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Over Expression of p16 in Squamous Cell Carcinoma of the Oral Cavity

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Abstract: ***Introduction: In recent years, there has been a worldwide increase in the incidence of squamous cell carcinoma of the oral cavity in young adults who are often without a history of smoking and who are often female. Alterations in p16INK4A, the tumor suppressor gene, have been regularly reported in this condition. Materials and methods: It is an interesting study of 14 cases of squamous cell carcinoma of the oral cavity collected in the department of stomatology and maxillofacial surgery. The term young is used to define patients aged 49 years or younger at the time of diagnosis. The expression of the p16INK4A protein was analyzed by immunohistochemistry. An invasive squamous cell carcinoma of the oral cavity with strong expression of p16INK4A was used as a positive control. Results: 28% were women and 43% were non-smokers. All tumors were squamous cell carcinomas, most of them moderately differentiated (65% of cases). No tumors were in stage I, while 44% and 28% were in stage III and IV respectively. The tumors were located on the lip (50% of cases), the anterior two thirds of the tongue (21% of cases), gum (14% of cases), the floor of the mouth (8% of the cases) IFJ (7% of cases). A strong and diffuse immunoreactivity of p16INK4A was observed in all cases of epidermoid carcinomas of the oral cavity. Discussion: Our results are consistent with those described in several studies and are in favor of overexpression of the p16INK4A protein in dysplastic cells of the oral cavity, an intense immunostaining with p16INK4A could constitute an effective marker of precancerous lesions of high grade and potentially lesions The overexpression of p16INK4A can be used to identify and confirm these lesions. Therefore overexpression of p16INK4A can be widely used routinely***

Keywords: *epidermoid carcinoma, oral cavity, p16INK4A*

1. Introduction

In recent years, there has been a worldwide increase in the incidence of squamous cell carcinoma of the oral cavity in young adults who are often without a history of smoking and who are often female [1,2]. Alterations in p16INK4A, the tumor suppressor gene, have been reported consistently in this condition, with a prevalence ranging from 25% to 83% [1].

In order to reduce mortality and improve the quality of management, new approaches including molecular biology methods have recently been studied.

The aim of this study, carried out by the Stomatology Department of Oral and Maxillofacial Surgery, is to determine the over expression of p16INK4A in squamous cell carcinoma of the oral cavity.

2. Materials and methods

2.1 Patients

It is an interesting study of 14 cases of squamous cell carcinoma of the oral cavity collected in the department of stomatology and maxillofacial surgery. The term young is used to define patients aged 49 years or younger at the time of diagnosis.

2.2 Methods

Study of the expression of the protein p16INK4A by immunohistochemistry. The expression of the p16INK4A protein was analyzed by immunohistochemistry. Cuts of 4 microns thickness were carried out. After dewaxing and rehydration, antigenic unmasking is carried out. The slides are incubated with the primary antibody: The anti-p16 monoclonal antibody (Dako REALTM EnVision® Detection System, Peroxidase / DAB +, Rabbit / MouseCode K5007). We used as positive control an invasive squamous cell carcinoma of the oral cavity with a strong expression of p16INK4A. The immunohistochemical labeling was evaluated semi-quantitatively. We defined a first score, ranging from 0 to 3 depending on the marking intensity (0: no marking, 1: low marking, 2: intermediate marking, 3: intense marking). Then we defined a second score of 1 to 5 according to the percentage of positively labeled tumor cells (score 0: no positive cell, score 1: <25%, score 2: 26-50%, score 3, 51-75% 76-99% and score 5: 100% of positive tumor cells). After multiplication of the two values, the immunohistochemical results are presented in a final score ranging from 0 (no reactivity) to 15 (100% of the tumor cells with an intense and diffuse marking) [Table 1].

1. Preparation of solutions:

- Unmasking solution:
- 280 ml of distilled water + 20 ml of citrate buffer PH = 6

- Diluted PBS Buffer:
- 900 ml of distilled water + 100 ml of PBS PH = 7.2

2. Procedure

- Dewax the slides in the oven at 60 ° C for 30 min.
- Dehydrate the slides in 3 toluene baths and then 3 min.
- Rinse the slides in warm water • Rinse with distilled water
- To unmask the antigen immerse the slides in the solution to unmask it is incubated at 95 ° C in a water bath for 45 min.
- Remove the slides and allow to cool in the unmasking solution for 20 min.
- Rinse in PBS solution.
- Block endogenous peroxidases by incubating the tissue in hydrogen peroxidase (H2O2) for 5 min.
- Rinse in PBS buffer.
- Apply the primary antibody (Dako REALTM EnVisionTM Detection System, Peroxidase/DAB+, Rabbit/MouseCode K5007)to the tissue for 30 min.
- Wash in PBS buffer.
- Add the DAB chromogen: buffer substance 1 ml + a chromogenic DAB drop (S 50), left for 10 min.
- Rinse with distilled water.
- Stain with hematoxylin for a few seconds.
- Wash with running water
- Dehydrate the tissue in 3 liquor baths
- Wash the fabric in 3 toluene baths
- Mount the blades for observation using the OlympusR X21 microscope.

3. Results

Clinical features of the cases are listed in Table 2. 28% were women and 43% were non-smokers. All tumors were squamous cell carcinomas, most of them moderately differentiated (65% of cases). No tumors were in stage I, while 44% and 28% were in stage III and IV respectively. The tumors were located on the lip (50% of cases), the anterior two thirds of the tongue (21% of cases), gum (14% of cases), the floor of the mouth (8% of the cases) internal face of the cheek (7% of cases) [Table 2]. Thirteen patients showed p16 immunoreactivity. An intense and diffuse immunoreactivity score between 8 and 15 was observed in 9 cases of squamous cell carcinomas of the oral cavity [Figure 1]..

80% of men have a high immunostaining score of p16INK4A. 64% of patients with a high p16 score were aged over 50 years and 42% were tobacco users [Table 3].

4. Discussion

In recent years, there has been a worldwide increase in the incidence of epidermal carcinoma of the oral cavity in young adults who are often without a history of smoking and who are often female [1,2].

In addition to known or suspected risk factors, a genetic predisposition for cancers of the oral cavity is discussed [6]. Indeed, the regulation of the cell cycle is explained by a series of events leading to the duplication of cells using two types of molecules that are cylindrical and CDK. In this process, two gene families, CIP / KIP and INK4a / ARF, act as tumor suppressors and prevent progression of the cell cycle [3,5].

At the level of the organism, the carcinogenic agents are metabolized by enzymes whose major role is their elimination. Some of the genes encoding these enzymes have a polymorphism. For an individual, inheriting an enzyme with reduced activity can lead to an excessive accumulation of toxic and has a decreased detoxification capacity [4].

Alterations in p16INK4A, a tumor suppressor gene, have been consistently reported in this condition, with a prevalence ranging from 25% to 83% [1].

The p16 protein is a cellular protein involved in the regulation of the cell cycle and its expression is then strictly controlled in normal cells. In dysplastic cells, p16 is clearly overexpressed. However, if in normal cells p16 is expressed at low levels and generally undetectable by immunochemical methods, it can also be transiently expressed and detected in normal metaplastic cells [1,6].

P16INK4A, a member of the family of cellular regulatory proteins INK4A, specifically inhibits the formation of the cyclin D / CDK4 complex. 6. Overexpression of p16INK4A could be an important tool for identifying precancerous lesions in the oral cavity and reducing variability Diagnosis observed during the evaluation of suspected lesions of the oral cavity [1,2].

Indeed, it has been shown that the loss of tumor suppressor genes p16 / p14 ARF plays a decisive role in the carcinogenesis of VADS. The product of the p16 gene (CDKN2A) has the function of inactivating the cdk4-cdk6-cyclin D complex, which prevents the phosphorylation of the retinoblastoma protein (pRb). In contrast, over expression of pRb is associated with inhibition of p16 [1,6].

In the majority of cancer lesions, expression of p16INK4A was strong and diffuse other studies concluded that the expression of p16INK4a has a significant correlation with the staging and progression of squamous cell carcinomas of the oral cavity [1]. A study of the expression of p16 INK4A by immunohistochemistry in cancers of the tongue showed that the loss of expression of p16 INK4A is associated with a significant reduction in survival and free interval without recurrence. Whereas p16 is most often overexpressed in higher grade and stage tumors [1,6].

Our results are consistent with those described in several studies and are in favor of overexpression of the p16INK4A protein in dysplastic cells of the oral cavity, an intense immunostaining with p16INK4A could constitute an effective marker of precancerous lesions of high grade and potentially lesions The overexpression of p16INK4A can be used to identify and confirm these lesions. Therefore, overexpression of p16INK4A can be widely used routinely [1,3].

Strong expression of cytoplasmic and nuclear p16INK4A was observed in our cases of squamous cell carcinomas.

Despite this small number, our results are consistent with most previous studies.

The CDKN2A gene encoding p16 is overexpressed in tumors associated with human papillomaviruses of high risk oncogenic due to the action of the viral oncoprotein E7 and can be used as a biomarker of tumors related to human papillomaviruses of high oncogenic risk. Teams propose to use the detection of the over expression of the p16 protein by immunohistochemistry [7,8]. Indeed, the CDKN2A gene encoding p16 is over expressed in tumors associated with human papillomaviruses of high risk oncogenic due to the action of the viral oncoprotein E7 and can be used as a biomarker of tumors related to human papillomaviruses of high oncogenic risk. This technique is easily performed on tumor samples in paraffin but lacks specificity with a risk of false positive. To circumvent the difficulty, Smeet et al. Proposed to associate the detection of the genome of human papillomaviruses of high risk oncogenic by PCR and immunohistochemistry, detecting p16. In this combination, tumors associated with high-risk human papilloma viruses were oncogenic where viral DNA and overexpression of p16 were simultaneously detected. This method would allow a sensitivity of 100% (in comparison with detection of the E6 / E7 transcript) and a false positive risk of 2% [9].

In cancers of VADS, overexpression of p16INK4A is correlated with a better prognosis [10].

Ang et al. They even propose to classify the risks of death of patients by taking into account their HPV status, their smoking consumption and the TNM status of the tumor and thus to introduce less invasive treatments for patients with a low risk of death. Positive HPV patients have a 5-year survival rate higher than HPV-negative patients [11].

Smith et al. Analyzed the individual and combined effect of the two markers: p16INK4A and HPV [10]. They showed that the positive p16INK4A-positive HPV group had the best survival rate but that the recurrence rate was significantly lower in p16INK4A negative-HPV positive or negative groups [12]. A similar study using p53 and HPV showed that the positive p53 negative-HPV group had the best prognosis with the lowest recurrence rate and a higher survival rate, while the p53 positive- HPV negative has the worst prognosis [10,13,14,15,16]. This could be due to a lack of field cancerization and / or a better radiation sensitivity of HPV-HR positive cancers. Overexpression of p16 (INK4A) induced by HPV-HR may play a role in this radiosensitivity [17]. P16 positive tumors were associated with better overall survival at 2 years than p16 negative tumors, and 'To better survival without fail. P16 was a significant prognostic factor for multivariate analysis. P16-positive patients had lower rates of locoregional failure and death from other causes [17].

Based on these results, overexpression of p16 would be a useful marker for squamous cell carcinoma of the oral cavity

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Table 1: Semi-quantitative immunohistochemical marking

patient	*score P16ink4a*	*Age*	*sexe*	*TABAC*	*site*	*degrés de différenciation*
1	8	68	w	no	lip	moderately
2	15	51	M	YES	floor	moderately
3	8	52	M	no	TONGUE	moderately
4	6	79	W	no	lip	LITTLE
5	8	61	M	no	lip	little
6	4	50	W	no	tongue	moderately
7	15	67	M	YES	lip	moderately
8	9	88	M	YES	lip	moderately
9	8	65	W	no	gum	moderately
10	12	55	M	YES	lip	moderately
11	8	50	M	YES	tongue	well
12	0	45	M	YES	lip	well
13	4	62	M	YES	Internal face of the cheek	well
14	3	41	M	YES	gum	moyen

Table 2: Clinicopathological characteristics of cases

Settings	n(%)
Age	
≤50	4 (28)
>50	10(72)
sexe	
men	10(72)
women	4(28)
site	
lip	7 (50)
Floor buccal	1(7)
tongue	3(21)
gum	2(15)
Internal face of the cheek	1(7)
smoking	
yes	8(57)
no	6(43)
stage	
I	0
II	4(28)
III	6(44)
IV	4(28)
Degrees of differentiation	
little	2(14)
way	9(65)
good	3(21)

Table 3: Correlation between clinicopathological characteristics of cases and P16 tests

	Overexpression	***positive***
Age		
≤ 50	1	3
> 50	9	1
Sex		
men	8	2
womem	2	2
Smoking		
yes	6	2
no	4	2

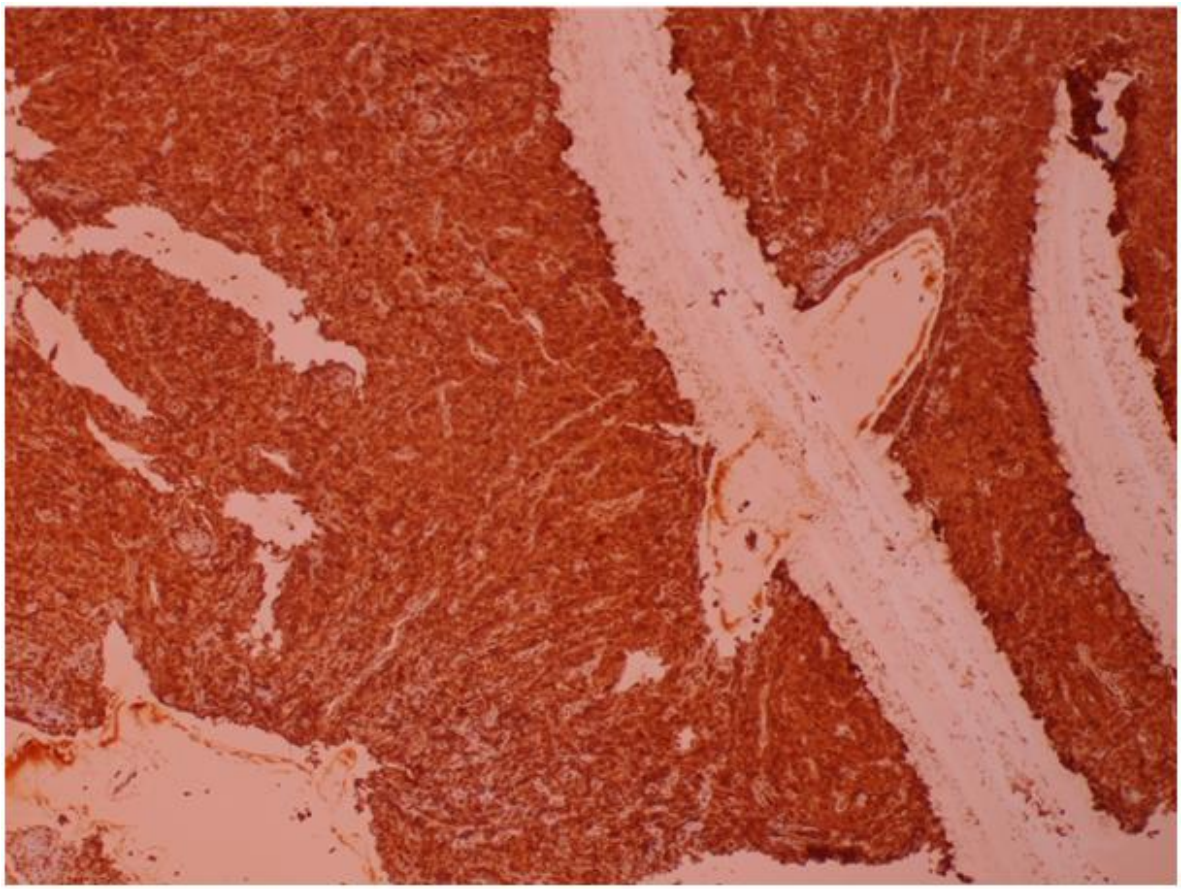

Figure 1 A: Immunohistochimie de carcinome épidermoïde de la cavité buccale: expression de p16 intensee et diffuse, cytoplasmique et nucléaire[grossissement x20]

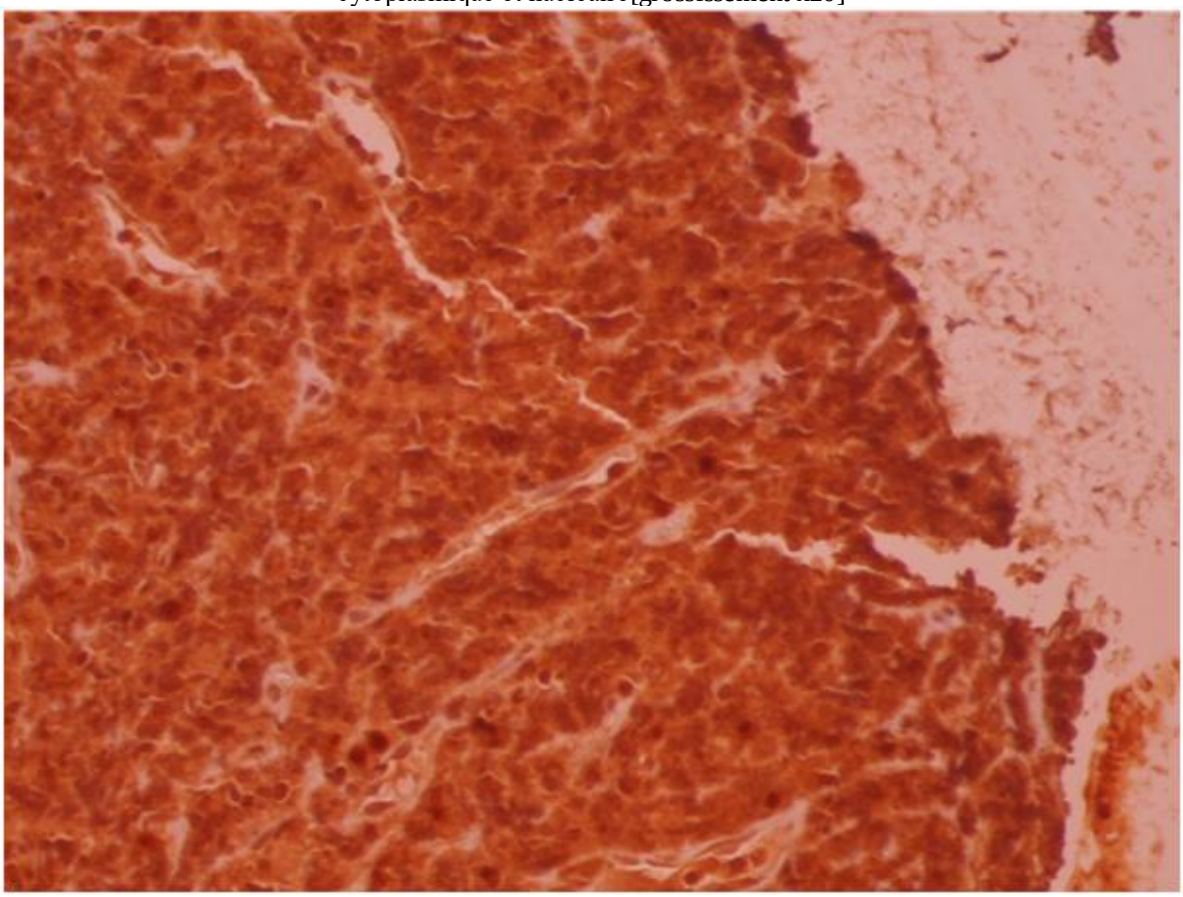

Figure 1 B: Immunohistochimie de carcinome épidermoïde de la cavité buccale : expression de p16 intensee et diffuse, cytoplasmique et nucléaire [grossissement x40]