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Investigation of Methylation-Related Expression Changes of GABRB3 Gene in Oral Malignant Lesions*

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ABSTRACT

Aim: Oral cancer constitutes 2-4% of all cancers and is the most common cancer in the head and neck region after laryngeal cancer. Oral squamous cell carcinoma (OSCC) constitutes more than 90% of all oral cavity carcinomas. While the 5-year survival rate in OSCC is 40%, this rate increases to 95% in early diagnosis. The GABRB3 gene was identified as a potential epigenetic biomarker candidate in the TUBITAK-SBAG-114S497 project conducted by Demokan et al., and in this study, the differing methylation and expression levels in OSCC patients were examined and its biomarker potential in early diagnosis and prognosis was investigated.

Methods: DNA and RNA were isolated from tissue samples taken from 15 patients diagnosed with OSCC, then the methylation status and gene expression levels of the GABRB3 gene were evaluated by Quantitative Methylation Specific PCR and Real-Time PCR methods, respectively.

Results and Conclusions: Methylation was detected in the promoter region of the GABRB3 gene in 60% of tumor samples from OSCC patients. When expression levels were evaluated, loss of expression of the GABRB3 gene was detected in 46.7% of samples compared to normal tissues in tumor tissues, while increased expression was observed as 13.3%. Methylation was detected in all patients with decreased expression levels. While decreased expression was observed in 33.3% of tumor tissues having no methylation, increased expression was observed in 50%. It is thought that GABRB3 gene may be responsible for a special subgroup of OSCC patients via methylation-related loss of expression and can be used as a potential biomarker candidate in early diagnosis and prognosis determination. Further studies in larger patient groups are needed to confirm these findings.

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

Oral Malign Lezyonlarda GABRB3 Geni Metilasyonla İlişkili Ekspresyon Değişimlerinin Araştırılması

ÖZET

Amaç: Oral kanserler, tüm kanserlerin %2-4'ünü oluşturur ve baş-boyun bölgesinde larenks kanserinden sonra en sık görülen kanser türüdür. Oral skuamöz hücreli karsinom (OSCC), tüm ağız boşluğu karsinomlarının %90'ından fazlasını oluşturur. OSCC'de 5 yıllık sağkalım oranı %40 iken, erken tanıda bu oran %95'e çıkmaktadır. GABRB3 geni, Demokan ve arkadaşları tarafından tamamlanmış TÜBİTAK-SBAG-114S497 kodlu projede potansiyel bir epigenetik biyobelirteç adayı olarak tanımlanmış ve bu çalışmada, OSCC hastalarındaki farklı metilasyon ve ekspresyon seviyeleri incelenerek erken tanı ve prognozdaki biyobelirteç potansiyeli araştırılmıştır.

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Yöntem: OSCC tanısı almış 15 hastadan alınan doku örneklerinden DNA ve RNA izole edildi, ardından GABRB3 geni metilasyon durumu ve gen ekspresyon seviyeleri sırasıyla Kantitatif Metilasyon Spesifik PCR ve Gerçek Zamanlı PCR yöntemleri ile değerlendirildi.

Bulgular ve Sonuçlar: OSCC hastalarından alınan tümör örneklerinin %60'ında GABRB3 geni promotör bölgesinde metilasyon tespit edildi. Ekspresyon seviyeleri değerlendirildiğinde, tümör dokularında normal dokulara kıyasla örneklerin %46,7'sinde GABRB3 gen ekspresyon kaybı tespit edilirken, %13,3'ünde ekspresyon artışı gözlendi. Ekspresyon seviyeleri azalmış olan tüm hastalarda metilasyon tespit edildi. Metilasyon gözlenmeyen tümör dokularının %33,3'ünde ekspresyonda azalma gözlenirken, %50'sinde ekspresyon artışı gözlendi. GABRB3 geni metilasyona bağlı ekspresyon kaybı yoluyla OSCC hastalarının özel bir alt grubundan sorumlu olabileceği ve erken tanı ve prognozun belirlenmesinde potansiyel bir biyobelirteç adayı olarak kullanılabileceği düşünülmektedir. Bu bulguları doğrulamak için daha geniş hasta gruplarıyla yapılacak ileri çalışmalara ihtiyaç vardır.

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

INTRODUCTION

Oral cancer is the sixth most common cancer worldwide, accounting for approximately 2–4% of all cancer cases and ranking second only to laryngeal cancer among head and neck malignancies (Barsouk, Aluru, Rawla, Saginala, & Barsouk, 2023; Tranby et al., 2022). Oral squamous cell carcinoma (OSCC) is the primary histological type, accounting for over 90% of oral cavity cancers (Tan et al., 2023). Because OSCC is usually diagnosed at an advanced stage, the 5-year survival rate is around 40%; however, this rate can be as high as 95% in cases diagnosed at an early stage (Mauceri et al., 2022). This highlights the critical prognostic importance of early diagnosis and necessitates the development of biologically based biomarkers with high sensitivity and specificity.

In recent years, studies to elucidate the molecular pathogenesis of OSCC have demonstrated that, in addition to genetic alterations, epigenetic regulations also play a significant role in tumor development and progression (Tan et al., 2023). These epigenetic changes, mediated by DNA methylation, histone modifications, and non-coding RNAs, can affect gene expression profiles, leading to the silencing of tumor suppressor genes or the activation of oncogenes (Kanwal & Gupta, 2012). Since changes in DNA methylation can occur at an early stage in cells capable of invasion and metastasis, they are considered promising targets for the development of both diagnostic and prognostic biomarkers (Song et al., 2025).

Demokan et al. (Unpublished data) conducted a project numbered TÜBİTAK-SBAG-114S497, which investigated potential epigenetic biomarkers using methylation and expression array analyses in tumor and matched normal tissues of OSCC patients, the GABRB3 (Gamma-Aminobutyric Acid Type A Receptor Beta 3 Subunit) gene was identified as a potential epigenetic biomarker candidate for OSCC. It is thought that methylation changes in the promoter region of the gene may affect expression levels and be associated with tumor development. This study evaluated the methylation and expression levels of GABRB3 in OSCC patients, demonstrating the gene's potential as a biomarker for early diagnosis and prognosis.

METHODS

Sample Collection

In our study, which received approval from the Istanbul University, Istanbul Faculty of Medicine Ethics Committee (2019/833), 0.5 cm primary tumor and conjugated normal tissue samples were obtained during surgery from patients with oral cavity cancer. All tissue samples were transported to the laboratory on ice and stored at -80°C until DNA and RNA extraction.

RNA Extraction and cDNA Synthesis

Total RNA isolation from tissue samples was performed using the Norgen Total RNA Purification Kit (Norgen, Ontario, Canada) according to the manufacturer's instructions. The obtained samples were measured spectrophotometrically with the Nanodrop 2000c (Thermofisher, USA), and cDNA conversion was performed

using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Germany) according to the manufacturer's instructions.

DNA Extraction and Bisulfite Treatment

DNA isolation from samples were performed using the phenol/chloroform method. Concentration measurements were performed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA). DNA samples of sufficient quality were subjected to bisulfite conversion using the Zymo Methylation Gold Kit (Zymo Research, CA, USA) according to the manufacturer's protocol to determine the methylation status in the promoter region of *GABRB3* gene.

Reverse Transcription and Quantitative Real-Time PCR

Expression levels of the *GABRB3* gene were assessed by quantitative real-time PCR (qRT-PCR). Tumor tissues obtained from patients with OSCC were analyzed in comparison with conjugated normal tissue samples (Guerrero-Preston R, et al., 2014). Ct values of each sample were normalized to the expression levels of the housekeeping gene (ACTB), which was determined as an internal control, using the "Basic Relative Quantification" analysis mode in LightCycler 480 software (Roche, Mannheim, Germany). Relative expression levels of the genes were calculated using the $2^{-\Delta\Delta Ct}$ method.

Quantitative Methylation-Specific PCR (QMSP)

DNA samples obtained from tumor and matched normal tissue samples taken from patients were analyzed using the QMSP method on the LightCycler 480 instrument after being modified according to the bisulfite conversion kit protocol (Demokan S., et al., 2014). Methylation levels in the gene promoter region were determined using the Absolute Quantification program. The methylation level in each sample was calculated using the formula Methylation (%) = (Methylated / [Methylated + Unmethylated]) × 100.

FINDING AND DISCUSSION

Clinical Parameters

Clinicopathological features of OSCC patients are shown in Table 1.

Table 1. Clinical Parameters of OSCC Patients

Parameter		**OSCC (n=15) n (%)**	**p values (Expression changes)**	**p values (Methylation status)**
Age (%n)	**≥ 50**	15 (100)		
Gender	**F** **M**	6 (40) 9 (60)	0.607	0.328

Anatomical Involvement Site **Buccal Mucosa** **Retromolar Trigon** **Tongue** **Floor of Mouth** **Inferior Alveolar Arch** **Hard Palate**	 6 (40) 1 (6,67) 3 (20) 2 (13.33) 2 (13.33) 1 (6.67)	0.747	0.479
Stage 1 **Stage 2** **Stage 3** **Stage 4**	2 (13.33) 3 (20) 4 (26.67) 6 (40)	0.895	1.000
Early Stage (I–II) **Advanced Stage (III–IV)**	5 (33.3) 10 (66.7)	0.679	0.768
Differentiation Grade **Low** **Moderate** **High**	 2 (13.3) 13 (86.7) -	1.000	0.933
Smoking **Yes** **No**	 7 (46.7) 8 (53.3)	0.281	0.463
Alcohol Consumption **Yes** **No**	 1 (6.7) 14 (93.3)	0.267	0.267
Lymph Node Involvement **Yes** **No**	 5 (33.3) 10 (66.7)	0.206	0.594
Metastasis **Yes** **No**	 4 (26.7) 11 (73.3)	0.851	0.949

Methylation was detected in the promoter region of the *GABRB3* gene in 60% of tumor samples from OSCC patients (Figure 1). Expression analysis revealed that *GABRB3* expression was decreased in 46.7% of tumor tissues compared to normal tissues and increased in 13.3% (Figure 2). Methylation was detected in all patients with low expression levels. Among tumor tissues without methylation, decreased expression was observed in 33.3% and increased expression was observed in 50%.

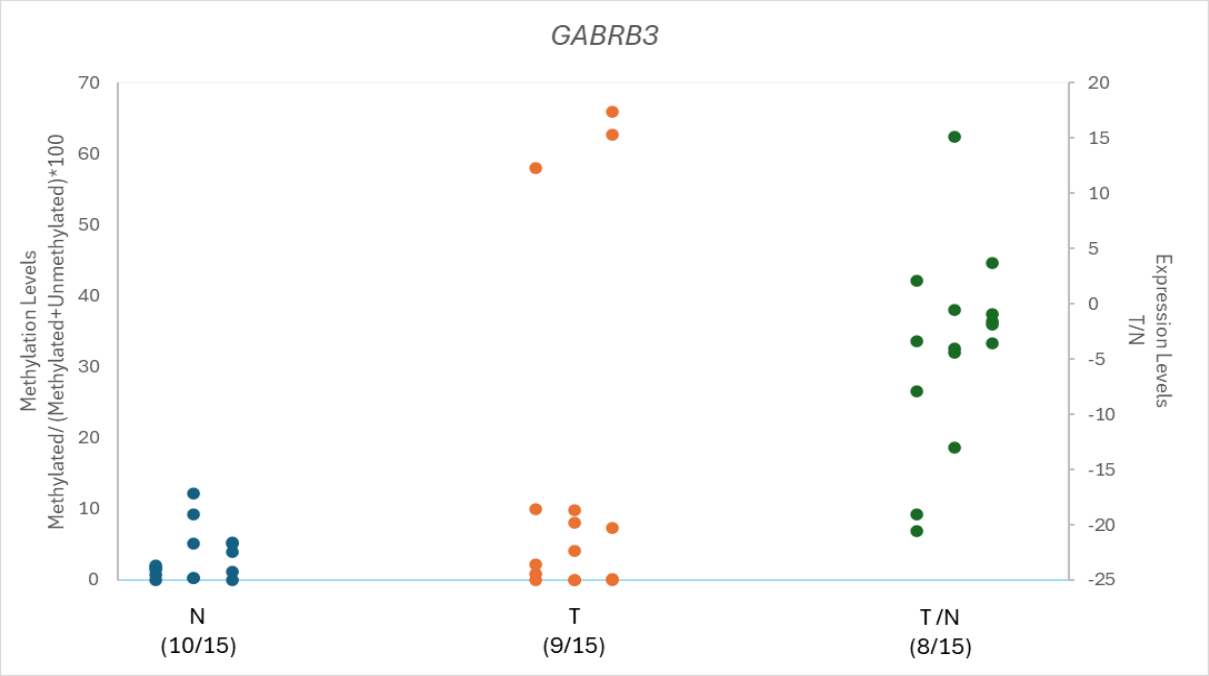

Figure 1: Comparison of methylation rates and T/N expression levels in normal and tumor tissues in OSCC patients

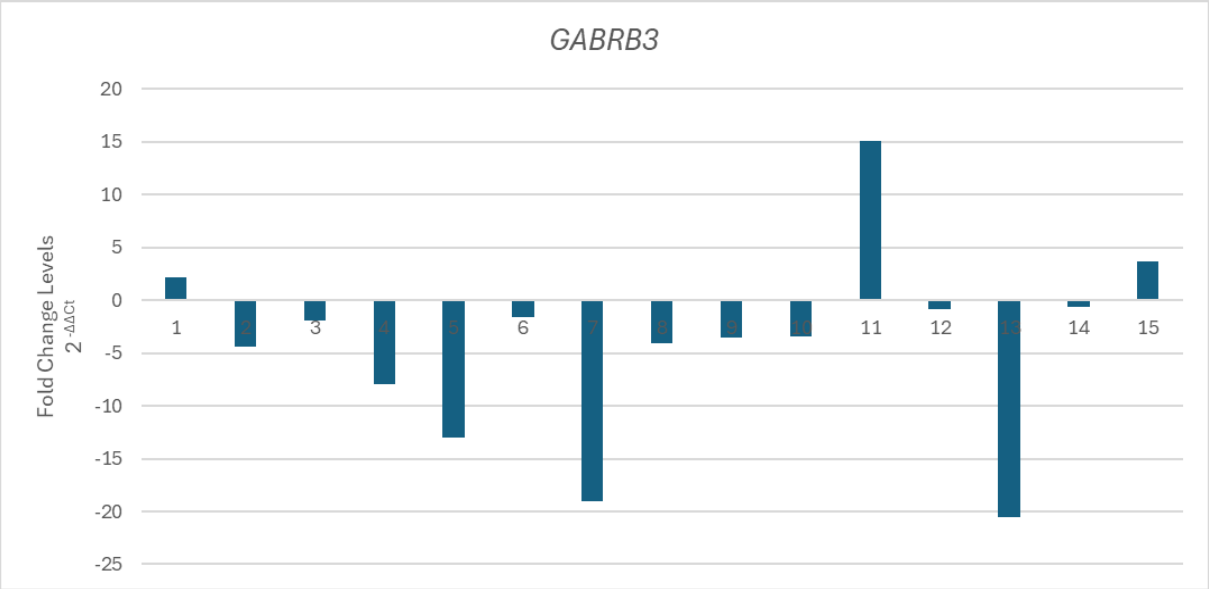

Figure 2: Distribution graph of GABRB3 gene expression levels according to differentiation in OSCC patients

Statistical analysis

All statistical analyses were performed using SPSS Statistics 20 software. Mann Whitney U test and Kruksal Wallis tests were used to compare gene expression levels and methylation status with clinical parameters, and $p<0.05$ was considered statistically significant.

DISCUSSION

There are two studies in the literature related to promoter methylation of the *GABRB3* gene. One of the studies examining DNA methylation profiles in brain metastases (PCBM) originating from primary prostate cancers, analysis of 42 PCBM and 17 matched primary tumor samples revealed significant epigenetic differences between PCBM and primary tumors. PCBM exhibited a CpG island hyper methylator phenotype, and promoter hypomethylation was detected in neuroactive ligand-receptor interactions and cell adhesion molecules (e.g., *GABRB3, CLDN8, CLDN4*) (Gallon J. et al., 2023). Another study in primary head and neck squamous cell carcinomas, 186 downregulated genes with cancer-specific promoter methylation were identified, along with 10 tumor suppressor genes (*GABRB3, HOXC12, PARP15, SLCO4C1, CDKN2A, PAX1, PIK3AP1, HOXC6, PLCB1, ZIC4*) that were inactivated through both promoter methylation and somatic mutation. A high frequency of genomic and epigenomic alterations was detected, particularly in the *PAX* gene family, affecting the NOTCH and TP53 pathways that regulate cell fate, survival, and genome integrity (Guerrero-Preston et al., 2014). Our study, in line with these studies in the literature, suggests that promoter methylation of the *GABRB3* gene may affect gene expression in OSCC.

CONCLUSION

It is thought that the *GABRB3* gene may be responsible for a specific subgroup of OSCC patients through methylation-associated loss of expression and could be used as a potential biomarker candidate for early diagnosis and prognosis. Further studies in larger patient groups are needed to confirm these findings.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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