Protein Expression of p53 and CD44 in Patients with Cancer of Buccal Mucosa

Trupti Trivedi, Suresh Prajapati**, Toral Kobawala***, Nandita Ghosh****, Prabhudas Patel******

Abstract :

Background: In India, the incidence of cancer of buccal mucosa is a serious public health issue where a large fraction is related to the poor treatment outcome. Identification of high-risk oral premalignant lesions could constitute as one of the key factors in reducing the morbidity, mortality and cost of treatment associated with oral squamous cell carcimnoma (OSCC). **Methods:** Total 62 subjects of buccal mucosa lesions were enrolled in this study. Using immunohistochemistry, expression of p53 and CD44 was studied from paraffin embedded tumor tissue blocks. Results: Nuclear p53 expression was significantly higher in premalignant lesions compared to malignant tumors (P=0.037), and also in early stage OSCC patients (P=0.012). On correlating CD44 protein expression with different clinicopathological parameters of OSCC patients, significant inverse correlation of CD44 was observed with lymphnode metastasis (P=0.042) and tumor stage (P=0.042). **Conclusion:** p53 expression is associated with patients having premalignant lesions & having significant higher risk of progressing to OSCC. Expression of both, p53 and CD44 were found to be early events in oral cancer. Thus, for identifying high risk OSCC patients for better patient management, p53 and CD44 might be useful significant biomarkers; however, further study in more number of patients is needed to identify their precise role in oral carcinogenesis.

Key words : Malignant transformation, Oral squamous cell carcinoma, Premalignant, p53 & CD44.

Introduction :

On a global scale, oral squamous cell carcinoma (OSCC) is one of the fastest rising and most lethal malignancies with severe disease-related morbidity and treatment-induced toxicity. Due to its unpredictable biological behavior, marked resistance to treatment and unfavorable prognosis of OSCC represent a major threat to the public health system and exerts a tremendous financial challenge for the society. The habit of tobacco chewing and/or smoking and alcohol consumption and premalignant lesions of the oral cavity are the most important etiological risk factors influencing in the prevalence of this heterogeneous tumor entity. The malignant transformation rates of 0.13-34% from leukoplakia to carcinoma, over a 10 year period have been reported.[1] Thus, identification of high-risk oral premalignant lesions could constitute as one of the key factors in reducing the morbidity, mortality and cost of treatment associated with OSCC.

p53 is the name of the tumor suppressor gene located on the short arm of chromosome 17, as well as the protein encoded by this gene. Many recent studies have focused on the p53 tumor suppressor gene, analyzing its gene and protein status. The immunohistochemical analysis of p53 protein is an uncomplicated method that has been broadly used; many studies have shown that p53 protein is implicated in oral carcinogenesis and its alteration occurs early in the progression of neoplastic transformation, frequently preceding identifiable histological alterations. [2]

CD44 is a multistructural and multifunctional cell surface molecule involved in cell proliferation, cell differentiation, cell migration, angiogenesis, presentation of cytokines, chemokines and growth factors to the corresponding receptors, docking of proteases at the cell membrane, as well as in signaling for cell survival. All these biological properties are essential to the physiological activities of normal cells, but they are also associated with the pathologic activities of cancer cells.[3] Moreover, previous data indicate that CD44 is a direct target of p53 mediated

* Senior Scientific Officer & In-Charge
Clinical Carcinogenesis Lab-3
** Studnet, M.Sc. Cancer Biology
Clinical Carcinogenesis Lab-3
*** Research Assistant, Tumor Biology Lab-2
**** Senior Scientific Officer, Tumor Biology Lab-2
***** Professor & Head, Department Cancer Biology
The Gujarat Cancer and Research Institute, Ahmedabad, Gujarat, India
Corresponding Author : Dr Trupti Trivedi
E-mail : trupti.trivedi@gcriindia.org

Table 1 A: Patient and tumor characteristics (N=62)

Characteristics	Premalignant Patients (N=12) (Age range: 32 yrs - 60 yrs		Malignant Patients (N=50) (Age range: 28 yrs - 85 yrs)	
Total patients	**N**	**%**	**N**	**%**
Age				
≤45	06	50	26	52
>45	06	50	24	48
Gender				
Female	00	00	06	12
Male	12	100	44	88
Anatomic site				
Right Buccal Mucosa	07	58	24	48
Left Buccal Mucosa	05	42	26	52
Tobacco habit				
No	03	25	03	06
Yes	09	75	47	94
Chewers	05	56	38	81
Chewers + smokers	04	44	09	19
Treatment				
Only surgery	09	75	08	16
Followed by	03	25	35	70
Radiotherapy	00	00	07	14
Radiotherapy + chemotherapy	12	100	50	100
Family history				
No	00	00	00	00
Yes	07	58	-	-
Histopathology type:				
HyperplasiaDysplasia	05	42	-	-
- Mild	02	40	-	-
- Moderate	02	40	-	-
- Severe	01	20	-	-
Oral squamous cell carcinoma	-	-	50	100

transcriptional repression in breast cancer. It was demontrated that p53 transcriptionally represses CD44 expression in both normal and tumor-derived mammary epithelial cells by direct binding to the CD44 promoter. The data indicated that CD44 is a key tumor-promoting agent in transformed tumor cells lacking p53 function. Importantly, downregulation of CD44 expression was found to be a prerequisite for p53-dependent growth regulation and induction of apoptosis in mammary epithelium.Thus, it was hypothesized that, a similar functional interplay between p53 and CD44 might take place in oral epithelial cells.[4] Thus, based on this information, in the present preliminary study we aimed to explore the correlation between p53 and CD44 and to evaluate their clinical significance in patients of buccal mucosa cancer.

Methods:

A total of 62 untreated patients with histologically confirmed premalignant lesions (n=12) and SCC of buccal mucosa (n=50) enrolled at the Gujarat Cancer and Research Institute, Ahmedabad, India, were enrolled in this study. Paraffin embedded tissue blocks of enrolled premalignant and SCC patients were retrieved from the histopathology department of our institute and were further processed for Immunohistochemical localization.

Patients and tumor characteristic

The patients' and tumor characteristics of premalignant and malignant patients are shown in Table 1A and 1B, respectively.

Out of 12 premalignant patients having leukoplakia of buccal mucosa, 50% (6/12) patients had age $\leqslant$ 45 years and rest 50% (6/12) were >45 years. All these 12 patients were males. Fifty eight percent (7/12) patients had leukoplakia on right buccal mucosa and 42% (5/12) had left buccal mucosa involvement. Seventy five percent (9/12) patients had the habit of tobacco consumption (chewing and/ smoking) and rest 25% (3/12) did not have any such habit (Table 1A).

Out of 50 OSCC patients enrolled in the present study, 52% (26/50) of patients were in the age group of less than 45 years and rest 48% (24/50) of the patients belonged to the above 45 years age group, with average age range of 28-85 years. 88 % (44/50) of patients were males and 12% (06/50) of patients were females. According to the anatomic site of tumor, out of 50 OSCC patients, 48% (24/50) patients were having cancer of right buccal mucosa and the remaining 52% (26/50) were found to be associated with cancer of left buccal mucosa. The habit of tobacco chewing and smoking of the respective patients were also noted, where 94% (47/50) of patients were having tobacco habits whereas 06% (03/50) of patients were not associated with any type of tobacco related habits. In the present study, 36% (18/50), 30% (15/50), 18% (09/50) & 16% (08/50) of patients had T1, T2, T3 and T4 tumor size, respectively. Thus, majority of patients, 66% (33/50) were having T1/T2 tumor size. According to the nodal status, 64% (32/50) of patients were lymph node negative and 36% (18/50) patients were showing lymph node metastatic conditions (Table 1B). The cancer was staged according to the criteria of the American Joint Committee on Cancer, (AJCC) TNM classification. The study included 28% (14/50) patients at stage I, 18% (09/50) patients at stage II, 30% (15/50) patients at stage III and 24% (12/50) patients at stage IV.

The tumors were histologically graded as well differentiated (70%, 35/50), moderately and poorly differentiated (30%, 15/50). Regarding keratin formation, 74% (37/50) of patients showed absence of keratin formation and 26% (13/50) of patients were having presence of keratin formation. With respect to ulcerative growth, 78% (39/50) of patients were seen to have ulcerative growth and 22% (11/50) of patients were having exophytic growth (Table 1B).

Immunohistochemistry

Immunohistochemistry was performed using ABComplex technique, described previously[5], with minor modifications. Tissue sections (4µm-thick) were mounted on 3-aminopropyltriethoxy silane (APES) coated glass slides. The sections were deparaffinized with xylene and rehydrated in graded ethanol. The endogenous peroxidase activity was then blocked by incubating the sections with hydrogen peroxide block for 10 mins. Antigenicity was retrieved by heating the sections in 10 mM sodium citrate buffer (pH, 6.0) for 20 mins in a pressure cooker and then allowed to cool at room temperature. The respective primary antibodies appropriately diluted in TBS [mouse monoclonal antibodies for p53 (clone DO-7, DAKO) and CD44

Table 1 B: Patient and tumor characteristics. (N=50)

Characteristics	N	%
Tumor Size		
T1 (≤2 cm)	18	36
T2 (>2 cm and ≤4)	15	30
T3(>4 cm and ≤6)	09	18
T4 (≥4 cm with invasion)	08	16
Tumor Stage		
I	14	28
II	09	18
III	15	30
IV	12	24
Nodal Status		
Negative	32	64
Positive	18	36
Tumor Differentiation		
Well differentiated	35	70
Moderately/poorly differentiated	15	30
Keratin Formation		
Absent	37	74
Present	13	26
Lymphatic Permeation		
Absent	33	66
Present	17	44
Vascular Permeation		
Absent	45	90
Present	05	10
Perineural Invasion		
Absent	35	70
Present	15	30
Growth Pattern		
Ulcerative	39	78
Exophytic	11	22

(Clone P2A1, Santacruz) at 1:50 dilution each] were added to the sections and incubated at 4°C, overnight, in a moist chamber. Next, streptavidin peroxidase was applied to the sections and incubated for 10 min at room temperature. The specific immune reaction was identified using DAB mixture. Sections were lightly counterstained with haematoxylene and in the final step, sections were dehydrated and cleaned by passing through graded alcohol and finally the stained sections were mounted with DPX and observed under a light microscope (Nikon, Japan). As positive controls, formalin-fixed paraffin-embedded tissue sections with intense staining for p53 and CD44 were included with each staining procedure. For negative control, the primary antibody was replaced with normal serum. All the sections were scored independently, by two individual observers, in a blinded fashion. Sections were examined for evidence of nuclear or cytoplasmic staining and for scoring, a semiquantitative score ranging from negative (no staining or <10% of cells) to 3+ (1+ staining in 11-30% of cells: weak, 2+ staining in 31 to 50% of cells: moderate and 3+ staining in > 50% of cells: intense) was used.

Statistical Analysis

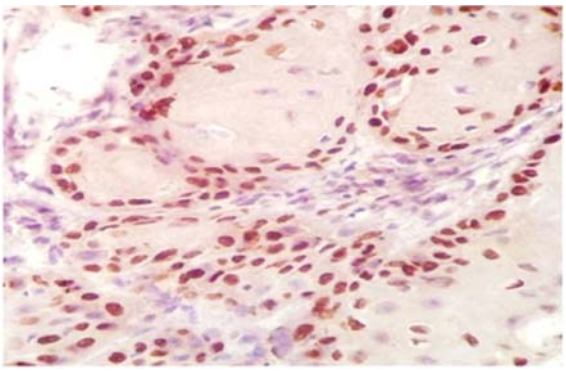
The data were analyzed statistically using SPSS software (release 19; Chicago, IL, USA, 1999). Two-tailed 2test was used to assess the associations between two parameters. Correlation between two parameters was calculated using Spearman's correlation coefficient (r) method. p value $\leqslant 0.05$ was considered significant.

Results:

Incidence of p53 in patients with premalignant and malignant lesions.

Nuclear pattern of p53 was observed in leukoplakia and OSCC patients. Expression of nuclear p53 was observed in 83% of leukoplakia patients. Amongst all, +1 and +2, staining was noted in 60% and 40% of patients, respectively (Table 2, Figure 1).

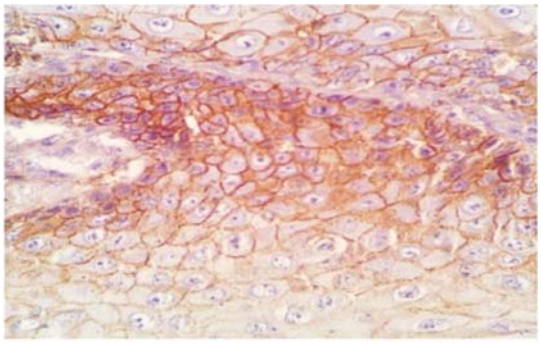
Figure 1: Nuclear expression of p53 in patients



According to histopathology classification, in hyperplasia, p53 expression was noted in 86% and in dysplasia expression of p53 was noted in 80% patients, respectively (Table 3).

In OSCC patients of buccal mucosa, the expression of p53 was observed in 50% whereas 50% patients showed absence of p53. Nuclear expression of p53 with +1, +2 and +3 staining was noted in 32%, 20% and 48%, respectively (Table 2). Thus, in premalignant patients, expression of p53 was observed in 83% and in malignant patients it was observed in 50%. This difference was statistically significant (χ^2=4.374, r=-0.266, P=0.037, Table 3).

Figure 2: Membranous expression of CD44 in patients with buccal mucosa of oral cavity



Protein expression of CD44 was reported in 68% of OSCC patients where 1+, 2+ and 3+ expression of CD44 was observed in 29%, 47% and 24% , respectively (Table 2, Figure 2).

Eighty-six percent of hyperplasia patients and 80% (04/05) of Dysplasia patients showed p53 expression, with no statistically significant difference in the incidence between the two groups of premalignant patients (χ^2=0.069 r=-0.076, P=0.815). When p53 protein expression was correlated between premalignant and early stage OSCC patients, it was observed that 83% (10/12) of premalignant patients showed higher p53 expression as compared to 61% (14/23) of early stage OSCC patients. This difference was statistically significant (χ^2=6.209, r=0.421, P=0.012). It indicates that the p53 positive premalignant patients had 7.778 times higher risk of developing OSCC as compared to p53 negative patients (OR=7.778, 95% CI=1.374-44.039) (Table 3).

Association between expression of p53 and CD44 with clinicopathological parameters of OSCC.

Protein expression of p53 and CD44 was correlated with clinicopathological parameters of OSCC patients and it is depicted in Table 4.

With clinicopathological parameters of SCC of buccal mucosa patients, protein expression of p53 did not show significant association with any of the clinicopathological parameters such as age, gender, habit of tobacco, anatomic site, tumor size, stage of disease, lymphnode status, histological grade of tumor or lymphatic permeation and keratin formation. However, membranous expression of CD44 showed significantly lower expression in patients having tumor on right anatomic site (P=0.025), presence of metastatic lymphnode (P=0.042), absence of keratin formation (P=0.050) and early stage disease (P=0.042) of buccal mucosa; than patients with their respective counterparts (Table 4). With rest of the clinicopathological parameters no significant association was observed with CD44.

Intercorrelation between p53 and CD44 in OSCC of buccal mucosa patients.

In the current study we have not noted significant correlation between p53 and CD44 with p value of 0.554. This finding demonstrated that inactivated p53 inhibits expression of the CD44 via binding to a noncanonical p53 binding sequence in the CD44 promoter.

Discussion:

Oral cancer remains a major cause of morbidity and mortality in patients with head and neck cancers. It is the first most common cancer in males among all the types of cancers being diagnosed at GCRI. Many oral carcinomas are preceded by clinically evident premalignant lesions. These lesions show varying degrees of epithelial dysplasia, from mild to severe. However, predicting the behavior of dysplasia is difficult, since histological characterization may not always provide prognostic information.[6] Molecular biological markers have been suggested to be of value in the diagnosis and prognostic evaluation of precancerous lesions. Thus, the development of OSCC is a multi-step genetic process involving specific alterations in oncogene and tumor suppressor genes.

Table 2: Protein Expression of p53 and CD44 in tumors of oral cavity

Markers	Premalignant patients (N=12)		Malignant patients (N=50)	
	N	%	N	%
P53				
Negative	02	17	25	50
Positive	10	83	25	50
+1	06	60	08	32
+2	04	40	05	20
+3	-	-	12	48
CD44				
Negative	-	-	16	32
Positive	-	-	34	68
+1	-	-	10	29
+2	-	-	16	47
+3	-	-	08	24

Table 3: Incidence of nuclear p53 expression in patients with hyperplasia, dysplasia, early and advance stage of OSCC (N=62)

p53 expression	Hyperplasia N=(07) N (%)	Dysplasia (N=05) N (%)	Early stage (N=23) N (%)	Advanced stage (N=27) N (%)
Negative	01 (14)	01 (20)	09 (39)	12 (44)
Positive	06 (86)	04 (80)	14 (61)	15 (56)

Hyperplasia vs Dysplasia: χ^2=0.069 r=0.076, P=0.815

Premalignant vs Early stage OSCC: **χ^2=6.209, r=-0.421, P=0.012**

Dysplasia vs Early stage OSCC: χ^2=2.758, r=0.314, P=0.104

Early vs Advance stage OSCC : χ^2=1.342, r=0.164, P=0.256

Premalignant vs Malignant: **χ^2=4.374, r=-0.266, P=0.037**

Many recent studies have focused on the p53 tumor suppressor gene, analyzing its protein status. The Immunohistochemical analysis of p53 protein is an uncomplicated method that has been broadly used; many studies have shown that p53 protein is implicated in oral carcinogenesis and its alteration occurs early in the progression of neoplastic transformation, frequently preceding identifiable histological alterations. However, the prevalence of p53 expression varies among reports; oral habits practiced in diverse geographical regions have been suggested to be responsible for this variability.[2] Moreover, recently it has been shown that a transmembrane cell-surface protein-CD44 is essential for the homing and stem cell properties of leukemic stem cells.[7, 8] It has also been found to support anchorage-independent growth in vitro and tumor growth and metastasis in experimental models of solid cancers[9], whereas it inhibited tumor growth in yet other models.[10, 11] The precise role played by CD44 in tumorigenesis has thus remained unclear.

p53 has been found to inhibit expression of the CD44 via binding to a noncanonical p53-binding sequence

Table 4: Relation between p53 and CD44 expression with clinicopathological parameters of OSCC patients (N=50)

Variables	Patients	p53		CD44	
	N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)
Age					
≤45	06 (12)	02 (33)	04 (67)	02 (33)	04 (67)
>45	44 (88	24 (55)	20 (45	14 (32)	30 (68)
		χ2=0.952, r=-0.138 P=0.339		χ2=0.006, r=-0.011 P=0.942	
Gender					
Female	06 (12)	02 (33)	04 (67)	02 (33)	04 (67)
Male	44 (88)	24 (55)	20 (45)	14 (32)	30 (68)
		χ2=0.952, r=-0.138 P=0.339		χ2= 0.006, r=-0.011 P=0.942	
Anatomic site					
Right BM	26 (52)	14 (54)	12 (46)	12 (46)	14 (54)
Left BM	24 (48	12 (50)	12 (50)	04 (17)	20 (83)
		χ2=0.074, r=0.038 P=0.790		χ2=4.987, r=-0.316, P=0.025	
Habit of tobacco					
None	09 (24)	20 (53)	18 (47)	12 (32)	26 (68)
Present	38 (76)	05 (56)	04 (44)	04 (44)	05 (56)
		χ2=0.642, r=0.113 P=0.433		χ2=0.006, r=0.011 P=0.939	
Lymphnode status					
Negative	32 (64)	19 (59)	13 (41)	07 (22)	25 (78)
Positive	18 (36)	07 (39)	11 (61)	09 (50)	09 (50)
		χ2=1.937, r=-0.197 P=0.171		χ2=4.188, r=-0.289 P=0.042	
Tumor stage					
Early stage	23 (46)	14 (61)	09 (39)	04 (17)	19 (83)
Advanced stage	27 (54)	12 (44)	15 (56)	12 (44)	15 (56)
		χ2=1.342, r=0.164 P=0.256		χ2=4.177, r=-0.289 P=0.042	
Keratin Formation					
Absent	37 (74)	20 (54)	17 (46)	09 (24)	28 (76)
Present	13 (26)	06 (46)	07 (54)	07 (54)	06 (46)
		χ2=0.241, r=0.069 P=0.632		χ2=3.853, r=-0.278 P=0.050	

Histological grade					
Well	35 (70)	18 (51)	17 (49)	13 (37)	22 (63)
Moderate/Poor	15 (30)	08 (53)	07 (47)	03 (20)	12 (80)
		χ2=0.015, r=-0.017 P=0.904		χ2=1.418, r=0.168 P=0.242	
Lymphatic permeation					
Absent	33 (66)	17 (52)	16 (48)	10 (30)	23 (70)
Present	17 (44	09 (53)	08 (47)	09 (53)	08 (47)
		χ2=0.009 r=-0.014 P=0.926		χ2=0.128, r=-0.051 P=0.727	

in the CD44 promoter. In the absence of p53 function, the resulting derepressed CD44 expression is essential for the growth and tumor-initiating ability of highly tumorigenic mammary epithelial cells. Immunohistochemical analysis have demonstrated that CD44 protein is expressed at high levels together with elevated levels of p53 and have implicated that CD44 might be repressed by wild-type p53.[12]

Thus, this study sought to determine the protein expression of p53 and CD44 in patients with premalignant oral lesions and squamous cell carcinoma of buccal mucosa using immunohistochemistry. In the present study, nuclear expression of p53 was observed in both, leukoplakia and OSCC patients. This was consistent with the results of Vora et al.[13] and Lee et al.[14] who also observed nuclear p53 staining pattern. Further, p53 expression was noted in 17% of premalignant patients as compared to 50% of OSCC patients showing presence of p53 expression. This difference was statistically significant (P=0.037). The data indicated that the premalignant patients with p53 positive expression had 5 times higher risk of developing OSCC as compared to p53 negative patients. Moreover, although not significant, the dysplasia patients showed higher p53 expression as compared to the hyperplasia patients. This result was similar to other reports which indicated that p53 expression increased from hyperplasia to dysplasia to OSCC.[15] Further, the current study revealed no significant correlation between the p53 expression and the clinicopathological parameters of OSCC patients. This was in accordance with many other studies.[13, 16, 17] Numerous studies have shown higher p53 expression in non-chewers and non-smokers than in chewers and smokers.[13,18,19] However, in the present study, the incidence of p53 expression between the chewers and chewers +smokers was similar. Hence, no such association was observed between p53 expression and tobacco consumption. Further, although not significantly, p53 expression was found to increase from early to advanced stage OSCC. However, there are reports that demonstrated that p53 expression was lower in advanced stage disease as compared to early stage disease. The low frequency of p53 expression in advanced stage carcinoma could be due to hypermethylation of p53 gene, deletion of both the alleles and unstable premature truncated protein products due to mutations resulting in loss of antibody recognition sites. Also, overexpression of dominant inhibitors of p53 might inhibit it at advanced stage disease. In relation to this, in a study by Lee et al.[14] positive mutant p53 (that is more stable than the wild type protein) expression was frequently observed in late stage tumors (P=0.049). They have explained that mutant p53 causes inactivation and dysfunction of wild-type p53 and plays an important role in development and progression of carcinomas.

A variety of observations indicate that the CD44 molecule is an important factor for the progression of acute myeloid leukemia,[7, 8] as well as for the growth of both primary and metastatic tumors.[20] Godar et al.[12] have noted that the CD44 is indispensable not only for tumor growth but also for tumor-initiating ability, which correlates with its critical role in fostering anchorage-independent growth. In the current study, membranous staining pattern for CD44 expression was observed which was consistent with other studies.[14,21] On correlating with the clinicopathological parameters

of OSCC patients, positive CD44 expression was found to be significantly associated with absence of lymphnode metastasis, early stage tumor and absence of keratin formation, which indicates the occurrence of this stem cell marker as an early event and its association with a less aggressive behaviour of oral cancer. Similar to our result, Lee et al.[14] also observed that weak expression of CD44 was significantly related to a higher frequency of negative lymph node metastasis. Further, present result indicated that CD44 expression was higher in moderate/ poorly differentiated tumors (80%) as compared to the well differentiated tumors (63%). But this difference was not statistically significant (P=0.242). This was consistent with the findings of Bidaud et al.[22] and Lee et al.[14], who also observed enhanced CD44 expression in poorly differentiated tumors. Moreover, Riddle et al.[23] also observed upregulated CD44 expression in poorly circumscribed neurofibroma.

Additionally, this study did not reveal any statistical significant relationship between p53 and CD44 expression in the squamous cell carcinoma of the buccal mucosa. However, the observations by Godar et al.[12] suggested that high CD44 expression, by opposing p53 function, could serve as an important growth-promoting and survival factor in early stages of tumor progression. Acting in the opposite direction, p53 represses CD44 expression. Their observations suggested that p53 and CD44 may establish a self-amplifying positive feedback loop, in which p53 represses CD44 expression, which results in suppression of growth receptor signalling and a resulting decrease in MDM2 activity, permitting, in turn, further increase in p53 levels and function.[24]

Conclusion:

p53 expression was found to be oncogenic in oral carcinogenesis, with premalignant patients having significant higher risk of progressing to OSCC. Expression of both , p53 and the stem cell marker-CD44, were found to be early events in oral cancer. Thus, for identifying high risk OSCC patients and better patient management, p53 and CD44 might be useful as significant biomarkers. However, further study in more number of patients is needed to identify their precise role in oral carcinogenesis.

References:

1. Warnakulasuriya S and Ariyavardana A. Malignant transformation of oral leukoplakia: systemic review of observational studies.J Oral Pathol Med 2015; 1-4.
2. Efrain A, Raul J, Medina A, Martin C. Immunoexpression of p53 in oral squamous cell carcinoma and oral dysplastic lesions in patients with the habit of reverse smoke. Int J Odontostomat 2013; 7:185-91.
3. Naor D, Nedvetzki S, Golan I, Melnik L, Faitelson Y. CD44 in Cancer. Crit Rev Clin Lab Sci. 2002;39:527–79.
4. Zeilstra J, Joosten S, Vermeulen L, et al. CD44 Expression in Intestinal Epithelium and Colorectal Cancer Is Independent of p53 Status. PLoS ONE. 2013; 8: 72849. doi:10.1371/journal.pone.0072849.
5. Trivedi T, Tankshali R, Goswami J, Shukla S, Shah P, Shah N. Identification of site-specific prognostic biomarkers in patients with oral squamous cell carcinoma. Neoplasma 2011: 58: 217-26.
6. Penfold CN, Rojas R, Langdon JD, Partridge M. The role of angiogenesis in the spread of oral squamous cell carcinoma. British Journal of Oral and Maxillofacial Surgery. 1996; 34: 37-41.
7. Jin L, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. Nature medicine. 2006; 12: 1167-74.
8. Krause DS, Lazarides K, von Andrian UH, Van Etten RA. Requirement for CD44 in homing and engraftment of BCR-ABL-expressing leukemic stem cells. Nature medicine. 2006; 12: 1175.
9. Barbour AP, Reeder JA, Walsh MD, Fawcett J, Antalis TM, Gotley DC. Expression of the CD44v2-10 Isoform Confers a Metastatic Phenotype. Cancer research. 2003; 63:887-92.
10. Gao AC, Lou W, Dong JT, Isaacs JT. CD44 is a metastasis suppressor gene for prostatic cancer located on human chromosome 11p13. Cancer research. 1997; 57: 846-49.
11. Schmits R, Filmus J, Gerwin N, Senaldi G, Kiefer F, Kundig T, Wakeham A, Shahinian A, Catzavelos C, Rak J, Furlonger C. CD44 regulates hematopoietic progenitor distribution, granuloma formation, and tumorigenicity. Blood. 1997; 90: 2217-33.
12. Godar S, Ince TA, Bell GW, Feldser D, Donaher JL, Bergh J, Liu A, Miu K, Watnick RS, Reinhardt F, McAllister SS. Growth-inhibitory and tumor-suppressive functions of p53 depend on its repression of CD44 expression. Cell. 2008; 134: 62-73.
13. Vora HH, Trivedi TI, Shukla SN, Shah NG, Goswami JV, Shah PM. p53 expression in leukoplakia and carcinoma of the tongue. The International journal of biological markers. 2005; 21: 74-80.
14. Lee SJ. Unraveling the model minority stereotype: Listening to Asian American youth. Teachers College Press; April 2015
15. Swaminathan U, Joshua E, Rao UK, Ranganathan K. Expression of p53 and Cyclin D1 in oral squamous cell

carcinoma and normal mucosa: An Immunohistochemical study. Journal of Oral and Maxillofacial Pathology : JOMFP. 2012; 16:172-77.

16. Garozzo, Domenico, *et al*. Studies on the primary structure of short polysaccharides using SEC MALDI mass spectroscopy. Carbohydrate research 1999; 139-46.
17. Chow, Yen-whei, *et al*. Automatic retrieval of changed files by a network software agent. U.S. Patent No. 6,029, 2000; 175.
18. Ibrahim KM, Nichols RA, Hewitt GM. Spatial patterns of genetic variation generated by different forms of dispersal. Heredity. 1996; 77: 282-91.
19. Yan Y, Zuo X, Wei D. Concise review: emerging role of CD44 in cancer stem cells: a promising biomarker and therapeutic target. Stem cells translational medicine. 2015; 4: 1033-43.
20. McFarlane S, Coulter JA, Tibbits P, *et al*. CD44 increases the efficiency of distant metastasis of breast cancer. Oncotarget. 2015; 6:11465-76.
21. Zeilstra J, Joosten SPJ, Vermeulen L, *et al*. CD44 Expression in Intestinal Epithelium and Colorectal Cancer Is Independent of p53 Status. Sabapathy K, ed. PLoS ONE. 2013; 8: e72849. doi:10.1371/journal.pone.0072849.
22. Bidaud S. Le problème du signifié des prépositions «à» et «de» en français et dans quelques langues romanes. Çédille. 2010: 6:29-41.
23. Riddle MC, Ambrosius WT, Brillon DJ, Buse JB, Byington RP, Cohen RM, Goff DC, Malozowski S, Margolis KL, Probstfield JL, Schnall A. Epidemiologic relationships between A1C and all-cause mortality during a median 3.4-year follow-up of glycemic treatment in the ACCORD trial. Diabetes care. 2010; 33: 983-90.
24. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N. Role of AMP-activated protein kinase in mechanism of metformin action. The Journal of clinical investigation. 2001; 108: 1167-74.