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Original article

VIMENTIN 3 AS A NEW POTENTIAL BIOMARKER IN ORAL SQUAMOUS CELL CARCINOMAS

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Abstract

Introduction: Oral squamous cell carcinoma (SCC) is the most common type of oral cancer and has a high mortality rate due to the lack of reliable prognostic and predictive biomarkers. Vimentin 3 (VIM3), a variant of vimentin, is an intermediate filament protein with a unique C-terminal domain. It plays a key role in the cytoskeleton and has been suggested as a differential marker in kidney tumors and genitourinary diseases. This study sought to evaluate the prognostic significance of VIM3 expression in oral SCC.

Materials and methods: This retrospective study included 75 cases of oral SCC diagnosed between 2018 and 2023. Immunohistochemical staining for VIM3 was performed on paraffin-embedded tumor tissue blocks. The relationship between VIM3 expression and clinicopathological parameters including age, sex, smoking status, tumor size, location, differentiation, lymphovascular invasion, lymph node metastasis, and pathological stage was analyzed.

Results: VIM3 expression showed a significant association with tumor differentiation and smoking status. Poorly differentiated tumors had the highest levels of VIM3 expression, indicating a strong link between increased VIM3 and loss of differentiation. Additionally, active smokers exhibited significantly higher VIM3 expression compared to non-smokers.

Discussion: These findings suggest that VIM3 expression correlates with tumor aggressiveness and may serve as a prognostic biomarker in oral SCC. The association with smoking status highlights its potential role in tumor biology influenced by tobacco exposure.

Key words: oral squamous cell carcinoma, vimentin 3, biomarker, ımmunohistochemistry, prognosis

Resumen

Vımentına 3 como nuevo bıomarcador potencıal en carcınomas orales de células escamosas

Introducción: El carcinoma oral de células escamosas (CCE) es el tipo más común de cáncer oral y presenta alta tasa de mortalidad debido a falta de biomarcadores pronósticos y predictivos confiables. Vimentina 3 (VIM3), una variante de vimentina, es una proteína de filamento intermedio con un dominio C-terminal único. Desempeña un papel clave en el citoesqueleto y se ha sugerido como marcador diferencial en tumores renales y enfermedades genitourinarias. Este estudio se propuso evaluar la importancia pronóstica de la expresión de VIM3 en el CCE oral.

Materiales y métodos: Estudio retrospectivo, incluyó 75 casos de CCE oral diagnosticados entre 2018 y 2023.

Se realizó inmunohistoquímica para VIM3 en bloques de tejido tumoral embebidos en parafina. Se analizó relación entre la expresión de VIM3 y parámetros clínico-patológicos como edad, sexo, estado de tabaquismo, tamaño y localización del tumor, diferenciación, invasión linfovascular, metástasis en ganglios linfáticos y estadio patológico.

Resultados: La expresión de VIM3 se asoció significativamente con diferenciación tumoral y estado de tabaquismo. Los tumores pobremente diferenciados mostraron los niveles más altos de expresión de VIM3, indicando una fuerte relación entre el aumento de VIM3 y pérdida de diferenciación. Además, los fumadores activos exhibieron una expresión de VIM3 significativamente mayor en comparación con los no fumadores.

Discusión: Estos hallazgos sugieren que la expresión de VIM3 se correlaciona con la agresividad tumoral y podría servir como biomarcador pronóstico en el CCE oral. La asociación con el estado de tabaquismo destaca su posible papel en la biología tumoral influenciada por la exposición al tabaco.

Palabras clave: carcinoma oral de células escamosas, vimentina 3, biomarcador, inmunohistoquímica, pronóstico

KEY POINTS

Current knowledge

- Vimentin-3 (VIM3) is a splice variant of the vimentin protein, primarily studied in prostate and renal cancers. It is known to play a role in epithelial-mesenchymal transition, a critical process in cancer invasion and metastasis. However, its role in oral squamous cell carcinoma (SCC) remains unexplored.

Contribution of the article to current knowledge

- This study demonstrates that VIM3 expression is significantly higher in poorly differentiated oral SCC and in smokers. It is also associated with larger tumor size and aggressive behavior. These findings suggest that VIM3 may serve as a novel prognostic biomarker and therapeutic target in oral SCC.

Oral cancers are neoplasms that include carcinomas of the lip, oral cavity, and oropharynx. Oral squamous cell carcinomas (SCCs), the most common type, account for 90% of oral carcinomas. They rank among the leading cancers worldwide in terms of prevalence and morbidity. In Asia alone, oral SCCs account for 74% of global oral cancer-related deaths. Despite recent advancements in chemotherapy and surgery, the survival rate for oral SCCs has remained low for years. Therefore, identifying new potential biomarkers for early diagnosis and prognosis is critical[1].

Vimentin is an intermediate filament protein predominantly expressed in mesenchymal cells, with low expression in epithelial cells under normal conditions. Oral SCCs, which originate from the epithelial cells of the oral mucosa, often show increased vimentin expression associated with epithelial-mesenchymal transition (EMT), a process enhancing tumor cell migration and metastatic potential[2].

Vimentin 3 (VIM3) is a variant of vimentin with a unique C-terminal domain that plays a key role in maintaining cellular structure and stability under mechanical stress. Although vimentin has been extensively studied, research on VIM3 is limited. Previous studies suggest that VIM3 is a distinctive structural protein, expressed more specifically and abundantly than vimentin, particularly in epithelial cells, and has potential as a differential marker in kidney tumors and genitourinary diseases[3,4]. However, its role in oral SCC has not been investigated.

This study aims to evaluate VIM3 expression in oral squamous cell carcinoma and to investigate its relationship with clinicopathological parameters including age, sex, smoking history, tumor size, differentiation, lymphovascular invasion, and lymph node metastasis. We also seek to determine whether VIM3 expression could serve as a biomarker guiding tumor invasion, metastasis potential, aggressiveness, and prognosis.

Materials and methods

Case selection

Seventy-five cases diagnosed with oral squamous cell carcinoma between 2018 and 2023 at the Department of Medical Pathology, Faculty of Medicine, Karadeniz Technical University, were included in this study. Of these, 35 cases were located on the tongue, 27 on the lip, six on

the floor of the mouth, and seven on the buccal mucosa. Cases referred to consultation, those who came for consultation only, or those with minimal tumor areas were excluded. The cases were retrieved from pathology laboratory archives, and paraffin blocks containing sufficient tumor tissue were selected for analysis. Clinicopathological data including age, sex, and tumor size were obtained from the hospital information management system. This study included only current smokers and non-smokers, excluding ex-smokers.

Immunohistochemical evaluation

Four-micron-thick sections were cut from tumor-containing paraffin blocks using a microtome and mounted onto poly-L-lysine-coated slides. After deparaffinization, immunohistochemical staining for VIM3 was performed using the Ventana Benchmark Ultra automatic system (Ventana Medical Systems, Inc.), according to the manufacturer's protocol. The primary antibody used was Anti-Vimentin, clone VIM 3B4 (SIGMA/CBL202). After staining, slides were coverslipped with Entellan solution and examined under an Olympus BX50 light microscope. Muscle tissue served as a positive control.

The percentage of cytoplasmic staining and staining intensity at 40x magnification were evaluated using the H-score method. Staining intensity was scored as 3+ (strong), 2+ (moderate), 1+ (weak), or 0 (no staining). The H-score was calculated as: (3 × percentage of 3+ cells) + (2 × percentage of 2+ cells) + (1 × percentage of 1+ cells) + (0 × percentage of unstained cells), resulting in a score ranging from 0 to 300 for each case[5]. Two pathologists independently scored the slides, and the final H-score was obtained by averaging their scores.

The association between VIM3 expression and clinicopathological parameters such as age, sex, smoking history, tumor size, tumor location, differentiation, lymphovascular invasion, lymph node metastasis, and pathological stage was investigated.

Statistical analysis

Statistical analyses were conducted using SPSS version 29. Descriptive statistics were presented as frequency and percentage for categorical variables, and mean ± standard deviation, median, minimum, and maximum for numerical variables. Normality was assessed with the Kolmogorov-Smirnov test. Comparisons between two independent groups were performed using Student's t-test for normally distributed data and Mann-Whitney U test otherwise. For three or more groups, ANOVA was used if data were normally distributed; otherwise, the Kruskal-Wallis test was applied. Post hoc analyses for Kruskal-Wallis were conducted using the Bonferroni method. Qualitative data were compared with the chi-square test. A p-value < 0.05 was considered statistically significant.

Ethical statements

All procedures involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and adhered to the 1964 Helsinki Declaration and its subsequent amendments or comparable ethical standards. Due to the retrospective nature of this study, the ethical committee waived the requirement for informed consent.

Results

A total of 75 cases diagnosed with oral squamous cell carcinoma between 2018 and 2023 were included. The mean age of the patients was 66.5 years (range: 37-98). Of these, 48% (n=36) were female, and 52% (n=39) were male. The most frequent tumor site among females was the tongue (52.7%), whereas in males, the lip was the most common site (46.1%).

Regarding smoking status, 50.7% (n=38) were active smokers, and 49.3% (n=37) had no history of smoking. Among females, 22.2% were smokers, while 76.9% of males were smokers. The average tumor size was 2.7 cm. Tumors larger than 2 cm were present in 64% of cases, and the rest had tumors smaller than 2 cm. Among active smokers, 62.5% had tumors exceeding 2 cm in size, which was significantly larger compared to non-smokers ($p = 0.006$).

Lymphovascular invasion was observed in 28% of cases (n=21), with 76.2% of these located on the tongue. Cases with lymphovascular invasion more frequently had tumors larger than 2 cm ($p = 0.015$) and showed significantly advanced pathological stages ($p < 0.001$). Key findings including average VIM3 H scores and corresponding p-values are presented in Table 1.

The mean VIM3 H score was 130.4, ranging from 6 to 300. Representative hematoxylin and eosin and immunohistochemical staining images of VIM3-positive cases are shown in Figures 1A, 1B, 2A, and 2B.

A significant association was found between VIM3 expression and tumor differentiation as well as smoking status. Poorly differentiated tumors showed the highest mean VIM3 H score

Table 1 | Association between clinicopathological parameters and average VIM3 H-score with corresponding p-values

Parameter	Average VIM3 H score	Statistical significance (p-value)
Tumor location		
Tongue	141.34	
Lip	130.67	0.269
Floor of the mouth	102.00	
Buccal	98.57	
Sex		
Female	124.58	0.328
Male	135.69	
Lymphovascular invasion		
Positive	152.57	0.168
Negative	121.72	
Pathological stage		
T1	109.04	
T2	129.00	0.085
T3	167.67	
T4	116.73	
Smoking status		
Active smokers	151.26	0.016***
Non-smokers	108.89	
Tumor size		
> 2 cm	135.06	0.338
≤ 2 cm	122.00	
Degree of differentiation		
Poorly differentiated	244.29	
Moderately differentiated	157.57	0.000***
Well differentiated	98.73	

VIM3: vimentin 3
**** Indicates a statistically significant result (p < 0.05)*

(244.3), indicating a strong correlation between elevated VIM3 expression and loss of differentiation ($p < 0.001$). Additionally, active smokers exhibited significantly higher VIM3 expression (mean H score 151.3) compared to non-smokers (mean H score 108. 9) ($p = 0.016$). These results suggest that VIM3 may be a useful biomarker related to tumor aggressiveness and smoking-related changes in oral squamous cell carcinoma.

Discussion

This study examined VIM3 expression in oral SCC and its association with clinicopathological parameters. Tongue SCCs were more common in younger patients, while lip SCCs were more frequent in older individuals. These findings align with studies suggesting that tongue SCCs may exhibit a more aggressive course in younger populations[6].

Lip SCCs were more prevalent in men, and tongue SCCs in women, possibly reflecting differing smoking habits. In our cohort, the smoking rate was significantly higher among men, and smokers had larger tumor sizes and higher VIM3 expression levels. This supports evidence that smoking contributes to tumor progression by promoting chronic inflammation, oxidative stress, and epithelial-mesenchymal transition

Figure 1 | A and B: Well-differentiated squamous cell carcinoma case (Hematoxylin & Eosin X400) and vimentin 3 positivity in scattered tumor cells in a well-differentiated squamous cell carcinoma case (vimentin 3 X400)

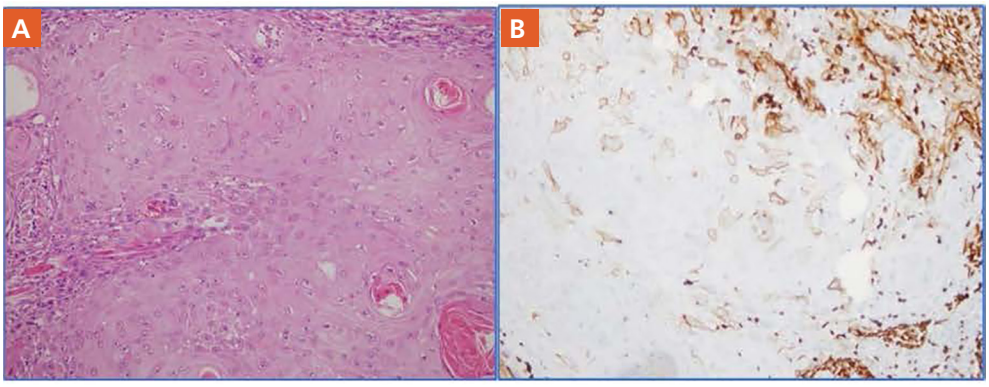
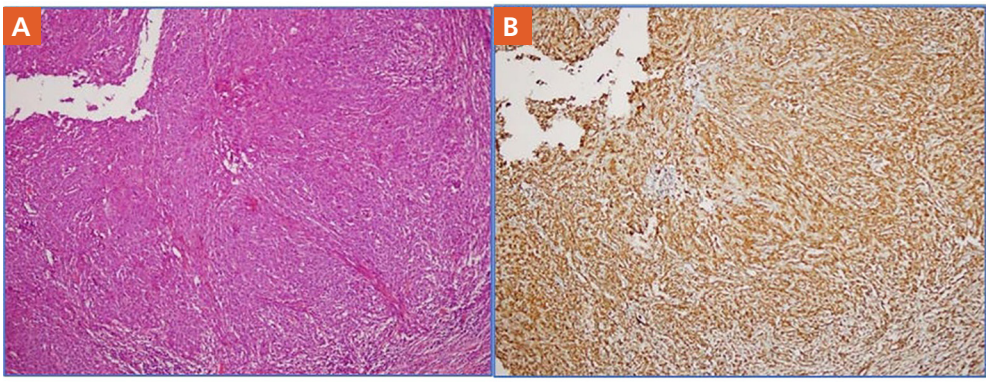


Figure 2 | A and B: Poorly differentiated squamous cell carcinoma case (Hematoxylin & Eosin X200) and diffuse vimentin 3 positivity in a poorly differentiated squamous cell carcinoma case (vimentin 3 X200)



(EMT), all of which facilitate tumor invasion and metastasis[7].

Lymphovascular invasion was more frequently observed in tongue SCCs, suggesting a more aggressive clinical behavior. Although VIM3 expression was higher in these cases, the difference was not statistically significant. However, previous studies have established a connection between vimentin expression and metastatic potential in various cancers[8].

A significant relationship was found between tumor differentiation and VIM3 expression. Poorly differentiated SCCs demonstrated markedly higher VIM3 expression, which is consistent with the role of vimentin in EMT and tumor dedifferentiation[9]. This suggests that VIM3 may serve as an indicator of biological aggressiveness.

Although VIM3 expression was not significantly associated with overall pathological stage, higher VIM3 H-scores were observed in T3 tumors. This trend suggests that VIM3 expression may increase with advancing tumor stage, though further studies with larger cohorts are needed to confirm this observation.

Our findings indicate that VIM3 may have prognostic value in oral SCC, particularly in smokers and in poorly differentiated tumors. Prior research has highlighted the role of VIM3 in prostate and renal cancers, where elevated expression was associated with tumor invasiveness and progression[3,4,9]. VIM3, a splice variant of vimentin lacking the typical C-terminal segment, has been proposed as a novel EMT-related marker, especially in aggressive malignancies[4].

To our knowledge, this is the first study to assess VIM3 expression in oral SCC. Although our results suggest that VIM3 is associated with tumor aggressiveness and smoking-related biological changes, further studies involving larger case series and multiple tumor types are necessary to validate these findings and explore its potential as a therapeutic target.

In conclusion, VIM3 expression correlates with poor differentiation and smoking history in oral SCC and may serve as a biomarker of tumor aggressiveness. These preliminary results support further investigation of VIM3 in clinical and research settings.

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Conflict of interest: None to declare

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