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Investigation of Methylation and Expression Levels in STK32C Gene in Oral Malignant Lesions*

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ABSTRACT

Aim: As a result of the array analyses obtained within the scope of the TUBITAK-SBAG-114S497 project conducted by Demokan and his colleagues, STK32C gene was determined as a potential epigenetic biomarker candidate. In this study, the methylation and expression levels of STK32C gene were examined in a larger patient group consisting of oral squamous cell carcinoma (OSCC) cases; the biomarker potential of this gene in terms of early diagnosis/prognosis evaluation was investigated.

Method: DNA and RNA were isolated from tissue samples of 15 OSCC patients, and the methylation and expression levels of the STK32C gene were examined using Quantitative Methylation Specific PCR and Real-Time PCR methods, respectively.

Results and Conclusions: In tumor samples of OSCC patients, 93.3% methylation was observed in the promoter region of the STK32C gene. When expression levels were evaluated, expression loss was detected in 53.3% of the samples in tumor tissues compared to normal tissues, while expression increase was observed in 13.3%. Hypermethylation was detected in all patients with decreased expression levels. It is suggested that the loss of expression observed in the STK32C gene due to hypermethylation may play a role in the molecular characterization of a certain subgroup of OSCC patients and that this gene may be a significant biomarker candidate for early diagnosis and prognosis. However, further studies covering larger patient populations are needed to confirm this.

Keywords: Oral squamous cell carcinoma, methylation, expression, biomarker

Oral Malign Lezyonlarda STK32C Genindeki Metilasyon ve Ekspresyon Düzeylerinin Araştırılması

ÖZET

Amaç: Demokan ve arkadaşları tarafından yürütülen TÜBİTAK-SBAG-114S497 kodlu proje kapsamında elde edilen array analizleri sonucunda, STK32C geni potansiyel bir epigenetik biyobelirteç adayı olarak belirlenmiştir. Bu çalışmada, oral skuamöz hücreli karsinom (OSCC) olgularından oluşan geniş bir hasta grubunda STK32C geninin metilasyon ve ekspresyon düzeyleri incelenmiş; bu genin erken tanı/prognoz değerlendirme açısından biyobelirteç potansiyeli araştırılmıştır.

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Yöntem: 15 OSCC hastasının doku örneklerinden DNA ve RNA izole edilmiş ve STK32C geninin metilasyon ve ekspresyon düzeyleri sırasıyla Kantitatif Metilasyon Spesifik PCR ve Gerçek Zamanlı PCR yöntemleri kullanılarak incelenmiştir.

Bulgular ve Sonuçlar: OSCC hastalarının tümör örneklerinde, STK32C geninin promotör bölgesinde %93,3 metilasyon gözlenmiştir. Ekspresyon seviyeleri değerlendirildiğinde, tümörlü dokulardaki örneklerin %53,3'ünde normal dokulara kıyasla ekspresyon kaybı, %13,3'ünde ise ekspresyon artışı tespit edilmiştir. Ekspresyon seviyeleri düşük olan tüm hastalarda hipermetilasyon saptanmıştır. Hipermetilasyona bağlı STK32C geninde gözlenen ekspresyon kaybının, OSCC hastalarının belirli bir alt grubunun moleküler karakterizasyonunda rol oynayabileceği ve bu genin erken tanı ve prognoz için önemli bir biyobelirteç adayı olabileceği düşünülmektedir. Ancak, bunu doğrulamak için daha geniş hasta popülasyonlarını kapsayan daha fazla çalışmaya ihtiyaç bulunmaktadır.

Keywords: Oral skuamöz hücreli karsinom, metilasyon, ekspresyon, biyobelirteç

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is common subtype of head and neck cancer and generally carries a poor prognosis (Imbesi Bellantoni et al., 2023; C. Zhang, Cai, & Ke, 2023). It is associated with risk factors such as tobacco and alcohol use, and HPV infection, and is usually diagnosed at an advanced stage (Galati et al., 2022).

Not only genetic mutations but also epigenetic changes play a significant role in cancer development (Kanwal & Gupta, 2012). DNA methylation is an epigenetic mechanism that involves the addition of a methyl group to the 5th carbon of the cytosine nucleotide and can suppress gene transcription (Moore, Le, & Fan, 2013). Promoter region hypermethylation can contribute to tumor development by silencing tumor suppressor genes (Liyanage et al., 2019). Therefore, examining methylation profiles is a promising approach for early diagnosis and prognostication (Li, Song, Chen, & Dai, 2025).

STK32C gene belongs to the serine/threonine kinase family and is thought to be involved in cellular signaling and various biological processes (Sun, Liu, Zhao, & Wang, 2019). While there are a limited number of studies in literature investigating the expression level of STK32C, no studies have been found investigating its hypermethylation status. High-resolution omics technologies' analyses conducted by Demokan and colleagues as part of the TÜBİTAK-SBAG-114S497 project identified the STK32C gene as a potential epigenetic biomarker candidate.

In this study, the methylation and expression levels of the STK32C gene were examined in a large patient group of OSCC patients, and its potential as a biomarker for early diagnosis and prognostic assessment was investigated.

MATERIALS-METHODS

Following approval from the Istanbul University Faculty of Medicine Ethics Committee (2019/833), primary tumor tissue and corresponding normal tissue samples were collected from 15 OSCC patients presenting to the Department of Otorhinolaryngology-Head and Neck Surgery, Istanbul Faculty of Medicine, Istanbul University. DNA and RNA were extracted from all tissue samples.

After DNA samples were subjected to bisulfite modification, the methylation levels of the STK32C gene were determined using the quantitative methylation-specific PCR (QMSP) method and Absolute Quantification software on the LightCycler 480. The percentage of methylation in the samples was calculated using the formula Methylation (%) = (Methylated / [Methylated + Unmethylated]) × 100. Expression analyses were normalized against the β-actin (ACTB) gene, selected as the reference gene, using the "Basic Relative Quantification" software on a LightCycler 480 (Roche, Mannheim, Germany). Fold change values for the STK32C gene were calculated using the $2^{-\Delta\Delta Ct}$ method.

RESULTS

In tumor samples of OSCC patients, 93.3% methylation was observed in the promoter region of the STK32C gene (Figure 1). Expression loss was detected in 53.3% of the samples in tumor tissues compared to normal tissues, while expression increase was observed in 13.3% (Figure 2). Methylation was detected in all patients with decreased expression levels. Table 1 shows the clinicopathological features of OSCC patients.

Table 1. Clinical and Pathological Features of OSCC Patients

Parameter		**OSCC (n=15) n (%)**
Age (%n)	**< 50**	1 (6.67)
	≥ 50	14 (93.33)
Gender	**F**	5 (33.33)
	M	10 (66.67)
Anatomical Involvement Site		
Buccal Mucosa		6 (40)
Retromolar Trigon		1 (6.7)
Tongue		4 (26.67)
Floor of Mouth		2 (13.3)
Hard Palate		2 (13.33)
Stage 1		-
Stage 2		3 (20)
Stage 3		4 (26.67)
Stage 4		8 (53.33)
Early Stage (I–II)		3 (20)
Advanced Stage (III–IV)		12 (80)
Differentiation Grade		
Low		5 (33.33)
Moderate		10 (66.67)
High		-
Smoking		
	Yes	9 (60)
	No	6 (40)
Alcohol Consumption		
	Yes	1 (6.7)
	No	14 (93.3)
Lymph Node Involvement		
	Yes	6 (40)
	No	9 (60)
Metastasis		
	Yes	4 (26.7)
	No	11 (73.3)

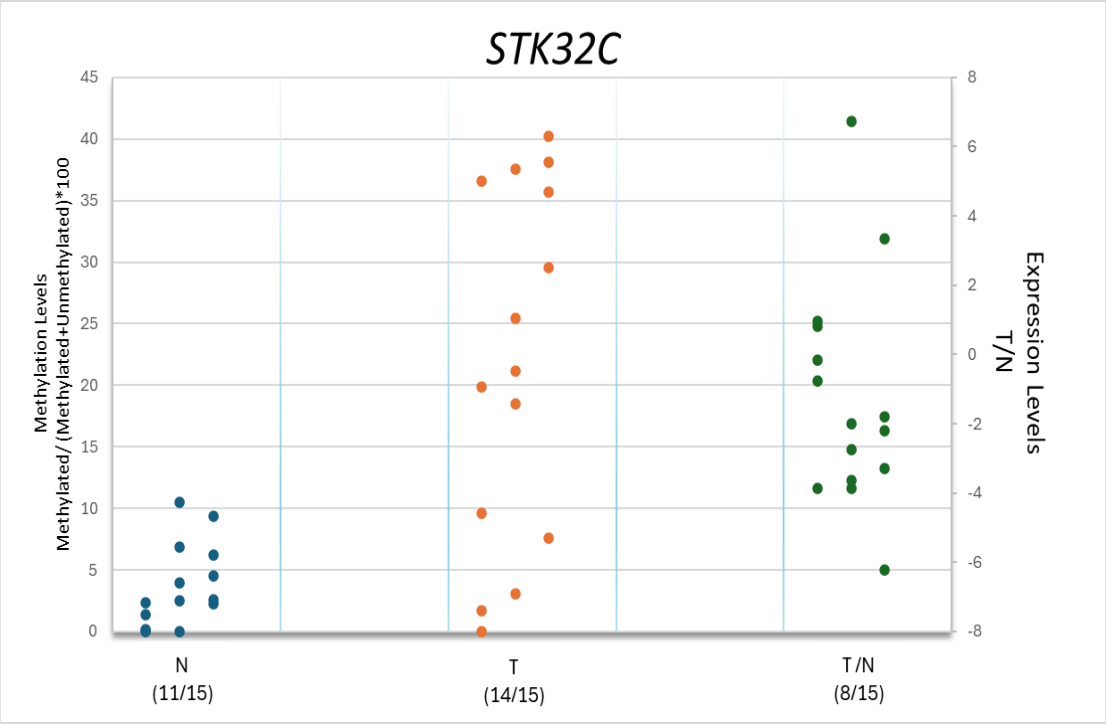

Figure 1: Methylation rates and T/N expression levels of OSCC normal and tumor tissues

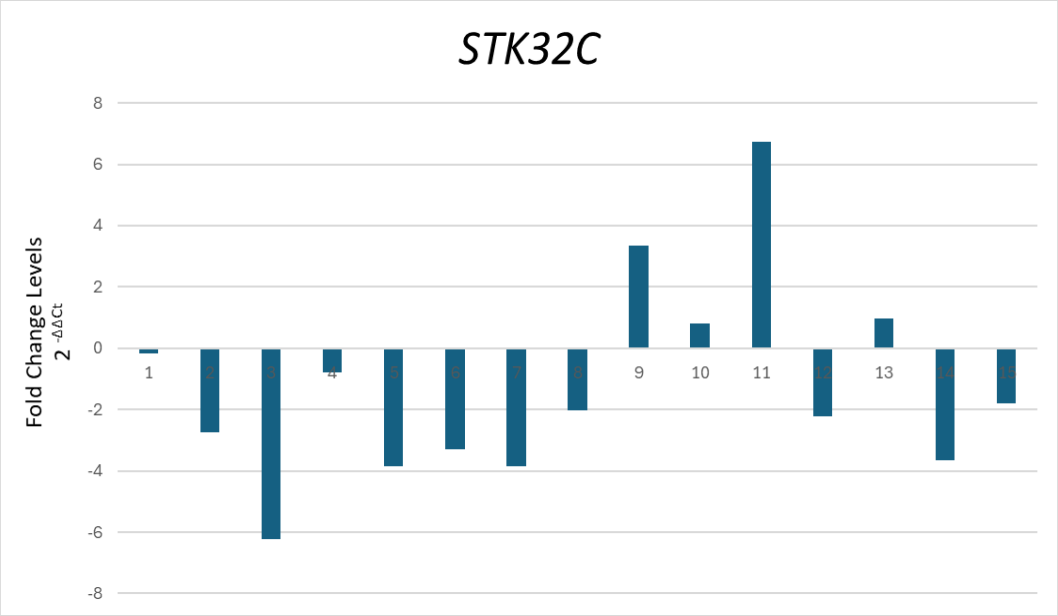

Figure 2: Differentiation expression graph of STK32C gene expression levels in OSCC patients

DISCUSSION

In a study examining the role of STK32C in colorectal cancer (CRC) progression and its relationship with the MYC signaling pathway, STK32C expression was found to be significantly elevated in CRC samples and was reported to be associated with poor prognosis (X. Zhang et al., 2025). A study examining the role of STK32C in doxorubicin resistance in triple-negative breast cancer (TNBC) showed that high STK32C expression was

associated with an unfavorable prognosis in TNBC patients treated with doxorubicin. Depletion of STK32C has been reported to increase drug sensitivity in doxorubicin-resistant cells (Xiao, Liu, & Huang, 2025). In another study examining the role of STK32C in bladder cancer (BC), immunohistochemistry analyses showed that high STK32C protein levels were associated with poor clinicopathological features and short recurrence-free survival (RFS), and suppression of STK32C reduced the proliferation, migration, and invasion of tumor cells in vitro and limited tumor growth in vivo models (Sun et al., 2019). While there are limited studies in literature investigating the expression level of STK32C in cancer, no study has investigated its methylation status. In our study, STK32C undergoes methylation-mediated gene silencing in OSCC and that expression changes may be related to epigenetic mechanisms.

CONCLUSION

It is suggested that the loss of expression observed in the STK32C gene due to methylation may play a role in the molecular characterization of a certain subgroup of OSCC patients and that this gene may be a significant biomarker candidate for early diagnosis and prognosis. However, further studies covering larger patient populations are needed to confirm the results.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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