Original Article

p16^{INK4a}, and p14^{ARF} Expressions in Carcinogenesis of Squamous Cell Carcinoma of the Lip

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Abstract

The goal of this study was to clarify the role of p16^{INK4a}, p14^{ARF,} and p53 protein expressions in carcinogenesis in squamous cell carcinomas of the lip. The expressions of the p53, p16^{INK4a}, and p14^{ARF} proteins were examined in 46 formaline-fixed paraffin embedded tissue specimens, which included 19 cases of squamous cell carcinoma of the lip, 14 cases of actinic cheilitis, and 13 cases of normal mucosa. Immunoreactivity in the peritumoral epithelium adjacent to squamous cell carcinomas was also evaluated. p16^{INK4a} expression was increased in actinic cheilitis in comparison with normal mucosa (p=0.001). p14^{ARF} expression progressively increased from normal mucosa to actinic cheilitis (p=0.001) and was observed to decrease significantly during the process of transition from actinic cheilitis to carcinoma (p=0.003). p53 values progressively increased from normal mucosa to actinic cheilitis (p=0.001) and carcinoma (p=0.008). A significant positive correlation was found between p14^{ARF} and p53 in the peritumoral epithelium adjacent to carcinomas. Our findings indicated that p16^{INK4a} and p14^{ARF} immunohistochemistry does not determine whether or not actinic cheilitis has the potential to develop carcinoma. The p14^{ARF} /p53 pathway is activated in the peritumoral epithelium adjacent to carcinoma; however, this activation would not be adequate to prevent carcinogenesis.

Keywords: *Actinic cheilitis, Carcinogenesis, Lip, p16^{INK4a}, p14^{ARF}*

Introduction

Squamous cell cancer of the lip is the most common form of oral cancer and squamous cell carcinoma (SCC) constitutes 90-95% of all malignant oral lesions. Actinic cheilitis (AC), which is a pathological condition that particularly affects the lower lip and appears due to chronic and excessive exposure to ultraviolet (UV) radiation in the sunlight, is a potentially malignant lesion that can transform into SCC of the lip.[1-5]

According to the 2017 Cancer Statistics Database of the Turkish Ministry of Health, the gender distribution of the age-standardized prevalence rate of lip cancer was 0.9 per 100000 for males and 0.2 per 100000 for females.[6] In a study representing the eastern region of Turkey, it was determined that the majority of lip cancers were encountered in males and that the predominant histopathological subtype was squamous cell carcinoma with a rate of 89%. Consistent with the literature, the mean age was higher than 50 years.[7] Similarly, in another study on oral cancers that was conducted in the west of Turkey, it was also determined that the prevalence rate was higher among males and that the mean age exceeded 50 years. Initiation of alcohol consumption at a young age, a low level of education, poor oral hygiene, and dietary habits was shown to be associated with the development of oral cancer.[8]

It is generally considered that head and neck SCCs originate from a common premalignant progenitor and the subsequent cumulative genetic aberrations that result in the overgrowth and phenotypic progression of the related clonal population to invasive malignancy.[9, 10] These genetic changes are products of the inactivation of multiple tumor suppressor genes and the activation of multiple proto-oncogenes including p16^{INK4a}, p53, cyclin D1, p14, FHIT, RASSF1A, EGFR, and Rb.[11, 12] Loss of chromosome 9p21 is encountered in 70-80% of head and neck cancers and this is the most common genetic change observed in squamous dysplasia.[9]

The locus of cyclin-dependent kinase inhibitor 2A (CDKN2A) on chromosome 9p21 is the second most commonly altered gene locus in human cancers following p53. The CDKN2A locus codes two different tumor suppressor proteins known as

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$p16^{INK4a}$ and $p14^{ARF}$.[13] $p16^{INK4a}$ is an important component of the Rb pathway that has a role in the progression of the cell cycle. Inactivation of cellular $p16^{INK4a}$ prevents the termination of the cell cycle and results in tumor development.[14] On the other hand, $p14^{ARF}$ regulates the cell cycle in the negative direction by inhibiting the MDM2 (murine double minute-2) oncoprotein, which regulates p53 at phases G1/S and G2/M. For this reason, $p14^{ARF}$ acts as a tumor suppressor gene in a p53-dependent pathway.[15]

While some studies suggest that increased expression of $p16^{INK4a}$ could be useful in the diagnosis of dysplastic lesions of the skin and the oral cavity, some studies highlight the diagnostic significance of decreased expression.[16] There are very few studies that have investigated $p14^{ARF}$ protein expression immunohistochemically.[17]

Although many studies have inspected the effect of UV light on the skin, very few studies have investigated the mechanisms of AC and SCC development.[18-22] Thus, it is important to understand the mechanism underlying the development of SCC. This study aims to investigate nuclear expressions of $p16^{INK4a}$, $p14^{ARF}$, and p53 in normal lip mucosa, AC, and SCC of the lip, and therefore, to determine the potential importance of these markers in early carcinogenesis, as well as their relationship with histopathological prognostic factors.

Materials and Methods

Study design

This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval was granted by the Ethics Committee of Kırıkkale University School of Medicine (IRB-2009/012). This study included specimens from three groups of Caucasians: i)AC group:14 patients with AC (mean age, 56.57 years; range, 40–84years; 4 female/10 male); ii) SCC group: 19 patients with SCC of the lip (mean age, 64.3 years; range, 50–82 years; 3 female/16 male); iii) control (C) group: histopathologically normal lip specimens from 13 healthy individuals (mean age 50.5 years; range, 38-72 years; 6 female/7 male). Archived hematoxylin-eosin stained preparations of all cases were reviewed by two pathologists (ANA, ESA) to select lesions.

A lesion was diagnosed as AC if the keratinocytes in the epidermis were arranged in a disorderly manner and demonstrated cytological atypia and increased mitotic activity. A diagnosis of SCC was made if invasive islands or groups of malignant squamous epithelial cells were present. For the SCC group, histologic tumor grade, the presence of ulceration, perineural and/or lymphovascular invasion, tumor thickness, diameter, and the presence of lymph node metastasis were recorded. Tumor grading was done according to Broders' classification.[23] Tumor thickness was measured from the granular cell layer to the deepest tumor cell using an ocular micrometer.

Immunohistochemistry

Immunohistochemical (IHC) staining was performed using the streptavidin-biotin peroxidase method with diaminobenzidine (DAB, Labvision) chromogen and counterstaining with Mayer's hematoxylin.

Five µm sections were prepared from each representative paraffin block. Each section was deparaffinized, rehydrated, and subjected to microwave antigen retrieval in ethylenediamine-tetraacetic acid (EDTA) buffer. The sections were incubated for 1 h with Anti-$p16^{INK4a}$ ($p16^{INK4a}$ Ab-4(16PO4), Neomarkers Ltd, dilution 1/100), Anti-p14ARF (GeneTex, GTX23642, dilution 1/50), and Anti-p53 (SP5, Neomarkers Ltd, dilution 1/100), which were used as primary antibodies.

Evaluation of immunohistochemical staining

Nuclear immunostaining for $p16^{INK4a}$ and p53 and nucleolar and nuclear immunostaining for $p14^{ARF}$ in keratinocytes were regarded as positive immunoreactivity. For each AC, and C specimen, the location of staining was recorded as the lower one-third, lower two-thirds, or upper one-third of the epidermis, while superficial areas and invasive fronts were also recorded for SCC cases. The modified immunohistochemical score (H-Score) was used for quantifying staining as previously specified.[24] An H-score was recorded for each marker ($p16^{INK4a}$, p53, and $p14^{ARF}$) in each AC, SCC, and C specimen. In addition to the H-scores for the invasive portions of the SCC lesions, H-scores were also calculated for the peritumoral dysplastic epidermis of each SCC. Positivity in stromal cells was not assessed.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS v. 15.0, Chicago, IL, USA). Mean ± standard deviation values were used as variables. The H-Scores for $p16^{INK4a}$, $p14^{ARF}$, and p53 were not normally distributed in any of the study groups; therefore, non-parametric tests were used to compare the H-score data. Kruskal-Wallis one-way analysis of variance (ANOVA) was used to analyze the differences between the study groups in terms of the mean H-scores for each marker. When a significant difference was observed, the Mann-Whitney U and Wilcoxon signed-rank tests were used to identify the pair(s) of groups that differed statistically. The H-scores calculated for the peritumoral epidermis and invasive islands of tumor cells in SCCs were compared using Wilcoxon signed-rank tests. Spearman's rank correlation coefficient was used to test for relationships between the three H-scores within each study group, and for associations between the H-scores for each marker and various histopathological parameters.

Results and Discussion

None of the SCC cases presented lymphovascular invasion and only two cases presented perineural invasion. Of the three cases that had lymph node dissection specimens, none had metastatic lymph nodes. Six cases manifested a tumor diameter larger than 2 cm, and seven tumors had a tumor

thickness of >5 mm. Invasion depth reached the muscle tissue in 10 cases, the adipose tissue in two cases, through the entire dermis in four, and the middle dermis in two cases. Of the cases in the SCC group, 74% were composed of Grade 1(n=13) and Grade 2(n=2) tumors and 26% of Grade 3(n=4) tumors. Ten cases (53%) were T1N0M0, and nine (47%) cases were T2N0M0 according to the AJCC 8th edition.

The patterns of p16^{INK4a}, p14^{ARF}, and p53 expression in the epidermis are presented in **(Table 1)** and **(Table 2)**. **Table 1** presents the staining distribution for p16^{INK4a}, p14^{ARF}, and p53 expression. **Table 2** demonstrates the immunostaining patterns according to localization in the epithelium. Regarding p16^{INK4a} positivity in the groups, four (30.8%) of the 13 C specimens showed staining for p16^{INK4a} in the lower two-thirds of the epidermis; the same staining pattern was detected in nine (64.3%) of the 14 AC lesions and the peritumoral epidermis of 18 (94.8%) of the 19 SCC lesions.

Table 1. Positive immunohistochemical staining percentages of the cases.

	C group (n:13) n(%)	AC Group (n:14) n(%)	SCC group (n:19) n(%)	PE of SCC* (n:19) n(%)
P16	4(30.8)	9(64.3)	12(63.1)	18(94.8)
P14	13(100)	14(100)	19(100)	19(100)
P53	13(100)	14(100)	16(84.2)	19(100)

*PE of SCC (Peritumoral epidermis of SCC)

Table 2. Immunohistochemical staining localizations of cases in the C, AC, and PE of SCC groups

Location in epidermis	C group n (%)	AC Group n (%)	PE of SCC n (%)
	P16 p14 p53	P16 p14 p53	P16 p14 p53
Lower third (basal)	4 6 13 (30.8) (46.1) (100)	8 3 10 (57.1) (21.4) (71.4)	13 4 17 (68.4) (21) (89.5)
Middle third	0 7 0 (53.9)	1 4 4 (7.1) (28.6) (28.6)	5 11 2 (26.3) (58) (10.5)
Upper third	0 0 0	0 7 0 (50)	0 4 0 (21)
Total cases	13	14	19

*PE of SCC (Peritumoral epidermis of SCC)

Regarding p14^{ARF} positivity in the groups, seven (50%) of 14 AC specimens showed staining for p14^{ARF} in the lower two-thirds of the epithelium, and the same staining pattern was observed in the peritumoral epidermis of 15 (78.9%) of the 19 SCC lesions. All specimens in the C group showed staining lower two-thirds of the epithelium. Seven (50%) specimens in the AC group and four (21%) specimens of the peritumoral epithelium of SCC lesions showed p14^{ARF} staining in the upper epithelial cells.

Regarding p53 positivity, all C and AC specimens showed staining for p53 in the lower two-thirds of the epidermis, and the same staining pattern was detected in the peritumoral epithelium in all SCC cases **(Figure 1)**.

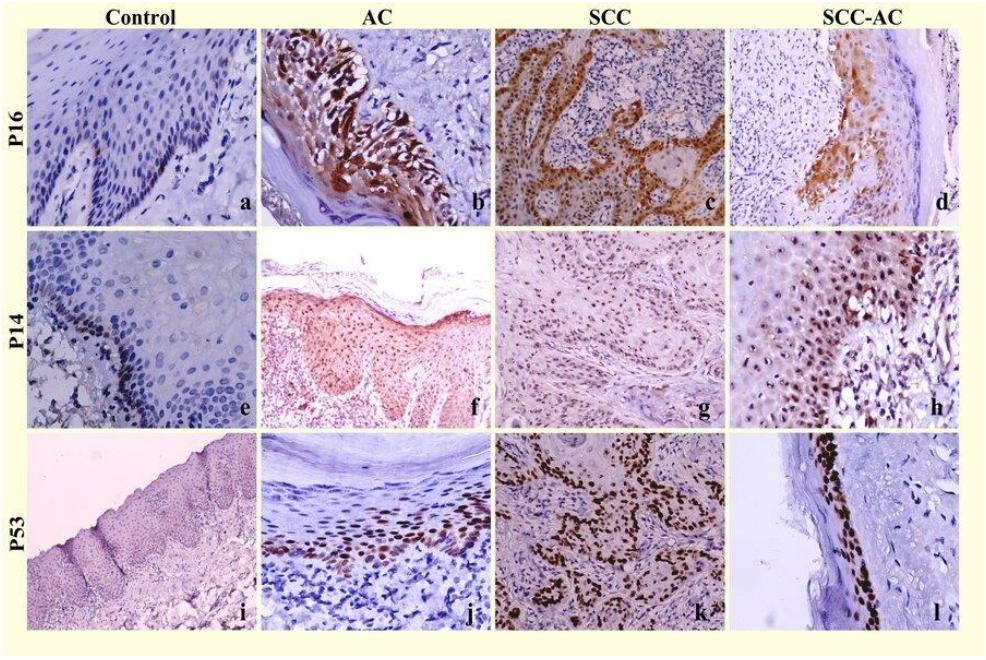

Figure 1. Representative images of p16, p14, and p53 expression patterns
Nuclear expression of p16 in (a)normal mucosa, (b)actinic cheilitis, (c)squamous cell carcinoma, (d) peritumoral epidermis (a,b: x400; c,d:x200, IHC) .Nuclear and nucleolar expression of p14 in (e) normal mucosa,(f)actinic cheilitis,(g)squamous cell carcinoma,(h) peritumoral epidermis. (e,h:x400; f,g:x200, IHC). Nuclear expression of p53 in (i) normal mucosa, (j)actinic cheilitis, (k)squamous cell carcinoma, (l) peritumoral epidermis (i:x100; j,l: x400; k:x200, IHC)

The patterns of p16^{INK4a}, p14^{ARF,} and p53 expression in invasive islands of SCC are summarized in **(Table 3)**. Four cases in the SCC group demonstrated immunostaining for p16 around invasive islands. We observed focal scattered staining in four cases, a diffuse staining pattern in one case, and immunostaining in the superficial areas in four cases for p16.

Table 3. Distribution of immunohistochemical stainings in the invasive areas of squamous cell carcinoma cases.

Staining pattern in invasive tumor islands	P16 n(%)	P14 n(%)	P53 n(%)
Peripheral	4(21)	16(84.2)	16(84.2)
Scattered	4(21)	3(15.8)	0
Diffuse	1(5)	0	0

Regarding p14^{ARF} positivity in the SCC group, 16 cases showed immunostaining at the basal aspect of the invasive islands and three cases showed a scattered staining pattern.

Regarding p53 positivity, 16 cases showed a staining pattern in the form of one to two lines in the basal layer at the periphery of the invasive islands, while three cases did not show immunoreactivity.

In the SCC group, all of the p16^{INK4a} negative cases demonstrated positive staining with p14^{ARF} and p53. The three p53 negative cases were found to show positive staining with p16 and p14^{ARF}.

Descriptive statistical values for the H-Scores of p16^{INK4a}, p14^{ARF}, and p53 expressions in all groups are presented in **(Table 4)**. The distribution characteristics of the H-Score values of antibody expressions are depicted by a box-and-whisker plot **(Figure 2)**.

The highest mean H-Score of p16^{INK4a} expression was higher in the AC and SCC groups compared to the C group (p=0.001, p=0.005).

Table 4. H-Score values of p16, p14, and p53 expressions

	C group H-scores (n:13)	AC Group H-scores (n:14)	SCC group H-scores (n:19)	PE of SCC H-scores (n:19)
P16	5.62(0-35)	32.79(0-101)	45.32(0-123)	53.89(0-120)
P14	52.92(10-68)	160.93(101-205)	119.95(64-178)	143.89(70-186)
P53	7.54(5-25)	59(12-150)	109.89(0-156)	59.84(15-169)

*PE of SCC (Peritumoral epidermis of SCC)

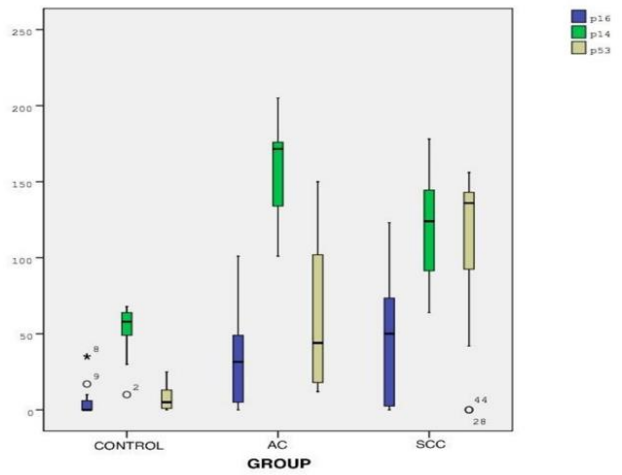

Figure 2. Box-and-whisker plot of H-Score values of p16, p14, and p53 in C, AC, and SCC groups

The highest mean H-score of p14^{ARF} expression was found to be higher in the AC and SCC groups compared to the C group (p=0.001, p=0.001). The highest mean H-score of p14^{ARF} expression was determined to be lower in the SCC group compared to the AC group with statistical significance (p=0.003).

The highest mean H-score of p53 expression was found to be higher in the AC and SCC groups. p53 expression was determined to be higher in the SCC group than in the AC group (p=0.008).

The comparison of p16^{INK4a}, p14^{ARF}, and p53 expressions between the peritumoral epidermis and the tumor in cases with SCC revealed no significant differences concerning p16^{INK4a}, a decrease in p14^{ARF} expression in tumoral areas compared with the higher levels in peritumoral areas, and a greater expression of p53 in tumoral areas (p>0.05, p=0.007, p=0.005).

The comparisons made between the peritumoral epithelium and cases of AC without an accompanying tumor revealed no significant differences in terms of the H-Score values associated with the expressions of the three antibodies (p>0.05).

Spearman's correlation analysis revealed no significant relationship between clinicopathological parameters and expressions of p16^{INK4a}, p14^{ARF}, and p53. No correlation was determined between the SCC, AC, and C groups in terms of p16^{INK4a}, p14^{ARF}, and p53 expressions. However, in SCC cases, a positive correlation was noted between p14^{ARF} and p53 expressions in the peritumoral epithelium (p=0.011).

The majority of studies conducted on head and neck cancers and oral SCCs using p16^{INK4a}, p14^{ARF} and p53 have focused on tumors in different localizations. SCCs originating from the lip were usually studied as a sub-group in these studies.[12, 25, 26] The literature on SCC of the lip contains studies showing positive correlations for p53/Ki67 and CD44/VEGF in the assessment of the prognosis and progression of tumor evolution.[27] Molecular changes such as homozygous deletion, promoter methylation and mutation have been found in INK4A-ARF genes in the tumor and tumor free margins of oral cancers.[28] In this study, we compared the proteins produced by the genes p53, p16^{INK4a}, and p14^{ARF} in cases of AC, and investigated the changes associated with the SCC of the lip. To the best of our knowledge, this study is the first that investigates all of these three proteins in SCCs originating from the lip.

Inactivation of the p16^{INK4a} gene is prevalent in head-neck and oral SCCs and this inactivation is believed to be brought about by a variety of mechanisms including homozygous deletion, DNA methylation, and point mutation.[28-31] Various ideas exist regarding the immunohistochemical evaluation of p16^{INK4a} gene inactivation. Sanchez-Cespedes *et al*. argue that immunohistochemical loss of p16^{INK4a} is connected to the inactivation of the p16^{INK4a} gene.[32] Losses in p16^{INK4a} expression have also been observed in premalignant oral lesions and oral cavity tumors.[30]

The relationship between the mutation in the p16^{INK4a} gene and immunohistochemical staining of protein expression continues to remain unclear. While p16^{INK4a} gene inactivation is the most prominent molecular change that is detected, various publications have reported different results such as an increase or a loss in p16^{INK4a} protein expression based on immunohistochemistry.[33, 34]

In their study investigating the transition from normal skin to SCC, Hodges *et al*. reported that p16^{INK4a} expression was higher in cases of squamous cell carcinoma in situ compared to actinic keratosis, that it manifested a full-thickness staining pattern, and that expression was correlated with progression from normal skin to SCC.[34] They showed in the same study that there was p16^{INK4a} staining in the lower half of the epidermis in actinic keratosis. Similar to their study, our results showed higher p16^{INK4a} expression in AC compared to normal mucosa. Although there are differences in the localizations when our study is compared with that mentioned above, it was determined that AC on the lips and actinic keratosis on the skin could manifest similar p16^{INK4a} expression.

Tokman and colleagues investigated p16^{INK4a} and p53 expression in oral SCC.[20] Squamous cell carcinomas of the lip, which they investigated as a sub-group in their study, were found to manifest positive staining with p16^{INK4a} at a rate of 58%.[26] Similarly, in our study, 63% of cases of SCC of the lip demonstrated positive staining.

The p16^{INK4a} expression patterns in cases of AC and SCC can be explained by an accumulation of inactive p16^{INK4a} proteins and an inability of the cells to avoid the cell cycle, or the failure or inadequacy of one of the other components of the Rb pathway, resulting in a functional overexpression of the p16^{INK4a} protein. p16^{INK4a} overexpression can support the hypothesis that UV radiation results in defective p16^{INK4a}

tumor suppressor gene activity, in concordance with the previous studies.[35]

There are very few studies that have investigated $p14^{ARF}$ protein expression in head and neck cancers using immunohistochemistry.[33, 36] Only a limited number of studies exist in the literature on AC.[35, 37] Most of these studies have focused on p53 expression. In a study done by Weber *et al*. to determine the genetic and epigenetic changes associated with $p16^{INK4a}$ and $p14^{ARF}$, it was shown that immunohistochemical expression was negative in cases of malignant and benign tumors of the head and neck area who had methylation in genes $p16^{INK4a}$ and $p14^{ARF}$, whereas almost all cases without methylation manifested gene product expression at moderate-strong levels.[36]

We observed a statistically significant increase in $p14^{ARF}$ expression in transition from normal mucosa to AC and lower expression in cases of SCC than in cases of AC. While SCC cases manifested more significant expression compared to the C group, it was noticed that this expression was lower than that seen in AC cases. This increase in $p14^{ARF}$ expression in the forming of the actinic cheilitis period can be considered as $p14^{ARF}$ coming into play as a protective mechanism as a result of its oncogenic activation. The overexpression of $p14^{ARF}$ causes MDM2-$p14^{ARF}$complex formation and serves to prevent it from binding to p53. The detected presence of higher $p14^{ARF}$ expression levels in SCC compared to normal mucosa may suggest that p14ARF maintains its effect, however, fails to prevent the development of carcinoma.

Mutations in TP53 constitute the most commonly detected genetic disruptions in head-neck squamous cell carcinomas and studies have shown that the mutational spectrum of TP53 is different in cancers of the lip and cancers of the oral cavity.[38]

Studies investigating p53 protein expression in AC, actinic keratosis, and SCCs have mostly used immunohistochemical markers that indicate both mutant and wild-type p53(wt-p53). Therefore, the p53 overexpression detected in these studies should not be considered entirely an indicator of mutation. While mutations in TP53 are the most commonly identified mutations in cancers, p53 mutations were not found in all skin tumors showing p53 expression.[38, 39] Wild-type p53 increases after exposure to UV radiation, and since mutated p53 degrades more slowly, accumulation occurs. Therefore, the increase in p53 expression determined in studies can be either due to an increase in mutated p53 protein during tumor progression or wt-p53 expression in response to instability and DNA damaging agents in other genes. Multiple studies performed on skin cancers have determined that p53 mutation is not correlated well with immunohistochemical overexpression of the p53 protein.[39, 40]

In this study, we determined the p53 protein at low levels in normal epithelial cells, in concordance with other studies.[41] We determined a change in p53 expression that followed a pattern of increase during progression from normal mucosa to AC and SCC. This increase that we determined in p53 suggests that the wt-p53 might potentially contribute to the expression of mutant p53 as stated in other studies.[41, 42] However, we believe that this argument should be supported by molecular studies detecting mutant type p53. In this study, we detected that 84.21% of our SCC cases had p53 expression and determined that p53 had increased expression in lip carcinogenesis.

Studies have determined p53 expression levels that increase in parallel with the progression from AC to SCC.[37, 43-45] Moreover, they did not determine any differences between cases of AC related to, and unrelated to the tumor in terms of p53 expression.[37] It has also been stated that some treatment modalities such as ingenol mebutate has no effect on histopathological response or p53 expressions on actinic cheilitis despite the clinical improvement.[46] When we compared p53 expression between the peritumoral epidermis of SCC cases and cases with AC that had no relation to the tumor, no significant differences were found. This result shows that p53 immunohistochemical staining would not serve as a useful marker alone in determining the transition from AC to SCC.

The studies that have investigated protein expression in the head and neck area have reported varying results in terms of clinicopathological parameters and prognosis. In their study, Kwong *et al*. aimed to determine the prognostic importance of $p14^{ARF}$ along with $p16^{INK4a}$, p53, p21, and E2F-1 markers in the SCC of the anterior tongue and found that the loss of $p14^{ARF}$ expression affected survival negatively[17] Immunohistochemical expression of CDKN2A/p16 revealed low values in the recurrent samples as compared to the non-recurrent ones of oral SCCs in another study.[47] In our study; for both AC and SCC groups, the cases were older than 50 years of age, and the most frequently affected site by SCC was the lower lip (n=18) in accordance with the literature.[7, 8, 43, 48] We determined no significant relationship between expressions of p53, $p16^{INK4a}$, $p14^{ARF}$ and histopathological factors in cases of SCC of the lip, consistent with the study done by Cheng *et al*.[45]

There are very few studies investigating the immunohistochemical features of the epithelium adjacent to SCC of the lip.[37, 49, 50] We observed a positive correlation between $p14^{ARF}$ and p53 protein expressions in the peritumoral epithelium of SCC cases, but no correlation was observed in AC cases without SCC. The presence of such a correlation may indicate that, during progression from AC to SCC, $p14^{ARF}$ -mediated p53 overexpression comes into effect at the stage of early carcinogenesis as a preventive factor, however, proves insufficient. In addition, the fact that p14ARF and p53 are overexpressed simultaneously may suggest that the oncogenic activation of $p14^{ARF}$ contributes as much as UV-induced carcinogenesis as a first step and perhaps that these mechanisms play an effective role in combination. We have determined that $p14^{ARF}$ expression continues although it is reduced after the appearance of SCC, and we think that this situation preserves wt-p53 activation. Therefore, we reason

that these protective mechanisms could explain the more favorable prognosis associated with the SCC of the lip compared to other oral cancers.

Rb and p53 pathways are the two main cell cycle control pathways that are frequently targeted in almost all human tumors. In our study, all cases who were $p16^{INK4a}$-negative manifested positive staining with $p14^{ARF}$ and p53. The picture presented by the cases who showed negativity in this study could indicate the inactivation of $p16^{INK4a}$ and therefore of the Rb pathway, the activation of $p14^{ARF}$ and wt-p53, and thus explain the cause of increased $p14^{ARF}$ and p53 expression in these cases.

Conclusion

In our study, no statistically significant differences were determined in antibody expression between cases of AC and the peritumoral epithelium of SCC cases. This shows that the expressions of antibodies $p16^{INK4a}$, $p14^{ARF}$, and p53 by themselves do not constitute useful markers for determining whether AC would transform to SCC. We found a positive correlation between $p14^{ARF}$ and p53 in the peritumoral epithelium of SCC. We think that, albeit inadequate, $p14^{ARF}$ and p53 work in coordination to prevent early carcinogenesis and that there are other mechanisms responsible for carcinogenesis, which also simultaneously activate the ARF/p53 pathway.

Although our study is small-scale in terms of the number of cases, it suggested that $p16^{INK4a}$ and $p14^{ARF}$, which are located in the same locus, were regulated by separate mechanisms in the development of AC and SCC of the lip. In this context, it represents a preliminary study showing that other studies can be conducted to investigate the relationship of the CDKN2A locus with various molecules in squamous carcinogenesis of the lip.

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Conflict of interest
None.

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None.

Ethics statement
This study was approved by the Ethics Committee of Kırıkkale University School of Medicine (IRB-2009/012).

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