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Araştırma/ Research Article

LÖKOPLAKİ VE ORAL SKUAMÖZ HÜCRELİ KARSİNOMDA IGF-1R, EZH2, LAMININ-5 EKSPRESYONU≠

EXPRESSIONS OF IGF-1R, EZH2, LAMININ-5 IN LEUKOPLAKIA AND ORAL SQUAMOUS CELL CARCINOMA≠

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ABSTRACT

Aim: The aim of this study is to investigate the expressions of IGF-1R, EZH2 and Laminin-5 antibodies in biopsy samples of leukoplakia and squamous cell carcinoma of the oral mucosa by immunohistochemistry method.
Materials and Methods: The study consisted of three groups; oral squamous cell carcinoma leukoplakia and control groups. The control group consisted of lesions with oral fibrous hyperplasia diagnosis. Biopsies of 38 patients for oral squamous cell carcinoma, 32 patients for leukoplakia and 15 patients for control group were examined and evaluated degree of staining of antibodies. Antibodies were evaluated as negative (0-10%):0, 1 positive (11-30%):+, 2 positive (31-60%):++, 3 positive (61-100%):+++ according to staining percentages. The staining degrees of antibodies were compared with Mann Whitney U test in study and control groups. Gender distribution between groups was compared with Chi-Square test and SPSS 21 program was used for calculations. The results were statistically significancant is $p < 0.05$.
Results: According to the findings obtained in this study antibodies revealed significantly higher staining in the oral squamous cell carcinoma group and leukoplakia group compared to with control groups.
Conclusion: In this study, it was shown that EZH2, IGF-1R and Laminin-5 may have roles in cancer development.
Key words: Squamous cell carcinoma, leukoplakia

ÖZ

Amaç: Çalışmanın amacı oral mukozada lökoplaki ve skuamöz hücreli karsinom biyopsi örneklerinde IGF-1R, EZH2 ve Laminin-5 antikorlarının immünhistokimyasal methodla ekspresyonlarının araştırılmasıdır.
Gereç ve Yöntem: Çalışmayı oral skuamöz hücreli karsinom, lökoplaki ve kontrol grubu olmak üzere üç grup oluşturmaktadır. Kontrol grubunu oral fibröz hiperplazi tanılı lezyonlar oluşturmuştur. Oral skuamöz hücreli karsinomda 38, lökoplakide 32 ve kontrol grubunda 15 biyopsi örneğinde IGF-1R, EZH2 ve Laminin-5 antikorlarının boyanma dereceleri incelendi ve değerlendirildi. Antikorlar boyanma yüzdelerine göre negatif (%0-10):0, 1 pozitif (%11-30):+, 2 pozitif (%31-60):++, 3 pozitif (%61-100):+++ olarak değerlendirildi. Çalışma ve kontrol gruplarında EZH2, IGF-1R ve Laminin-5 antikorları ile boyanma dereceleri Mann Whitney U testiyle karşılaştırılmıştır. Gruplar arasındaki cinsiyet dağılımı karşılaştırılması Ki-kare testiyle, hesaplamalar SPSS 21 programıyla yapılmıştır. Anlamlılık sınırı $p<0.05$tir.
Bulgular: Bu çalışmada elde edilen bulgulara göre IGF-1R, EZH2 ve Laminin-5 antikorlarının kontrol grubuyla karşılaştırıldığında lökoplaki ve oral skuamöz hücreli karsinom gruplarında önemli derecede yüksek boyanma göstermiştir.
Sonuç: Bu çalışmada EZH2, IGF-1R ve Laminin-5'in kanser gelişiminde rolleri olabileceği gösterilmiştir.
Anahtar Kelimeler: Skuamöz hücreli karsinom, lökoplaki

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INTRODUCTION

Oral mucosa, as well as skin and lips, is covered by squamous epithelium and most of malignant tumors in the head and neck region originate from this type of epithelium.[1] Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity. [2,3,4]

Smoking and alcohol consumption play an important role in the development of OSCC.[2] Early diagnosis of oral cancers is improving the survival and reducing the morbidity associated with the disease [4].

Oral leukoplakia (OL) is recognized a precancerous lesion of oral mucosa. OL is white plaque or patches that don't belong to any clinical or pathological disease group. [5] The ethiological factors of OL is various as human papilloma virüs (HPV), tobacco smoking. [6]

Enhancer of zeste homolog 2 (EZH2) is a protein involved in the regulation of cell cycle. Researches on normal oral mucosa, dysplasia and OSCC showed progressively higher expressions of EZH2, which was found to be associated with the potential of malignancy and poor prognosis.[7]

Insulin growth factor-1 receptor (IGF-1R) is well defined in malignant tumors. Increased IGF-1R associated with tumor cell migration, proliferation, invasion, metastasis, poor prognosis, treatment resistance and shortened survival.[8]

Laminin-5 is one of the extracellular matrix proteins, and plays an important role in cell migration and invasion. High expressions of Laminin-5 were detected in various tumors.[9]

The aim of this study is to investigate the expressions of EZH2, IGF-1R and Laminin-5 in the biopsy samples of leukoplakia and squamous cell carcinoma of the oral mucosa by immunohistochemistry method.

Material and Methods

The study involved patients with clinical diagnoses of squamous cell carcinoma and leukoplakia with dysplasia of the oral mucosa in the study groups. Biopsies sent for histopathologic diagnosis to the laboratory of Istanbul University, Institute of Oncology, Department of Tumor Pathology were evaluated. The study consisted of three groups; OSCC, leukoplakia with dysplasia and control groups. The control group was composed of oral fibrous hyperplasia cases. Biopsises of 38 patients for OSCC and 32 patients for leukoplakia, and 15 patients with oral fibrous hyperplasia for control group were stained, examined and evaluated under light microscope for EZH2, IGF-1R and Laminin-5 expressions. The sections were deparaffinized at alcohol series and then pretreated with citrate buffer solution in microwave oven for 20 minutes. Endogenous peroxidase activity was done 3% hydrogen peroxide. The slides were incubated with primary antibodies EZH2 (Abcam, Cambridge, USA) IGF-1R (Abcam, Cambridge, USA) and Laminin-5 (Abcam, Cambridge, USA) respectively. Immunoreactions were detected by the labeled streptavidinbiotin method and visualized with AEC chromogen solution, followed by counterstaining with Mayer hematoxylin.

In the microscopic evaluation of the slides, staining of epithelial tissue areas were graded as; 0 - 10% detectable staining: negative (-), 11 - 30% detectable staining: 1 positive (+), 31 - 60% detectable staining: 2 positive (++), 61 - 100% detectable staining: 3 positive (+++).

The degree of staining of EZH2, IGF-1R and Laminin-5 antibodies were compared with Mann Whitney U test. The gender distribution of the patients were compared with Chi- square test and the age distribution of the cases were compared with Oneway Anova test. The calculations were made with SPSS 21(IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. program). The significance limit was $p < 0.05$ for each test.

RESULTS

From a total of 85 cases in the study and control groups, 40 were female and 45 were male. 9 of the cases in the control group, 13 of the cases in leukoplakia group and 18 of the cases in squamous cell carcinoma group were females. 6 of cases in the control group, 19 of the cases in leukoplakia group and 20 of the cases in squamous cell carcinoma group were males. The statistical evaluation for gender distribution between the study and control groups showed no significance ($p = 1.541$) (Table 1).

Table 1. Gender distribution among groups

Gender	Female	Male	Total	p value[a]
Control group	9 (60%)	6 (40%)	15(100%)	P=1.541[b]
Leukoplakia group	13(40.6%)	19 (59.4%)	32(100%)	
Squamous cell carcinoma group	18(47.4%)	20 (52.6%)	38(100%)	
Total	40(47.1%)	45(52.9%)	85(100%)	

[a] *Statistically significant at the level p<0.005 (Chi-square test)*
[b] *In the statistical analysis between three groups p=1.541*

In examining the age distribution of the groups, it was found that ages of the patients were between 22 - 80 years in the control group, between 24 - 77 years in the leukoplakia group, and between 25 - 86 years in the squamous cell carcinoma group. The statistical analysis for age distribution between the three groups, with a p-value of 0.007. (Table 2).

Table 2. Distribution of age among groups

Age	N	n	SD	SE	95% confidence interval for mean		Min.	Max.	p-value[c]
					LB	UB			
Control group	15	51.27	16.007	4.133	42.40	60.13	22	80	p= 0.007 [d]
Leukoplaka group	32	47.25	11.170	1.975	43.22	51.28	24	77	
Squamous cell carcinoma group	38	58.32	16.005	2.596	53.05	63.58	25	86	
Total	85	52.91	15.083	1.636	49.65	56.16	22	86	

n= Average SD= Standard Deviation, SE= Standard Error, LB= Lower Bound, UB= Upper Bound, Min= Minimum, Max=Maxiumum
[c]Statistically significant at the level p < 0.05 (Oneway Anova test)
[d]In the statistical analysis between three groups p = 0.007.

In the squamous cell carcinoma group, 27 lesions were located in mandible, 5 lesions in maxilla, 2 lesions in buccal mucosa, 2 lesions in tongue and 2 lesions in sublingual region. In leukoplakia group, 17 lesions were located in buccal mucosa, 10 lesions in mandible, 2 lesions in maxilla, 1 lesion in tongue, 1 lesion in sublingual area and 1 lesion in labial mucosa. In the control group 5 lesions were located in mandible, 3 lesions in buccal mucosa, 3 lesions in labial mucosa, 2 lesions in maxilla and 2 lesions in tongue.

Cases were shown in staining scores with EZH2, IGF-1R and Laminin-5 antibodies (Table 3) (Figure 1-6). EZH2, IGF-1R, Laminin-5 antibodies showed significantly higher staining in leukoplakia and squamous cell carcinoma groups compared to the control group ($p < 0.001$). In addition, there was a statistically significant difference between the IGF-1R expressions of leukoplakia and squamous cell carcinoma groups ($p < 0.001$).

The comparative statistical values of the study and control groups are shown in Table 4.

Table 3. Findings of immunohistochemical staining among groups

Immunohistochemical staining	EZH2				IGF-1R				Laminin-5			
	-	+	+	+++	-	+	++	+++	-	+	++	+++
Control group	4	5	6	-	13	2	-	-	12	3	-	-
Leukoplakia group	1	2	10	19	1	3	19	9	-	4	17	11
Squamous cell carcinoma group	-	-	10	28	1	3	12	22	2	2	14	20

= negative, +=one positive, ++=two positive, +++=three positive

Table 4. p value in comparison between groups

p-value[e]	Control group/leukoplakia group	Leukoplakia group/squamous cell carcinoma group	Control group/squamous cell carcinoma group
EZH2	<0.001	0.143	<0.001
IGF-1R	<0.001	<0.001	<0.001
Laminin-5	<0.001	0.190	<0.001

[e] Statistically significant at the level p <0.05 (Mann Whitney U test).

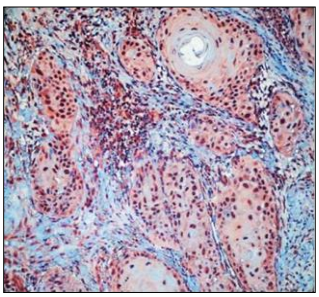


Figure 1. +++ positive EZH2 staining in squamous cell carcinoma group (immunohistochemistry stain, x200)

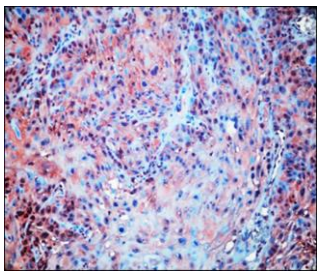


Figure 2. +++ positive IGF-1R staining in squamous cell carcinoma group (immunohistochemistry stain, x400)

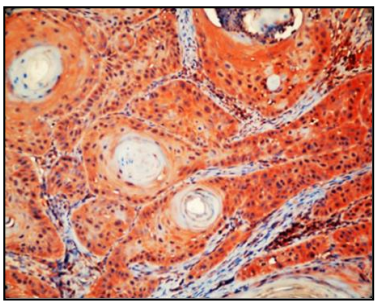
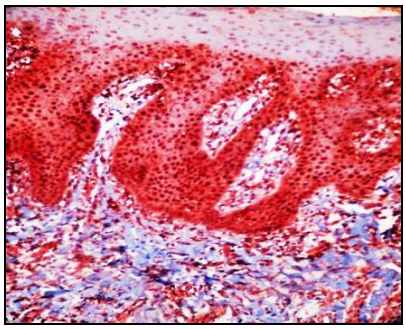
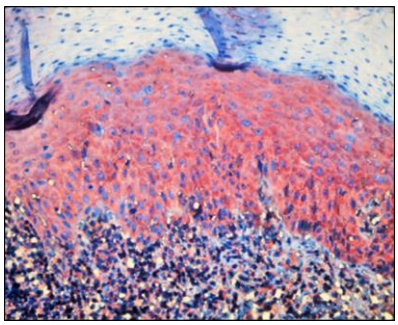
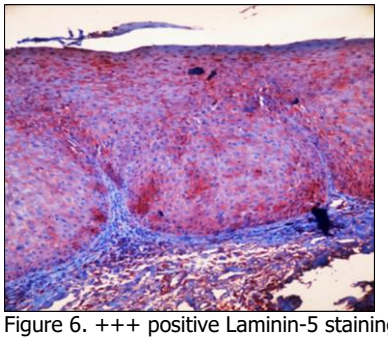


Figure 3. +++ positive Laminin-5 staining in squamous cell carcinoma group (immunohistochemistry stain, x200)


Figure 4. +++ positive EZH2 staining in leukoplakia group (immunohistochemistry stain, x200)


Figure 5. +++ positive IGF-1R staining in leukoplakia group (immunohistochemistry stain, x400)


Figure 6. +++ positive Laminin-5 staining in leukoplakia group (immunohistochemistry stain, x200)

DISCUSSION

EZH2 was found to be related to malignancy; tumor development, proliferation of tumor cells, metastatic process, drug resistance and preservation of stem cells.[10] In many studies high expression of EZH2 was correlated with poor prognosis inmammary, prostate and bladder cancers. [11-13] In the present study EZH2 expressions were significantly high in leukoplakia and squamous cell carcinoma groups compared to control group ($p < 0.001$). The observation of EZH2 expression in the epithelial papillae in the control group suggested that the marker indicates cell proliferation. Furthermore, the progressively increasing expressions of EZH2 in leukoplakia group showing tendency for malignant transformation, and carcinomas, demonstrated that EZH2 can be used to indicate progressive malignancy. EZH2 expression was found to be higher in poorly differentiated areas than well differentiated regions of squamous cell carcinoma specimens in the present study. Therefore it may be put forward that EZH2 antibody may be associated with poor differentiation and rapid growth. More studies should be made to show its correlation with the prognosis of the disease.

IGF-1R is found to have a function in neoplastic cell proliferation, cancer development and metastasis.[14-18] It was studied in head and neck tumors and found to show a high expression in precancerous lesions and squamous cell carcinomas.[19-23] In the present study IGF-1R expression was found to be significantly high in leukoplakia group and squamous cell carcinoma groups compared to control group ($P < 0.001$). In addition statistical significance was observed between the expressions of leukoplakia and squamous cell carcinoma cases.

Laminin-5 has many important functions such as holding the squamous cells together and providing the migration of epithelial cells in wound healing.[24-26] Laminin-5 is found to be related to malignant cell proliferation in a few studies.[27-29] In cervical cancers, in oral dysplastic lesions and oral squamous cell carcinoma, Laminin-5 expressions were showed to be related to the malignancy and invasion of the lesion. [30,31] In the present study Laminin-5 expressions were found to be significantly higher in leukoplakia group and squamous cell carcinoma groups compared to control group ($P <0.001$). Laminin-5 showed a more prominent expression in well differentiated and keratinized regions of squamous cell carcinomas. Therefore it may be concluded that although Laminin-5 is a marker for malignancy, it is not associated with poor differentiation, on the contrary it may especially be related to the keratinization of malignant squamous cells.

CONCLUSION

As a result EZH2 can be used as a marker showing the progressive malignancy in, potentially malignant, minimal cancerous and invasive cancerous lesions. IGF-1R can be used as a marker showing the cancer development and malignancy. Laminin-5 can be used as a malignancy marker especially in well

differentiated squamous cell tumors. In the present study, all of the three antibodies are revealed to be efficient in the differential diagnosis of leukoplakia and squamous cell carcinomas from hyperplastic epithelial proliferations of the oral mucosa.

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Conflicts of interest statement
The authors declare no conflict of interest

REFERENCES

1. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009;45:309-16
2. Pan H, Gu L, Liu B, Li Y, Wang Y, Bai X, Li L, Wang B, Peng Q, Yao Z, Tang Z. Tropomyosin-1 acts as a potential tumor suppressor in human oral squamous cell carcinoma. PLos One 2017; 12:e0168900
3. Akmansoy BP, Pekiner FN, Şakır MD, Alatlı C. Oral Skuamöz Hücreli Karsinom: 3 Olgu Sunumu ve Literatür Derlemesi. Atatürk Üniv Diş Hek Fak Derg 2018;28:239-44
4. Chaudhari V, Pradeep GL, Prakash N, Mahajan AM. Estimation of salivary sialic acid in oral premalignancy and oral squamous cell carcinoma. Contemp Clin Dent 2016;7:451-6
5. Bewley AF, Farwell DG. Oral leukoplakia and oral cavity squamous cell carcinoma Clin Dermatol 2017;35:461-7
6. Ramya AS, Majumdar S, Babu TM, Uppala D, Srinivas B, Rao AK. Expression of humanpapillomavirus DNA and p53 polymorphisms through polymerase chain reaction in normal mucosa and oral leukoplakia individuals with deleterious oral habits Int J Appl Basic Med Res 2017;7:134-8
7. Kidani K, Osaki M, Tamura T, Yamaga K, Shomori K, Ryoke K, Ito H. High expression of EZH2 is associated with tumor proliferation and prognosis in human oral squamous cell carcinomas. Oral Oncol 2009;45:39-46
8. Sun Y, Sun X, Shen B. Molecular imaging of IGF-1R in cancer. Mol Imaging 2017;16:1-7
9. An SJ, Lin QX, Chen ZH, Su J, Cheng H, Xie Z, Zhang XC, Zhou HY, Huang Y, Chen SL, Guo WB, Wu YL. Combinations of laminin-5 with PTEN, p-EGFR and p-Akt define a group of distinct molecular subsets indicative of poor prognosis in patients with non-small cell lung cancer. Exp Ther Med 2012;4:226-30
10. Zeng J, Kirk BD, Gou Y, Wang Q, Ma J. Genome-wide polycomb target gene prediction in Drosophila melanogaster. Nucleic Acids Res 2012;40: 5848-63
11. Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, Ghosh D, Sewalt RGAB, Otte AP, Hayes DF, Sabel MS, Livant D, Weiss SJ, Rubin MA, Chinnaiyan AM. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. Proc Natl Acad Sci USA 2003;100:11606-11
12. Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RGAB, Otte AP, Rubin MA, Chinnaiyan AM. The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 2002; 419:624-9
13. Hinz S, Kempkensteffen C, Christoph F, Hoffmann M, Krause H, Schrader M, Schostak M, Miller K, Weikert S. Expression of polycomb group protein EZH2 and its relation to outcome in patients with urothelial carcinoma of the bladder. J Cancer Res Clin Oncol 2008;134:331-6
14. Dziadziuszko R, Camidge DR, Hirsch FR. The insulin-like growth factor pathway in lung cancer. J Thorac Oncol 2008;3:815-8
15. Xiong L, Kou F, Yang Y, Wu J. A novel role for IGF-1R in p-53 mediated apoptosis through translational modulation of the p53-Mdm2 feedback loop. J Cell Biol 2007;178:995-1007
16. Brady G, Crean SJ, Naik P, Kapas S. Upregulation of IGF-2 and IGF-1 receptor expression in oral cancer cell lines. Int J Oncol 2007;31:875-81
17. Larsson O, Girnita A, Girnita L. Role of insulin-like growth factor 1 receptor signalling in cancer. Br J Cancer 2005;92:2097-101
18. Leroith D, Roberts Jr CT. The insulin-like growth factor system and cancer. Cancer Lett 2003;195:127-37
19. Barnes CJ, Ohshiro K, Rayala SK, El-Naggar AK, Kumar R. Insulin-like growth factor receptor as a therapeutic target in head and neck cancer. Clin Cancer Res 2007;13:4291-99
20. Slomiany MG, Black LA, Kibbey MM, Tingler MA, Day TA, Rosenzweig SA. Insulin-like growth factor-1 receptor and ligand targeting in head and neck squamous cell carcinoma. Cancer Lett 2007;248:269-79

21. Papadimitrakopoulou VA, Brown EN, Liu DD, El-Naggar AK, Jack Lee J, Hong WK, Lee HY. The prognostic role of loss of insulin-like growth factor-binding protein-3 expression in head and neck carcinogenesis. Cancer Lett 2006;239:136-43
22. Reszec J, Duraj E, Koda M, Musiatowicz B, Sulkowska M. Insulin-like growth factor-1 receptor in human oral cancer. Rocz Akad Med Bialymst 2004;49:58-60
23. Lara PC, Bordon E, Rey A, Moreno M, Lloret M, Henriquez-Hernandez LA. IGF-1R expression predicts clinical outcome in patients with locally advanced oral squamous cell carcinoma. Oral Oncol 2011;47:615-9
24. Sonnenberg A, de Melker AA, Martinez de Velasco AM, Janssen H, Calafat J, Niessen CM. Formation of hemidesmosomes in cells of a transformed murine mammary tumor cell line and mechanisms involved in adherence of these cells to laminin and kalinin. J Cell Sci 1993;106:1083-102
25. Goldfinger LE, Hopkinson SB, deHart GW, Collawn S, Couchman JR, Jones JC. The alpha3 laminin subunit, alpha6beta4 and alpha3beta1 integrin coordinately regulate wound healing in cultured epithelial cells and skin. J Cell Sci 1999;112:2615-29
26. Zhang K, Kramer RH. Laminin 5 deposition promotes keratinocyte motility. Exp Cell Res 1996;227:309-22
27. Pyke C, Romer J, Kallunki P, Lund LR, Ralfkiaer E, Dano K, Tryggvason K. The gamma2 chain of kalinin/laminin 5 is preferentially expressed in invading malignant cells in human cancers. Am J Pathol 1994; 145:782-91
28. Pyke C, Salo S, Ralfkiaer E, Romer J, Dano K, Tryggvason K. Laminin-5 is a marker of invading cancer cells in some human carcinomas and is coexpressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. Cancer Res 1995; 55: 4132-9
29. Sordat I, Bosman FT, Dorta G, Rousselle P, Aberdam D, Blum AL, Sordat B. Differential expression of laminin-5 subunits and integrin receptors in human colorectal neoplasia. J Pathol 1998;185:44-52
30. Imura J, Uchida Y, Nomoto K, Ichikawa K, Tomita S, Iijima T, Fujimori T. Laminin-5 is a biomarker of invasiveness in cervical adenocarcinoma. Diagn P 2012;7:105
31. Rani V, McCullough M, Chandu A. Assesment of laminin-5 in oral dysplasia and squamous cell carcinoma. J Oral Maxillofac Surg 2013;71:1873-79

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