and AC, no association or correlation were confirmed statistically between MC densities and the number of blood vessels. In contrast, a significant correlation between MC and the density of blood vessels in the dermis (Eady et al., 1979) and in the oral cavity SCC (Iamaroon et al., 2003) has been previously found.

MCs are important cells for the maintenance of structural integrity of tissues, due to their production of growth factors for epithelial and fibroblastic cells, including histamine, tryptase, chymase, IL-3, -4, -6, -8, TGF- β , EGF, FGF and leukotrienes (Grimaldeston et al., 2006). In this context, it has been speculated that the increased MC numbers in skin following chronic UV exposure contribute to the tissue remodeling of photodamaged skin (Grimaldeston et al., 2006). In accordance with this, chronic UV exposure (exposure to 18 J cm⁻² over 11 weeks, three times weekly) stimulated

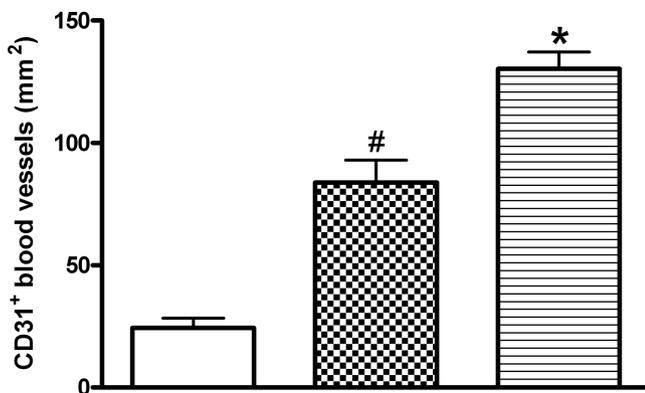


Fig. 4: Densities of CD31⁺ blood vessels in microscopically normal lip mucosa (control) (white bar), actinic cheilitis (AC) (checked bar) and lip squamous cell carcinoma (Lip SCC) (striped bar). Results are expressed as mean CD31⁺ blood vessels/mm² \pm SD (n=6 for control samples, n=15 for AC samples and n=37 for Lip SCC samples). (#*) P<0.01 when compared control with AC and lip SCC (Kuskall Wallis and Dunn test).

a 3.6-fold increase in dermal elastin in BALB/c mice, but did not have any effect on MC-deficient Kit^W/Kit^{W-v} mutant mice (Gonzalez et al., 1999). Recently, a member of our group demonstrated that the grade/degree of solar elastosis is a morphologic marker of neoplastic changes in human sun-damaged skin (Silva et al., 2007). Considering this aspect, we also investigated the grade of elastosis in AC and in lip SCC samples; the extent of elastosis has been correlated with an increasing density of dermal MCs in sun-exposed hand skin (Grimbaldeston et al., 2006), but this correlation was not demonstrated by our data.

The correlation between MCs and extracellular matrix (ECM) degradation was also investigated, since these cells are a source of MMPs and UV light induces the release and synthesis of several mediators involved in direct or indirect ECM remodelling (Rojas et al., 2004). In the present study, although the groups with a higher density of CD31⁺ blood vessels and a higher degree of collagen degradation presented a higher density of MCs, these associations were not confirmed statistically.

Considering the influence of MC mediators in keratinocytes (Huttunen et al., 2001), the correlation between MC densities and the proportion of Ki-67⁺ neoplastic cells was also investigated (proliferative index) in lip SCC, but this was not present in our samples. However, it has been suggested that MC accumulation may inhibit the proliferation and the dissemination of gastric carcinoma cells (Jiang et al., 2002). The exact functional role of the accumulated MCs in photodamaged diseases is still a matter of some discussion and of several studies.

The increase in MCs in AC and in lip SCC, observed by us, and by others (Rojas et al., 2004, 2005), may reflect the first changes in the sun-damaged microenvironment. Our study demonstrated that the migration of MCs is not significantly affected in AC and in lip SCC; this could contribute to the increase in MCs in these lesions. The overall impact of the increase in MC in lip SCC might reflect an important modification in the microenvironment during squamous photocarcinogenesis.

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Mast cells in lip squamous cell carcinoma

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