Actinic cheilitis and squamous cell carcinoma of the lip: clinical, histopathological and immunogenetic aspects *

Queilite actínica e carcinoma espinocelular do lábio: aspectos clínicos, histopatológicos e imunogenéticos

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Abstract: Actinic cheilitis is the main precancerous lesion of the lip. Squamous cell carcinoma of the lip is reported together with oral carcinomas in the Brazilian official statistics. Overall, they account for 40% of the head and neck carcinomas. In general, physicians and dentists know little about what causes oral tumor development and progression. Tumor suppressor genes and cell proliferation regulatory proteins play a role in the progression of actinic cheilitis to squamous cell carcinoma and in its biological behavior. Knowledge on prognostic and diagnostic markers has a positive impact on the follow-up of these patients.
Keywords: Carcinoma, squamous cell; Cheilitis; Cyclin-dependent kinase inhibitor proteins; Cyclin D1; Proto-oncogene proteins c-bcl-2; Tumor suppressor protein p53

Resumo: Queilite actínica é a principal lesão pré-neoplásica do lábio. O carcinoma espinocelular do lábio é incluído nas estatísticas brasileiras junto com os cânceres de boca e, em conjunto, somam 40% dos cânceres de cabeça e pescoço. Há certo desconhecimento médico e odontológico em geral quanto aos fatores relacionados à carcinogênese e à progressão de tumores de boca. Genes de supressão tumoral e proteínas regulatórias de proliferação celular exercem papel na evolução da queilite actínica para carcinoma espinocelular e no comportamento biológico deste. O conhecimento de marcadores de diagnóstico e prognóstico e sua investigação têm utilidade no acompanhamento de tais pacientes.
Palavras-chave: Carcinoma de células escamosas; Ciclina D1; Proteína supressora de tumor p53; Proteínas inibidoras de quinase dependente de ciclina; Proteínas Proto-oncogênicas c-bcl-2; Queilite

INTRODUCTION

Cancers of the head and neck represent 10% of all malignant tumors diagnosed and approximately 40% of them correspond to oral cancer. [1] Oral cancer includes those of the lip and oral cavity. The Brazilian Ministry of Health has estimated 14,120 new cases of oral cancer in the country for 2010, with 10,330 cases in men. [1]

Actinic cheilitis (AC) is considered a potentially malignant disorder likely to develop into invasive squamous cell carcinoma (SCC) of the lip. There is no agreement in relation to the frequency with which AC becomes SCC. Morphological analysis of this type of lesion is subjective and is not enough to predict with certainty which lesions will progress to oral carcinoma.[2,3]

Due to the high incidence of AC and SCC of the lip in the general Brazilian population, lack of early clinical diagnosis and to the difficulty in obtaining an accurate histopathological diagnosis, this article aims

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at reviewing clinical and histopathologic features and the role of tumor suppressor genes involved in the etiopathogenesis of AC and SCC of the lip. It also seeks to contribute to better understanding of the mechanisms involved in the process of progression of AC into SCC of the lip.

ACTINIC CHEILITIS

Actinic cheilitis (AC) is a chronic inflammatory process that affects the lower lip in 95% of the cases. It is caused by chronic exposure to sunlight or artificial ultraviolet radiation. [4] It is considered a potentially malignant lesion of high morbidity which can develop into invasive squamous cell carcinoma. [4-6] It affects fair-skinned individuals with a history of chronic sun exposure and can occur at any age. [7-9] However, clinical changes are frequently observed from the 5th decade of life, since the damage caused by solar radiation accumulates over the years. Smoking increases the likelihood that actinic cheilitis will progress into a frank neoplasm. [8]

In comparison with the skin, which reflects from 5% to 10% of ultraviolet radiation and absorbs 70%, especially through melanin, the lip is less protected because of its thinner epithelium, thin layer of keratin, lower amount of melanin and lower secretion from sebaceous and sweat glands, which makes the lip more vulnerable to damage by radiation. [7]

CLINICAL PRESENTATION

There is loss of the usually sharp border of the lip, atrophy of the vermilion border and darkening of the lip at the border between the lip and the skin of the face. As the lesion progresses, rough and scaly areas develop, which become thick, especially when they extend to the wet line of the lip. Chronic focal ulceration can occur in one or more sites, as well as leukoplakic lesions. [10] (Figure 1).

Leukoplakia, the most common form of AC and the most common change that precedes squamous cell carcinoma of the oral mucosa, is defined as a white patch of undefined risk of transformation. [10,11] There is disagreement in the literature regarding the frequency with which it transforms into SCC, with estimates ranging from 1.4% to 36% at an interval of 1 to 30 years.[10-12]

Despite the preventive means available and its early clinical diagnosis being easy, several factors are attributed to the late clinical diagnosis of AC: not knowing about the lesion, no pain, initially harmless clinical appearance and lack of knowledge on the part of many professionals who consider it a simple chronic inflammatory process. [13] Since there are acute episodes of occurrence of the condition followed by remission of the signs and symptoms, many patients associate their lip changes to the burden of their occupation, not identifying them as a chronic inflammatory process and accepting them as part of the context of their occupational activity, just as having callused hands or tanned skin. Biopsy is recommended for cases of AC in which there is loss of the usually sharp line marking the transition between the red of the lip and the normal skin, change in texture of the lip on palpation, change in the thickness of the semimucosa and presence of ulceration. [13]

HISTOPATHOLOGY

Hyperkeratosis (or parakeratosis) and acanthosis are universal aspects, although areas of atrophy are not rare (Figure 2). The lamina propria shows blood vessels, often dilated, and vascular prominence due to the increased volume of endothelial cells and not to their proliferation. Presence of solar elastosis is a frequent and important sign (Figure 2). Still in the dermis, there is presence of inflammatory infiltrate of intensity varying from mild to severe and which is composed predominantly of lymphocytes, but also with distinct participation of plasma cells and eosinophils. Keratinocyte atypia is the most important aspect and it gradually settles in the epithelium. Mild to very severe degrees of epithelial dysplasia can be found, and the potential for development of invasive carcinoma increases with the severity of this change (Figure 3). More severe cellular atypias may be associated with intense inflammatory conditions, often observed in ulcerated AC. In these situations, the diagnostic distinction between AC and carcinoma *in situ* becomes even more difficult.[14] It is important to note that the histological changes of AC are not evenly distributed through the vermilion of the lip, even in cases where clinical presentation is homogeneous. [14]

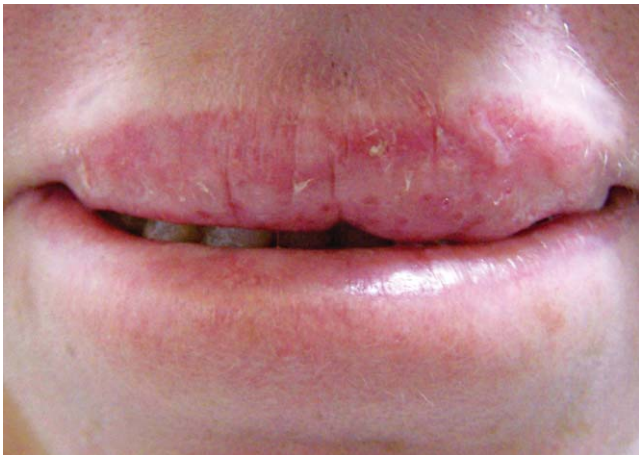

Figure 1: Actinic cheilitis: edema, exulceration and desquamation of the upper lip. Edema and loss of definition of the lower lip vermilion border

TREATMENT

The main AC therapies aim to remove or destroy the abnormal epithelium. They include vermillionectomy, topical application of 5-fluorouracil (5-FU), chemical peel with trichloroacetic acid, cryotherapy, electrocauterization, carbon dioxide laser ablation, follow-up with intense local photoprotection and, more recently, the use of imiquimod, photodynamic therapy and YAG-laser.[15-20]

Vermillionectomy is the treatment of choice, since it allows for a histopathological review of all of the tissue removed. It is considered an excisional biopsy. All other treatment options do not generate a surgical specimen that allow for anatomopathological evaluation and consequent detection of a possible invasive tumor. [14-17] However, any treatment will only be effective if the patient becomes aware of the importance of sun protection as well as of adherence to regular consultations for effective clinical control of the disease.

LIP SQUAMOUS CELL CARCINOMA

Worldwide, more than 300,000 new cases of squamous cell carcinoma (SCC) are diagnosed each year, which represents approximately 90% of the mouth neoplasms. SCCs have high rates of morbidity and mortality due to advanced clinical stage at the time of diagnosis. [21,22]

In Brazil, cancer of the lip plays an important role in the epidemiological indices; however, there are no national data showing its isolated incidence. The statistical data available and which should be considered for this type of tumor are those seen for cancer of the mouth. [1]

Cancer of the lip corresponds to 25% to 30% of all diagnosed mouth cancers. Squamous cell carcinoma (SCC) is the most common histological type, accounting for 95% of the cases. It is twenty times more prevalent in the lower lip than in the upper lip. [1,21,22] Its incidence is higher among males, with a ratio of 6:1 in relation to women and primarily affects people over 50 years old, of fairer skin types and who perform professional and/or leisure activities related to chronic sun exposure.[10,21,22] It is uncommon among young individuals, but has been observed in young people, those who have undergone kidney transplant or who are infected with HIV. [10,23,24]

When diagnosed at an early stage, it has a cure rate of 80 to 90% and a mortality rate between 10% and 15%. [25] When there are metastases, they occur at a late stage and range from 11 to 18%; in these cases, the mean survival in five years drops to 25%. [25-27]

The incidence of UV radiation on Earth has progressively increased, and it is considered the primary factor inducing changes in the lower lip due to its intense absorption by nucleic acids. Most of the damage caused by UVB is due to changes that prevent transcription of genetic information to the mRNA, blocking the mechanism of DNA replication, which, from a molecular point of view, leads to decreased mitotic activity and, from a clinical point of view, to atrophy of the epithelium and reduced photoprotective capacity of the lip. [4]

The damage triggered by ionizing radiation is caused by direct interaction with target molecules or indirectly by formation of free radicals, which have high affinity for electrons and hydrogen bonds, with the DNA being the primary target. [5]

When a tissue is continually exposed to ionizing radiation, it may undergo both chromatid and chromosome aberrations involving two or one of the chromatids, respectively, since the relative amount of these

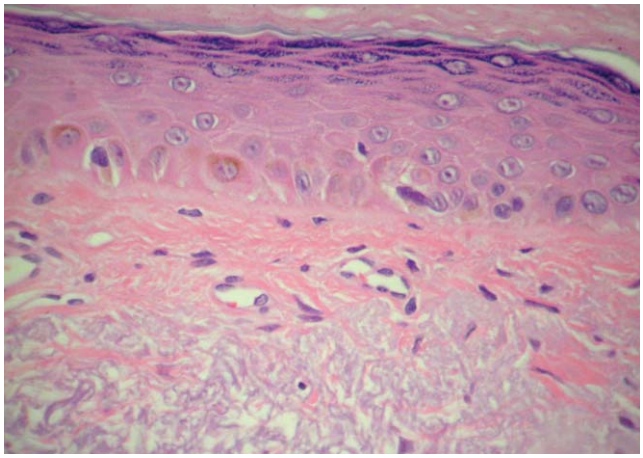

Figure 2: Actinic cheilitis, hyperkeratosis and atrophy of the epidermis. Discrete dysplasia of the epidermis. Elastosis of the dermal collagen (HE 200X)

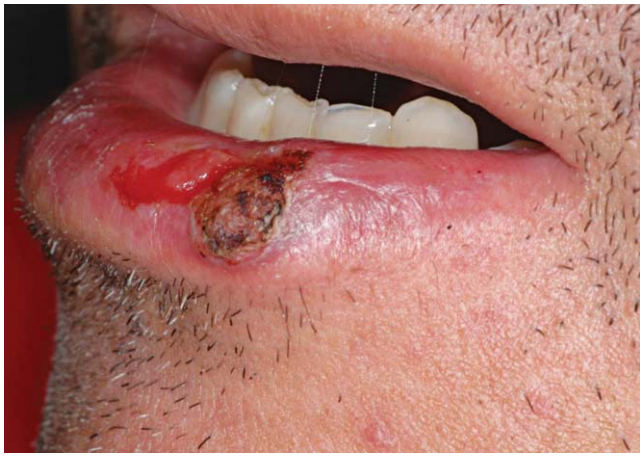

Figure 3: Squamous cell carcinoma of the lip: shallow ulcer with infiltrated border and covered with scales and crusts. Borderline ulcerated lesion (ulcerated actinic cheilitis, histologically)

changes depends on the type, dose, time and rate of exposure, as well as on the sensitivity of the affected cells in various stages of the cell cycle and on DNA repair capacity. [5, 6]

Although solar radiation is the most important risk factor for development of lesions on the lip, it is not the only one. Some factors associated with it are smoking, alcohol abuse, HPV, race, family and genetic predisposition, immunosuppressive state, poor diet and socio-economic situation. [28-32]

CLINICAL PRESENTATION

SCC of the lip is considered a disease of low aggressiveness and favorable prognosis due to its tendency to progress slowly. It is initially asymptomatic and it may be difficult to distinguish its lesions from AC. It manifests as leukoplakic or erythroplakic, atrophic plaques showing persistent fissures with scales or crusts. An ulcerated lesion with infiltration at the base, clear limits and variable growth rate is gradually formed (Figure 3). In advanced stages, there is pain associated with exudative ulcer, covered with scales and crusts, with indurated borders and infiltrated base that does not heal. It may present a bleeding, friable, verrucous, exophytic mass. [10,32] Concomitant palpation of the internal mucosal surface and the external skin surface helps to define the real extent of the lesion in terms of clinical depth of invasion[32]

HISTOPATHOLOGY

Squamous cell carcinoma of the lip is histopathologically characterized by invasive cords or islands of malignant squamous epithelial cells. The tumor cells have a glassy eosinophilic cytoplasm and enlarged nuclei, often hyperchromatic, as well as an increased nucleus-cytoplasm ratio. Varying degrees of nuclear and cellular pleomorphism are observed, as well as presence, diameter and intensity of keratin pearls are variable. Also, there might be keratinization in individual cells.

Invasion is characterized by irregular extension of the tumor through the basement membrane toward the connective tissue in the dermis. Squamous epithelial cells and nests or islands of cells are seen growing detached from the surface epithelium. Invasion of the dermis may occur in an expansive or infiltrative manner going deep into the underlying adipose tissue, muscle, bone, blood and lymph vessels, causing tissue destruction (Figures 4 and 5). Often there is an intense inflammatory cell response, predominantly lymphocytic, to the invading epithelium and focal areas of necrosis. The tumor growth can induce angiogenesis and occasionally produce a desmoplastic response, of dense fibrosis. [32]

The histopathological classification of malignancy proposed by WHO is based on the degree of cell differentiation or anaplasia, grouping these malignancies in three categories: poorly, moderately and well differentiated. [33-35] Low-grade, grade I or well differentiated SCC correspond to those carcinomas with tissue architecture similar to normal squamous epithelium. Well-differentiated tumors grow slowly and metastasize late. On the other hand, poorly-differentiated SCC is characterized by predominance of immature cells, numerous typical and atypical mitoses, expressive and frequent nuclear and cellular pleomorphism, little or no production of keratin, exhibit rapid growth and early metastases. SCC with such characteristics is called high-grade or grade III, poorly differentiated or anaplastic carcinoma. A tumor with morphological appearance between these two extremes is called grade II or moderately-differentiated carcinoma. [33-35]

Considering that the histopathologic pattern of SCC of the mouth varies widely within the same lesion, Bryne et al (1989) proposed a new histological classification, beginning the histological grading system of invasive tumor front. This name is reserved for the tumor area considered the most invasive. [36]

The invasive tumor front corresponds to the point of greatest invasion of neoplastic cells in the connective tissue, ie, the interface between tumor cells and the stroma at the deepest limit of tumor invasion. It is the place where neoplastic cells are more aggressive and where molecular changes are more evident, allowing us to study them more accurately (Figures 6, 7 and 8). This region is characterized by increased neoplastic proliferation, loss of cohesion of the matrix proteins and local invasion. [37]

The invasive front characterizes the degree of histological differentiation of the tumor by altering

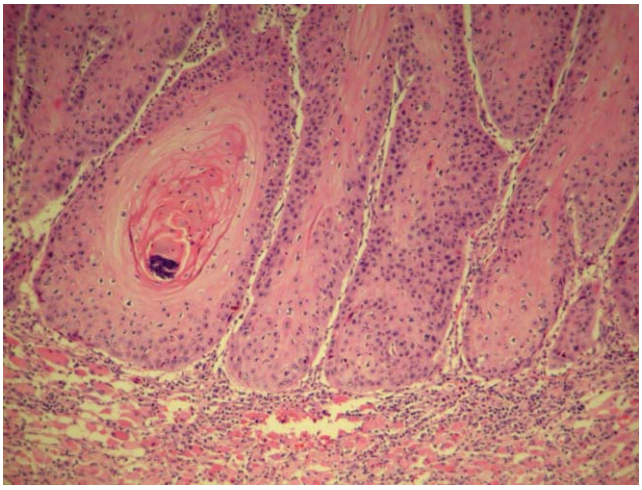

Figure 4: Squamous cell carcinoma of the lip: expansive pattern of tumor invasion (HE 200X)

the structure of the extracellular matrix. Characteristics of the neoplastic invasion and of the microenvironment of the site of invasion are taken into account. There are several types of cells that make up this infiltrate. The predominant ones are inflammatory cells as well as myofibroblasts and endothelial cells involved in neovascularization. The aggressiveness of the cells in the invasive front and their interaction with the extracellular microenvironment may be considered together with other prognostic factors. [37]

TREATMENT

The TNM-based staging system (according to which T represents the tumor size; N, the presence or absence of metastasis in regional lymph nodes; M, the presence or absence of distant metastasis) is what determines the type of treatment. [35]

Stage I (tumors smaller than 2 cm) and II (between 2 and 4 cm) are curable with surgery or radiotherapy, and treatment choice is based on the functional and aesthetic results expected. The presence of positive margins or tumor invasion depth greater than 5 mm suggests that a combination of the two forms of therapy should be considered for better results. Stage III (tumors larger than 4 cm) and IV (which invade adjacent structures) are the major challenges. Treatment consists of surgery combined with radiotherapy, chemotherapy combined with radiotherapy or surgery following the Mohs method (Table 1). Regional lymph node excision is done when it is suspected to have been affected and done by routine in stages III and IV.

Follow-up studies after Mohs surgery showed a recurrence rate of 2.3% and 7.6% of metastases in five years. [25-27] Conventional surgical methods and radiotherapy showed recurrence rates of 10.5% and 13.7% of metastases for the same time period of follow-up.[26,38] Local recurrence is associated with initial diameter of the tumor, positive surgical margins, histological invasion greater than 5 mm, invasive front pattern and presence of perineural invasion.[38] Survival rate after five years is 90% to 100% for early stages without detectable metastases. When there is presence of metastases at diagnosis, survival rate drops to 25% to 70% of the cases. [26,35-38]

Tumor markers

Disorders in the induction of apoptosis (programmed cell death) are considered the initial event for the development of cancers and lymphoproliferative diseases, since they allow malignant cells to survive and give them a chance to multiply. [39-40]

Several apoptosis regulatory pathways show to be altered in premalignant and malignant lesions. The disordered relationship between cell proliferation and cell death can often predict the progression and behavior of these lesions, in addition to providing information on tumor progression and response to cancer therapies.

Thus, identification of tumor markers is important and necessary to define the prognosis of the disease more accurately. However, there is not a universally accepted marker, but a panel with a limited number of genes and proteins.

p53 Protein

p53 protein is one of the proteins responsible for maintaining DNA integrity. Mutations in the *TP53* gene make p53 unable to control cell proliferation,

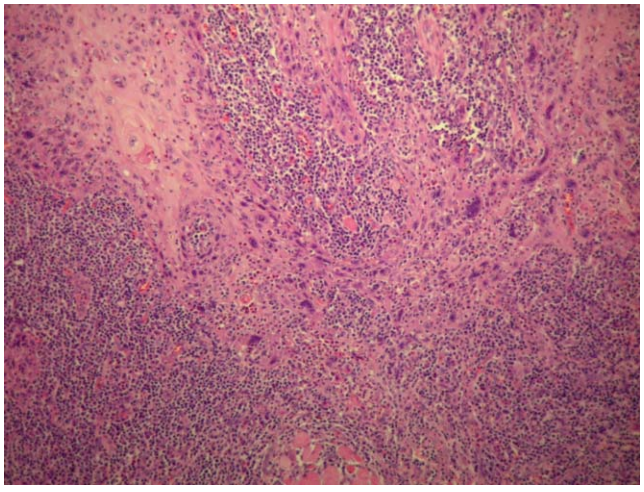

Figure 5: Squamous cell carcinoma of the lip: infiltrative pattern of tumor invasion and intense associated inflammatory infiltrate (HE 200X)

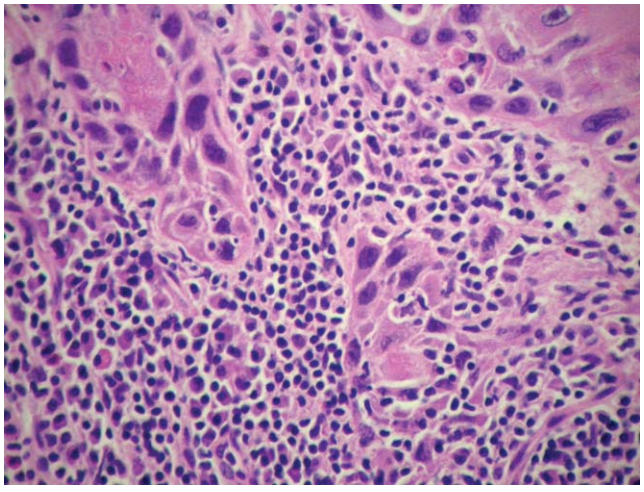

Figure 6: Squamous cell carcinoma of the lip: infiltrative pattern of tumor invasion with isolated tumor cells and blocks. Associated lymphoplasmacytic infiltrate. (HE 400X)

Table 1: Classification of neck and head tumors according to stage and form of treatment

Tumor stage	Tumor size	Lymph node metastasis	Distant metastasis	Forms of treatment
I	T_1	N_0	M_0	Surgery or radiotherapy
II	T_2	N_0	M_0	Surgery or radiotherapy
III	T_3	N_0	M_0	**Resectable,** surgery + radiotherapy
	T_1	N_1	M_0	**Unresectable:** radiotherapy, salvage surgery if sal
	T_2	N_1	M_0	necessary, radiotherapy + chemotherapy,
	T_3	N_1	M_0	vage surgery if necessary.
IV	T_4	N_0, N_1	M_0	Same as stage III
	Qualquer T	$N_{2a,b,c,}$, N_3	M_0	
	Qualquer T	Qualquer N	M_1	

T_1 - tumor with 2 cm or less in greatest dimension; T_2 – tumor with more than 2 cm up to 4 cm; T_3 - tumor with more than 4 cm; T_4 - tumor invades adjacent structures. N_0 - absence of metastasis in regional lymph nodes; N_1 – single homolateral lymph node = 3 cm; N_{2a} – single homolateral lymph node > 3-6 cm; N_{2b} – multiple homolateral lymph node = 6 cm; N_{2c} – contra-lateral, bilateral lymph node = 6 cm; N_3 - lymph node > 6 cm. M_0 - absence of distant metastasis; M_1 presence of distant metastasis.

resulting in inefficient DNA repair and allowing many cells exposed to mutagens to replicate damaged genetic material, spreading changes incorporated into the genome. Although a single mutation is not enough to transform cells, loss of p53 function predisposes cells to additional mutations and malignant transformation. [41]

The genes regulated by p53 are those involved in cell cycle arrest, apoptosis and angiogenesis. Under conditions of stress, particularly induction of DNA damage, wild-type p53 promotes cell cycle arrest in the G1 phase, thus allowing for repair of the genetic material damaged by the mutagen. If for some reason this mechanism fails, p53 will signal for the cell to get into apoptosis, thus preventing cells that have mutated from dividing. The functions of p53 are due to its transcriptional ability, which allows activation of a number of genes involved in cell cycle regulation. Therefore, when a cell is exposed to genotoxic attack by chemicals or UV radiation, the *TP53* gene transcriptionally activates the *TP21* gene, inducing the synthesis of the protein with the same name, whose function is to inhibit the action of cyclin-dependent kinases (CDK), causing cell cycle arrest in the G1 phase until the DNA is repaired. Next, the p53 protein activates the GADD-45 gene, which acts fixing the DNA damage. Once it is repaired, the p53 protein is degraded by the action of the MDM-2 protein. [42] Wild-type p53 has a short half-life (6-20min), does not accumulate in normal cells and is not detected by immunohistochemical methods.

Mutations in the *TP53* gene lead to production of altered p53 protein, which has a long half-life, is unable to combine with DNA and accumulates in the

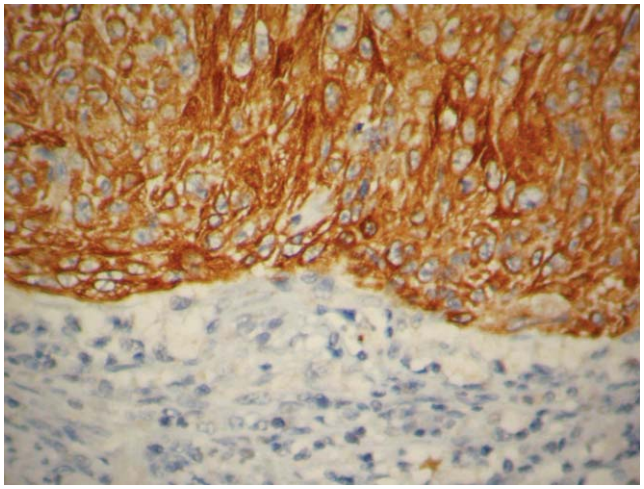

Figure 7: Squamous cell carcinoma of the lip: invasive front with expansive pattern. Immunostaining for cytokeratin (400X)

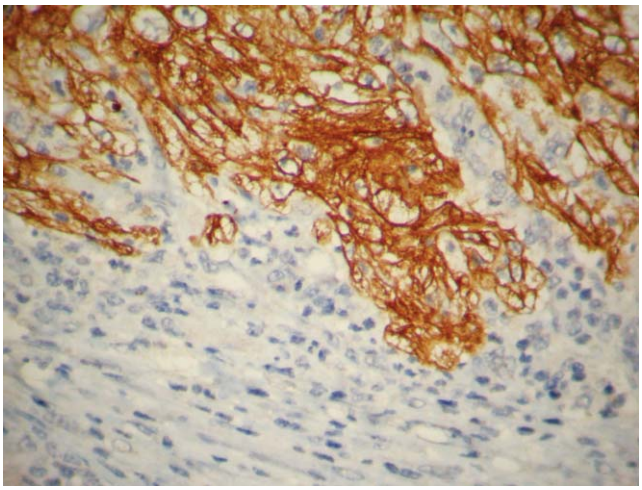

Figure 8: Squamous cell carcinoma of the lip: tumor front with infiltrative pattern. Immunostaining for cytokeratin (400X)

cell nucleus. It is easily visualized by immunohistochemical methods.

Abnormalities in the *TP53* gene are the most common molecular changes found in human neoplasms. It is suggested that *TP53* is mutated in more than 90% of SCC of the skin and in more than 50% of basal cell carcinomas.[43] Expression of p53 in the tissue, revealed by immunohistochemistry, is positively associated with the degree of epithelial dysplasia and progression to squamous cell carcinoma of the mouth. [43-45] Also, increased expression of p53 has been reported in SCC of the lip and lesions of actinic cheilitis. [44-46]

p16 Protein

The p16 protein is encoded by the *TP16* gene (CDKN2a/INK4a), also known as a CDK-inhibitor, tumor suppressor gene. In a normal cell cycle, this protein is able to bind to CDK 4 and 6, inhibiting phosphorylation of retinoblastoma protein (pRb). Hypophosphorylated pRb inhibits cell entry in the S phase of the cell cycle by physical association with members of the E2F family of transcription factors. The activated E2F-PRB complex represses transcription of target genes that regulate DNA synthesis with consequent deactivation of the checkpoint in the G1-S transition of the cell cycle.[47]

When there is dysfunction of p16, CDK4/6 can bind to cyclin D1 and form the cyclin D1-CDK4/6 complex, promoting pRb phosphorylation and releasing E2F transcription factor, which accelerates the G1-S phase transition of the cell cycle, leading to genomic instability (Figure 9). [47] There is then uncontrolled cell proliferation. It also contributes to evasion of senescence *checkpoints* and apoptosis. The expression of p16 in lesions of SCC of the lip and actinic cheilitis and its meaning has been little studied.

p21 Protein

The p21 protein, a product of the *TP21*/WAF1 gene, is a member of the family of tumor suppressor genes. It acts as an inhibitor of CDK, which is essential for cell growth, differentiation and apoptosis. The expression of p21 is regulated by p53 in response to DNA damage. Then, p21 associates with CDK, preventing phosphorylation of its substrates and blocking cell cycle progression. This arrest gives the cell time to repair DNA, thus preventing replication of damaged genetic material. [48]

The p21 protein is activated in response to low doses of UVB radiation. Increased expression of p21 associated with increased p53 has been detected in skin exposed to UVB rays and at the invasive front of lip carcinoma, as well as in normal lips; however, this association was lost in AC.[49] In SCC of the mouth, an increased expression of p21 is considered a marker of poor prognosis and tumor invasion. When it is associated with increased tissue expression of p53, it suggests a correlation with occurrence of lymph node metastases.[50]

Survivin

Survivin is part of the family of IAPs *(inhibitor of apoptosis proteins)*. Since its discovery in 1997, it is considered an important marker of malignancy and also a prognostic indicator of cancer response to therapy.[50] It is believed that survivin is important in the period of cell replication but not essential for cell survival. Adult cells do not die in the absence of survivin; however, it is essential to the process of cell prolifera-

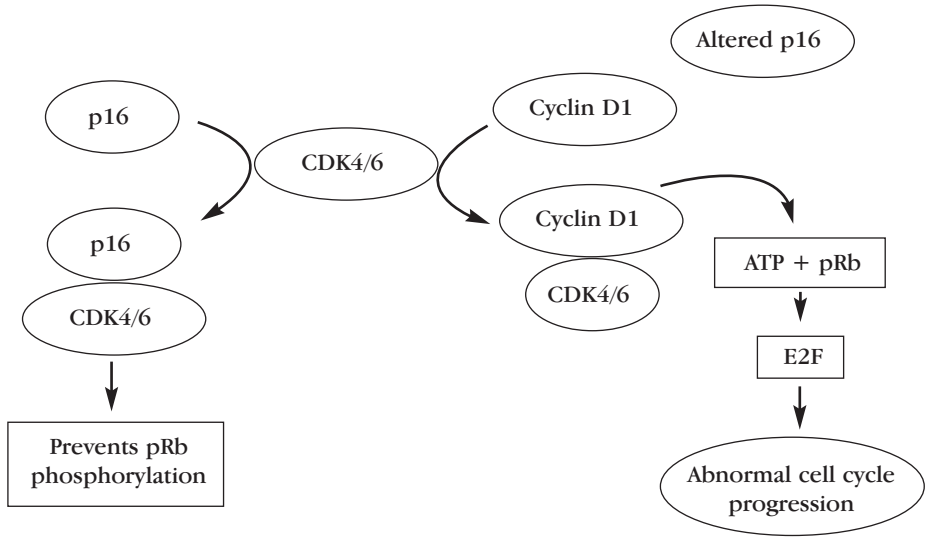

Figure 9: Role of p16 and CDK4/6 in cell-cycle regulation

tion, contributing to the separation of sister chromatids in mitosis. On the other hand, increased survivin immunostaining has already been observed in many cancers, such as nasopharyngeal carcinoma, squamous cell lung cancer, pancreatic adenocarcinoma, breast cancer, colorectal cancer, prostate and gastric cancer and high-grade non-Hodgkin's lymphomas. [51] Studies on the expression of survivin in oral cancer associate immunostaining with more aggressive and invasive tumor phenotypes. Nevertheless, no association was found between immunostaining for survivin in SCC of the mouth and tumor size, degree of dysplasia, presence of affected lymph nodes and distant metastases. [52] There are no specific studies on tissue immunostaining and the role of survivin in actinic cheilitis and lip squamous cell carcinoma.

iNOS

Endogenous production of nitric oxide may play an important role in cell progression, from dysplasia to frank malignancy. Nitric oxide is a reactive radical gas synthesized by the nitric oxide synthase enzyme from the L-arginine amino acid. Three isoforms of calcium-dependent nitric oxide synthase have been identified, two of which are physiologically present: endothelial and neuronal nitric oxide synthase, responsible for maintaining homeostasis of the nervous and cardiovascular systems, respectively. The third one, a protein of molecular weight of 130 kDa, is synthesized by specific stimuli, such as inflammatory processes and tumor progression, being called inducible nitric oxide synthase (iNOS). [53] Once synthesized, iNOS produces high levels of nitric oxide, which reacts rapidly with the superoxide anion present in eukaryotic cells, resulting in a peroxynitrite anion, which is a highly reactive molecule and, therefore, capable of generating a wide spectrum of lesions in DNA. [54]

iNOS synthesis was shown to be absent in normal mucosa of the oral cavity. However, increased expression of iNOS was found in dysplastic lesions of the oral cavity, which is positively correlated with the degree of epithelial dysplasia. [55] In human head and neck squamous cell carcinoma and experimental carcinoma of the cheek pouch of hamsters, the mRNA expression of iNOS was predominantly observed in frank tumor cells. [56] These results allow us to verify that nitric oxide favors tumor growth, angiogenesis and promotes metastasis. However, the importance of iNOS expression in actinic cheilitis and its role during the process of lip carcinogenesis has not yet been reported.

Bcl-2

The bcl-2 *(B-cell lymphoma* 2) family consists of anti-apoptosis proteins (Bcl-2, Bcl-XL, Bcl-w, Bfl-1, Brag-1, Mcl-1, A-1) and pro-apoptotic proteins (Bax, Bak, Bcl-XS, Bad, Bid, Bik, Hrk), which are located in the mitochondrial membrane, in the nuclear envelope or in the endoplasmic reticulum. [57] Thus, bcl-2 participates in the intrinsic pathway of apoptosis, and it is the relative expression of these proteins inside the cell that determines whether apoptosis will occur. [57,58] Since high levels of bcl-2 expression prevents the occurrence of apoptosis, increased immunoexpression of bcl-2 is associated with aggressive clinical course of cancer, resistance to chemotherapy or radiation therapy and shorter survival. [57,58] On the contrary, high levels of bax result in induction of apoptosis and prevention or better clinical behavior of cancer. [57,58] In the skin, the dysplastic epithelium and the epithelium around lesion borders of SCC show strong immunostaining for bcl-2 while bax is poorly expressed. Significant immunostaining for bcl-2 is also present in cases of undifferentiated SCC, while it is the opposite in well-differentiated SCC, which shows more significant immunostaining for bax. [57,58]

The expression of bcl-2 is more pronounced in actinic cheilitis than that observed in normal mucosa. In the same study, bax did not show to be more expressed in actinic cheilitis than in normal mucosa or in normal semimucosa of the lip, despite the statistical trend of showing more pronounced immunostaining in dysplastic epithelium of actinic cheilitis. These findings suggest that there is, within certain limits, pro-apoptosis phenomena in actinic cheilitis in an attempt to eliminate the harmful effects of solar radiation.

Maspin

Maspin *(mammary serine protease inhibitor)* or serpin B5 is a protein that belongs to the serpin *(serine protease inhibitor)* superfamily, which comprises protease inhibitor proteins.[59] Its function is related to tumor suppression, inhibition of angiogenesis and inhibition of tumor invasion and metastasis. That is, the expression of maspin in the tissue is inversely correlated with tumor malignancy. In principle, maspin is expressed in normal cells and has reduced or absent expression in dysplastic cells and tumor cells, respectively, and could be used as a prognostic marker of tumor invasion. Immunodetection of maspin is related to better prognosis in breast and prostate cancer. The same has been observed in relation to head and neck SCC and SCC of the tongue.

In actinic cheilitis, maspin immunostaining is inversely proportional to the degree of dysplasia of epidermal cells. [59] Therefore, maspin could be useful in the prognostic evaluation of actinic cheilitis. With regard to SCC of the lip, there was intense immunostaining for maspin both in the neoplasm itself, in virtually all cells, and in the epithelium adjacent to the SCC. [59] This finding, which is somewhat paradoxical,

suggests the need for further investigation as to immunostaining and the role of maspin in actinic cheilitis and SCC of the lip.

CONCLUSION

From what was mentioned above, the authors stress the frequency and importance of actinic cheilitis as a potentially malignant disorder, which is often neglected by patients and medical and dental practitioners. They demonstrate the connection between lip cancer and prior existence of actinic cheilitis, the importance of early diagnosis to prognosis and draw attention to the severity of lip SCC when it is diagnosed in stages III-IV. They also stress the importance of knowing and studying tumor markers for understanding the natural course of SCC of the lip. In addition, they suggest immunostaining in cases of AC with a higher grade of histological dysplasia as an aid in the prognosis of this disease. ❑

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