Research Article

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Expression levels of *BAP1*, *OGT*, and *YY1* genes in patients with eyelid tumors

Göz Kapağı Tümörlü Hastalarda *BAP1*, *OGT* ve *YY1* Genlerinin İfade Düzeyleri

Abstract

Objectives: The aim of this study was to investigate *BAP1*, *OGT* and *YY1* genes and protein levels in 12 samples (8 males, 4 females) of eyelid tumor tissue with basal cell carcinoma (BCC) and 12 normal control subjects (8 males, 4 females).
Methods: The expression levels of these genes were determined with RT-PCR and the protein levels and expression using ELISA and IHC methods, respectively.
Results: In RT-PCR analysis, statistically significant upregulated expression was determined of 1.84-fold of *BAP1*, 2.85-fold of *OGT* and 3.06-fold of *YY1* genes (p < 0.05). In the patient group, compared to the control group, there was a similar statistically significant strong correlation between the proteins (BAP1 and YY1; r = 0.850, BAP1 and OGT; r = 0.811, OGT and YY1; r = 0.755) (p < 0.05). In the ELISA and IHC analysis methods, a significant increase in BAP1 and YY1 protein expression levels was observed compared to the control group (p < 0.05).
Conclusions: The study results demonstrated that *BAP1* and *YY1* genes and protein levels were upregulated in eyelid tumor tissue with BCC.

Keywords: *BAP1*; basal cell carcinoma; expression; eyelid; *OGT*; tumor; *YY1*.

Öz

Amaç: Bu çalışmanın amacı, bazal hücreli karsinomlu (BHK) göz kapağı tümör dokusuna ait 12 örnek (8 erkek, 4 kadın) ve 12 normal kontrol deneklerinde(8 erkek, 4 kadın) *BAP1*, *OGT* ve *YY1* genlerini ve protein düzeylerini araştırmaktı.
Yöntemler: Bu genlerin ekspresyon seviyeleri RT-PCR ile, protein seviyeleri ve ekspresyonu sırasıyla ELISA ve IHC yöntemleri kullanılarak belirlendi.
Bulgular: RT-PCR analizinde, *BAP1*'in 1.84 katı, *OGT*'nin 2.85 katı ve *YY1* genlerinin 3.06 katı için istatistiksel olarak anlamlı yukarı regüle edilmiş ekspresyon belirlendi (p < 0.05). Hasta grubunda kontrol grubu ile karşılaştırıldığında, proteinler arasında istatistiksel olarak anlamlı benzer güçlü bir korelasyon vardı (BAP1 ve YY1; r = 0.850, BAP1 ve OGT; r = 0.811, OGT ve YY1; r = 0.755) (p < 0.05). ELISA ve IHC analiz yöntemlerinde kontrol grubuna göre BAP1 ve YY1 protein ekspresyon düzeylerinde anlamlı artış gözlendi (p < 0.05).
Sonuçlar: Çalışma sonuçları, BHK'lı göz kapağı tümör dokusunda *BAP1* ve *YY1* genlerinin ve protein düzeylerinin yukarı regüle düzenlendiğini göstermiştir.

Anahtar Kelimeler: *BAP1*; *OGT*; *YY1*; Bazal Hücreli Karsinom; Ekspresyon; Göz Kapağı; Tümör.

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Introduction

Benign or malignant lesions may be observed around the eyelid [1]. Malignant eyelid tumors are rarely observed, but the diagnosis and treatment of such tumors are the focus of interest of eye specialists [2]. The main eyelid malignant tumors affecting the eye are basal cell carcinoma (BCC), sebaceous gland cell carcinoma, squamous cell carcinoma, and malignant melanomas [3]. BCC is the most common malignant eye tumor, especially in western countries [4].

Some DNA damage, which may be hereditary, constitutes a risk factor for many diseases. For example, the risk of eye melanoma has been reported to increase in some people with mutations in the *BAP1* tumor suppressor gene [5]. BAP1 is a deubiquitinating enzyme involved in the control of the cell cycle, such as deubiquitination activity and nuclear localization [6]. Four ubiquitin C-terminal hydroxylases (UCHs) have been identified in mammals, namely BAP1, UCH-L1, UCH-L3, and UCH-37 [7]. UCHs are a small subclass of deubiquitinating enzymes that catalyze the elimination of additive products at the C-terminal of ubiquitin [8]. Recent studies have shown that mutations in the BAP1 gene are associated with various types of cancer [9]. Many genes are known to interact with the *BAP1* gene, primarily *OGT* and *YY1* genes [10]. Protein glycolysis catalyzed by the OGT enzyme is a post-translational modification by O-linked N-acetylglycoamination (O-GlcNAc). OGT catalyzes the addition of a single N-acetylglucosamine group to amino acid residues, such as serine and threonine, of nuclear, cytoplasmic, and mitochondrial proteins [11]. Proteins have been shown to cause abnormal O-GlcNAc in the pathological progression of metabolic diseases such as diabetes, heart disease, neurodegenerative disorders and cancer [12–14]. The clinical significance of changes in the O-GlcNAc signal and OGT abnormal expression in cancer is not yet fully known. However, some studies of cancer patients have shown the overexpression of both OGT and O-GlcNAc modifications in cancer tissues [15]. *YY1* is another gene that interacts with *BAP1*. YY1 is a ubiquitous transcription factor, which is a member of the polycomb group protein family [16]. The YY1 protein contains four C2H2-type zinc-finger motifs with two specific domains characterized by the activator or suppressor function [17]. YY1 is known to play a key role in normal biological processes, such as embryogenesis, differentiation, replication, and cell proliferation. Lu et al. [18] determined the *YY1* gene expression levels to be very low in the lungs, colon, and small intestine, and very high in skeletal and cardiac muscles. The aim of the current study was to determine the expression levels of BAP1, OGT, and YY1 genes and proteins in eyelid tumor tissue with BCC.

Materials and methods

Patient and control groups

The study patient group was formed of patients diagnosed with eyelid tumors in the Ophthalmology Department of Sivas Cumhuriyet University (SCU), Faculty of Medicine, Research and Application Hospital. As there was no similar study in literature regarding the determination of the number of patients, the sample size was established with the support of the Department of Biostatistics. Approval for the study was granted by the Local Ethics Committee (decision no: 2014-05/18) and all procedures were applied in compliance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines. Of patients who underwent surgery within 1 year of the date of ethics committee approval, 20 suitable cases were identified. Of these 20 patients, 12 had BCC and the other 8 had different types such as small cell malignant tumor, trichoepithelioma, malignant fibrous histiocystoma, squamous cell carcinoma, melanoma, chronic calcified granuloma, and sebaceous gland carcinoma. Therefore, to obtain meaningful results, only the 12 patients with BCC were evaluated in this study. No restrictions were placed in terms of gender, age, or the histopathological type and degree of cancer, but there were no patients younger than 50 years old in the study. Informed consent for participation in the study was provided by all the patients before surgery. Patients were included in the study after determination of the histopathological type of the tumor in the pathology report of the sample excised during surgery. The study was conducted on the tumor tissue taken from all patients during the surgery and control samples [19–21] obtained from the normal tissue around the tumor in accordance with the recommendation of the pathologist (Table 1). The patients included in the study were those who had not received any preoperative radiotherapy or chemotherapy as that can significantly affect the expression of biomarkers. Blood samples were taken from these patients into citrated tubes for ELISA analysis of the protein levels. A control group was formed of 12 age and gender-matched healthy individuals with no history of cancer, from among those who presented at SCU, Faculty of Medicine, Research and Application Hospital, Department of Physical Therapy and Rehabilitation. The experimental stage of this study was conducted in the Biochemistry Department and the Histology Department of the Faculty of Medicine.

Gene expression analysis with the real-time PCR

RNA isolation from control and tumor tissue was performed in accordance with the protocol recommended by the manufacturer, using the RNeasy Fibrous Tissue (Qiagen) catalogue number 74704 and RNeasy (Qiagen) mini-isolation kit with catalogue number 74104, respectively.

The RT2 First Strand cDNA synthesis kit (Qiagen, cat. no: 330404) was used for the synthesis of cDNA. The concentrations of RNA and cDNA samples throughout the experiments were quantitatively measured by nanodrop (Maestro Nanodrop [Green Bioresearch, USA]).

RT-PCR analysis was performed using the RT^2 SYBR Green qPCR Mastermix kit (Qiagen). For precipitate that might occur in Mastermix tubes, it was kept at 42 °C for 1 min and then vortexed. A 25 µL qPCR mixture was prepared so that it contained 1 µL of diluted cDNA. In the experiments, all cDNA samples were examined 3 times under the same conditions, and the average of these three measurements was used in the analyses. The housekeeping gene (*GAPDH*) was used as an internal control to determine expression level differences between the control and study groups. The *BAP1* (Qiagen, cat. no: PPH02153C), *OGT* (Qiagen, cat. no: PPH19166A), *YY1* (Qiagen, cat. no: PPH00440F), and GAPDH (Qiagen, cat. no: PPH00150F) primers used in the study were optimized by the manufacturer, which minimized all the negativities in terms of creating a specific product because of the presence of guaranteed primers.

All the RT-PCR analysis results of the gene expression experiments were quantified using the Rotor-gene 6000 Series Software Version 1.7. Statistical analysis of the data was performed using the ΔΔCT method in "RT2 profiler RT-PCR Array Data Analysis version" software (https://geneglobe.qiagen.com/de/analyze/).

Protein levels analysis with ELISA

From the patients diagnosed with eyelid tumor and the control group, 4 mL blood samples were withdrawn into sterile citrate tubes. Plasma was obtained from the collected blood samples (1,000×*g*, 15 min) and stored at −80 °C in Eppendorf tubes.

The BAP1, OGT, and YY1 protein levels were determined using the ELISA method. The detectable ranges of human BAP1 (Sunred Biological Technology, cat. no: 201–12–3062), OGT (Sunred Biological Technology, cat. no: 201–12–7461), and YY1 (Sunred Biological Technology, cat. no: 201–12–7436) were 20–6,000 mg/L, 12.5–3,200 ng/L, and 0.05–15 ng/mL, respectively. The human BAP1, OGT, and YY1 ELISA kits had a sensitivity of 18.663 mg/L, 12.314 ng/L, and 0.045 ng/mL respectively. An automatic washer Bio-Tek ELX50 (BioTek Instruments, USA) device was used to wash the plates, and the Microplate Reader device (BioTek, Epoch, USA) was used for absorbance readings.

Protein expressions analysis with immunohistochemistry

The study group tissues were fixed in 10% buffered neutral formalin for 30–36 h. The tissues were stored at +4 °C after the dehydration, making them transparent, and then embedded in paraffin blocks. Sections of 5 µm were cut with a microtome and placed on a poly-L-lysine-coated slide, then fixed at room temperature and stored at +4 °C until staining. In the sections taken, BAP1 (cat. no: EPP10790), OGT (cat. no: EAP0752), and YY1 (cat. no: EPM12542) protein expressions were determined using immunochemistry markers. In summary, following deparaffinization in xylene and rehydration with graded series of ethanol, the sections were washed in distilled water. The endogenous peroxidase activity was blocked using 3% hydrogen peroxide in methanol for 15 min at RT. After washing in phosphate-buffered saline with Tween-20 (PBS-Tween-20), antigen retrieval was performed on the sections by boiling twice for 10 min in 10 mM sodium citrate buffer (pH 6). To prevent non-specific binding, sections were incubated for 10 min in Ultra V Block (Thermo Scientific, USA) at RT, and then treated with anti-BAP1 antibody (rabbit polyclonal IgG,

EPP10790, Elabscience, USA) (1:100 dilution), anti-OGT antibody (rabbit polyclonal IgG, EAP0752, Elabscience, USA) (1:100 dilution), and anti-YY1 antibody (rabbit polyclonal IgG, EPM12542, Elabscience, USA) (1:100 dilution) overnight at +4 °C. After washing, these sections were incubated with HRP-streptavidin labeled, a biotinylated secondary antibody (TP-015-HA; Thermo Scientific, USA) and visualized with AEC and counterstained with hematoxylin. All the steps of the immunohistochemistry staining processes were applied to the negative control sections without the primary antibody incubation.

The intensity of the proteins was determined at ×10, ×20 and ×40 magnification with semi-quantitative scoring. For each patient, three randomly selected fields in non-consecutive sections (five for each tissue) were analyzed. Dye intensity was scored as weak (1+), medium (2+), and strong (3+) immunolabelling. Weak staining (1+) was used for preparation only selectable of primary antibody under ×40 microscope magnification, moderate staining (2+) was used for preparation only selectable of primary antibody under ×20 microscope magnification, and strong staining (3+) was used for preparation only selectable of primary antibody under ×40 microscope magnification [22]. Staining intensities 2 and 3 were considered as positive staining of the expression of *BAP1*, *OGT*, and *YY1*. Representative fields from each section were digitally photographed under light microscope (Olympus BX 51, Japan). All images were captured using the same light filter settings. While cell nuclear staining in the anterior stroma adjacent to the invasive tumor and within the tumor center, was regarded as positive for BAP1 staining, cytoplasmic positivity was evaluated as a non-specific reaction. The staining scoring of *YY1* and *OGT*, cytoplasmic and nuclear expressions was based on positive staining in the nucleus and cytoplasm or both. For each patient, at least 500 cells in 5–10 representative high power fields were evaluated as previously described [23]. BAP1, OGT, and YY1 immunostaining were evaluated both in stroma adjacent to the invasive BCC tumor anterior and within the BCC tumor center. The percentage of positively stained cells was calculated.

Statistical analysis

Data obtained in the study were analyzed statistically using SPSS vn. 22.0 software (IBM Corp., Armonk, NY, USA). Data showing normal distribution were evaluated using analysis of variance, Tukey's test, and the RT-PCR data were evaluated with the significance test of the difference between two means, the Student's t-test. The error level was taken as 0.05. Data not conforming to normal distribution were evaluated with the Kruskal-Wallis, Mann-Whitney U, and X^2 tests. The error level was taken as 0.05. The immunochemistry data were analyzed using the GraphPad Prism 7 (GraphPad Software, CA, USA) program. Conformity of the data to normal distribution was assessed with the D'Agostino-Pearson omnibus normality test. Results were presented as mean ± standard deviation (SD) values.

Results

Patient information

Evaluation was made of 12 patients diagnosed with eyelid tumor, with no restriction in terms of gender, age, or

histopathological type and grade of cancer. The histopathological type of the tumor was added to the data based on the pathology report of the tissue sample taken from the patients during surgery (Table 1). The characteristics of the patients with eyelid tumors, such as gender, age range, mean age, family history of cancer, and sun exposure, are presented in Table 1. The patients comprised 8 (66.7%) males and 4 (33.3%) females with a mean age of 65.30 ± 13.31 years (range, 50–88 years). Family history of cancer was determined in 6 (50.0%) patients and long-term exposure to the sun because of occupation was determined in 6 (50.0%) patients (Table 1).

Analysis of *BAP1*, *OGT*, and *YY1* genes expression results

The expression levels of the *BAP1*, *OGT*, and YY1 genes in the eyelid tumor and control tissues were analyzed using

the RT-PCR method. *GAPDH* was used as a housekeeping gene. The expression levels of working genes were normalized with *GAPDH*. The statistical analysis of the RT-PCR results was performed using the RT2 profiler RT-PCR Array Data Analysis version 3.5 software, Rotor-Gene 6000 software. The data obtained from the experiment were analyzed based on the ΔΔCt method. The RT-PCR data were evaluated using the Student's t-test as the significance test of the difference between the two means.

A 1.84-fold increase was observed in the *BAP1* gene expression levels in the tumor tissue group, which was statistically significant (p = 0.005). A 2.85-fold increase was observed in the *OGT* gene expression levels in the tumor tissue group, which was statistically significant (p = 0.002). There was a 3.06-fold increase in the *YY1* gene expression levels in the tumor tissue compared to the control group, which was statistically significant (p = 0.001). All the differences in the expression levels of all the genes evaluated in this study were statistically significant (Figure 1).

Table 1: Demographic information of cases with eyelid tumors.

	Cases with BCC n (%)
Sample size	12
Sex	
Males	8 (66.7)
Females	4 (33.3)
Age, years	
Range	50–88
Mean ± SD	65.30 ± 13.31
Histopathological type	Basal cell carcinoma
Family history of cancer	6 (50.0)
Exposure to the sun	6 (50.0)

Analysis of BAP1, OGT, and YY1 plasma protein levels results

The characteristics of patients with eyelid tumors and the control group, such as gender, age range, and mean age, are presented in Table 2. The patient group comprised 8 (66.7%) males and 4 (33.3%) females with a mean age of 65.30 ± 13.31 years (range, 50–88 years). The control group comprised 8 (66.7%) males and 4 (33.3%) females with a mean age of 65.66 ± 12.17 years (range, 51–87 years) (Table 2).
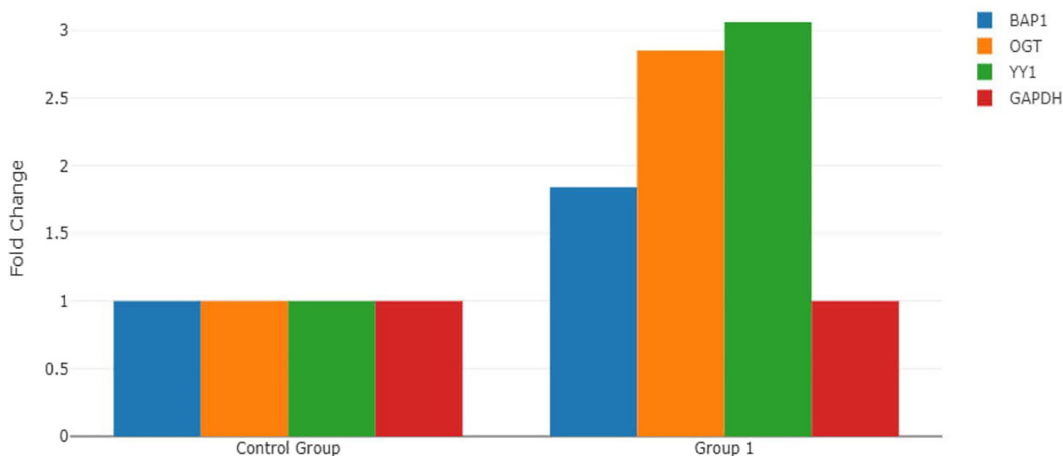

Figure 1: Comparison of the expression levels of *BAP1*, *OGT* and *YY1* genes in the tumor group and the control group.

Table 2: Demographic information of eyelid tumor cases and controls.

	Cases	Control
Sample size	12	12
Age, years		
Range	50–88	51–87
Mean ± SD	65.30 ± 13.31	65.66 ± 12.17
Sex		
Males	8 (66.7)	8 (66.7)
Females	4 (33.3)	4 (33.3)

The BAP1, OGT, and YY1 plasma levels in the patient and control groups were determined. The BAP1 levels in the patient group were found to be statistically significantly higher than those in the control group (p = 0.001). The OGT plasma levels were found to be similar in the patient and control groups (p = 0.319). The YY1 levels were statistically significantly higher in the patient group than in the control group (p = 0.008) (Table 3).

Pearson correlation analysis was applied to find relationships between variables. The correlation coefficients (correlations) between BAP1, OGT and YY1 in the control group were found to be very small and statistically insignificant (p > 0.05) (Table 4). In the patient group, there was determined to be a statistically significant positive relationship between BAP1 and YY1 (r = 0.850, p < 0.05). A statistically significant positive correlation was determined between BAP1 and OGT (r = 0.811, p < 0.05). A statistically significant positive correlation was determined between OGT and YY1 (r = 0.755, p < 0.05). The correlation coefficients were high in the correlation measures for all three proteins.

Using ROC curve analysis, the area under the curve (AUC), sensitivity and specificity values were calculated for cut-off points of BAP1, OGT, and YY1 protein levels. The cut-off point for BAP1 was 206.00 mg/L (95% confidence interval (CI): 0.746–1.000), OGT was 821.51 ng/L (95% CI: 0.393–0.857) and YY1 was 2.40 ng/mL (95% CI: 0.632–0.993). The cut-off points, and the sensitivity, specificity, and AUC values of those cut-off points are shown in Table 5. The ROC curves of the levels of *BAP1*, *OGT*, and *YY1* in patients with eyelid tumors are shown in Figure 2.

Analysis results of BAP1, OGT, and YY1 proteins expression

Immunohistochemical evaluation was performed on the 12 tissue samples of the patient group diagnosed with BCC. In immunohistochemical evaluation, it is known that the staining patterns of tissues taken from different patients may be different. Therefore, BAP1, OGT, and YY1 protein expressions in the tissue samples diagnosed with BCC in this study, were comparatively evaluated with the control
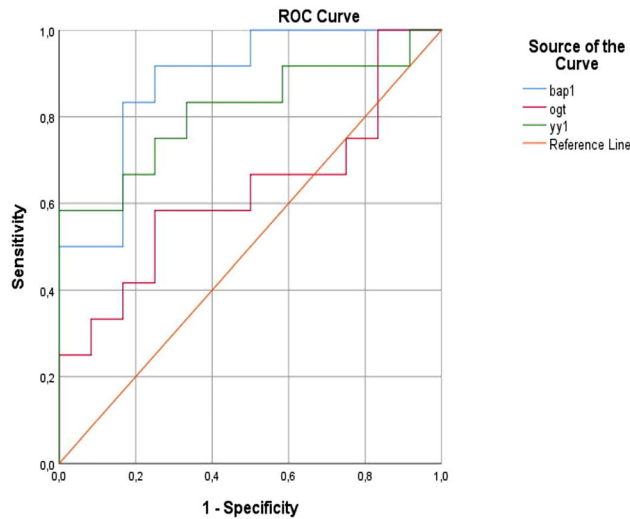
Table 3: BAP1, OGT and YY1 protein levels between groups.

	Case median (Min–Max)	Control median (Min–Max)	p-Value
BAP1, mg/L	875.50 (134.00–4.130.00)	171.54 (20.00–446.00)	**0.001**
OGT, ng/L	1,405.19 (630.67–5.728.67)	818.33 (509.67–1.313.67)	0.319
YY1, ng/L	4.22 (1.43–10.93)	2.05 (1.32–2.78)	**0.008**

p < 0.05 value is statistically significant. Bolded *p* values are significant.

Table 4: Correlations of BAP1, OGT and YY1 levels between groups.

		Cases			Control		
		BAP1	**OGT**	**YY1**	**BAP1**	**OGT**	**YY1**
BAP1	r	0.850**	1	0.755**	−0.023	1	0.392
	p	−	0.001	0.001	−	0.943	0.771
OGT	r	0.811**	0.755**	1	−0.094	0.392	1
	p	0.001	−	0.005	0.943	−	0.208
YY1	r	1	0.850**	0.811**	1	−0.023	−0.094
	p	0.001	0.005	−	0.771	0.208	−

**Correlation coefficients significant at $\alpha = 0.01$.

Figure 2: ROC curves of the levels of BAP1, OGT and YY1 in patients with eyelid tumors.

tissues. In tumor areas with BCC, BAP1 immunolocalization was observed to be positive at different intensities in tumor cells (Figure 3A–B arrow). OGT immunolocalization in BCC was observed in D–E and F control samples (Figure 3). The immunoreactivity of OGT protein in the BCC samples was expressed in tumor cells but the staining intensity was weak. YY1 (G–H) immunolocalization in BCC patient tissues was observed to be positive at different staining intensities in tumor cells (Figure 3: G–H arrowhead).

The staining intensity of BAP1, OGT and YY1 protein expression

The staining intensity of protein expression in BCC and control tissues was determined. The BAP1 protein staining score in the BCC tumor areas was high (Figure 4, p = 0.0001). The OGT protein was observed to be expressed at a much lower rate compared to BAP1 protein in BCC tumor areas (p = 0.182). The YY1 protein was observed to be expressed similarly to BAP1 in BCC tumor areas (Figure 4, p = 0.0001).

Discussion

BCC is the most common malignant tumor of the eyelid and accounts for 80% of all malignant eyelid tumors [1, 24]. The progression of BCC is slow, and metastasis to distant tissues is rare [25]. There is limited information on how changes in gene expression are related to eyelid BCC pathogenesis, so it is important to examine the molecular mechanism underlying carcinogenesis at the gene profile level [26]. Therefore, the expression levels of *BAP1*, *OGT* and *YY1* genes and proteins in eyelid tumor tissue with BCC were investigated in this study. When the eyelid tumor tissue with BCC was compared with the control group, the increase in the expression level of *BAP1*, *OGT* and *YY1* genes in the eyelid tumor tissue with BCC was found to be statistically significant. A 1.84-fold increase was determined in the BAP1 gene expression level in the eyelid tumor tissue as a result of RT-PCR analysis. This result may indicate that there is no mutation in the BAP1 gene in tumor tissue with BCC, because, *BAP1* gene mutations, insensitive or frameshift mutations lead to truncated BAP1 protein which lacks the nuclear localization signal, or to decreased protein or mRNA stability, and often leads to loss of nuclear expression [27–29]. Recent studies have shown that germline mutations in the *BAP1* gene cause tumor susceptibility syndrome and new types of cancer, such as mesothelioma, uveal melanoma, renal cell carcinomas, and cutaneous melanoma. Loss of *BAP1* expression has been determined as a result of these mutations [28, 30–35]. In contrast, *BAP1* expression among prostate cell lines has been found to be high in tumorigenic and metastatic cell lines, but low in normal prostate cell lines [36]. Therefore, the literature confirms the *BAP1* gene and BAP1 protein expression results. It can be concluded that BAP1 gene expression may differ in different cancer types. In the current study, a significant increase was determined in protein level and protein expressions due to the increase in *BAP1* gene expression. To the best of our knowledge, this is the first study to have identified BAP1 immunolocalization in BCC carcinoma eyelid tumors. *BAP1* immunolocalization was observed to be strong in tumor areas with BCC. In contrast

Table 5: Cut-off points for BAP1, OGT, and YY1 levels in patients with eyelid tumors of the BCC type, and AUC, sensitivity, and specificity for the cut-off points.

	Cut-off point	Sensitivity, %	Specificity, %	AUC	95% Confidence interval		p-Value
					Lower bound	Upper bound	
BAP1	206.00	83.0	81.0	0.882	0.746	1.000	**0.001**
OGT	821.51	58.0	56.0	0.625	0.393	0.857	0.299
YY1	2.40	75.0	76.0	0.813	0.632	0.993	**0.009**

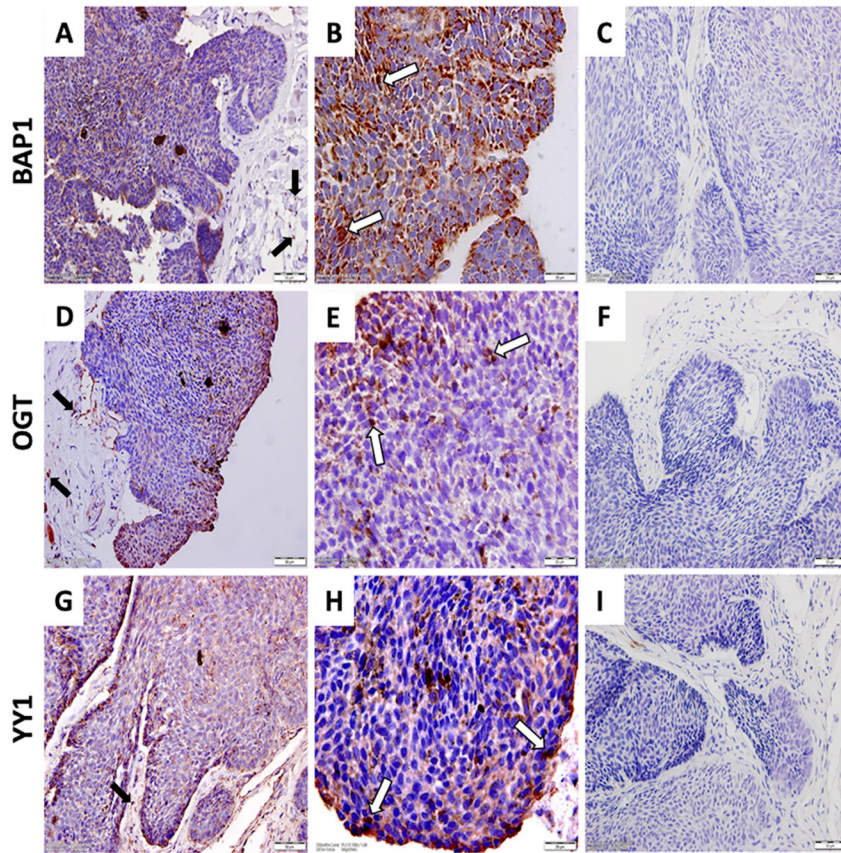*p* < 0.05 value is statistically significant. Bolded *p* values are significant.

Figure 3: BAP1(A–B), OGT (D–E), and YY1 (G–H) immunolocalization in BCC. Negative control (C–F–I). White arrow: positive cells in tumor area and black arrow: positive cells in stromal cells. Chromogen; AEC, contrast dye; Hematoxylin. The intensity of the proteins was determined at ×10, ×20 and ×40 magnification with semi-quantitative scoring.
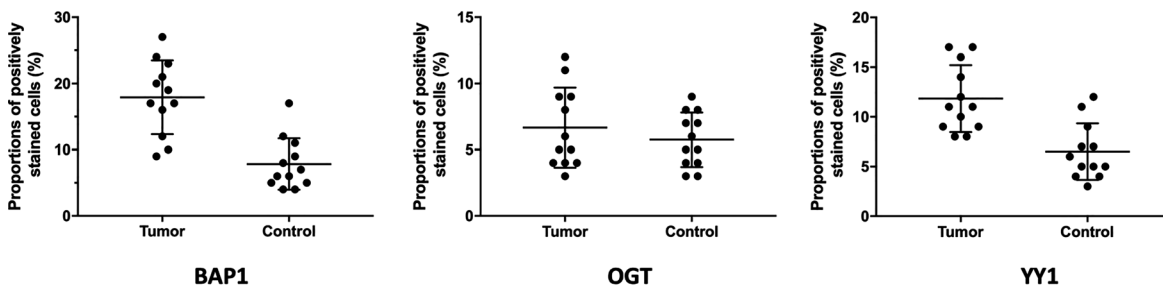

Figure 4: BAP1, OGT and YY1 staining scores in BCC tumor and control areas.

to the current study, loss of nuclear *BAP1* expression has been associated with an increased risk of metastasis and an increased number of T cells and macrophages in dysomia 3 tumors [37–39]. In another study, *BAP1* was found to be frequently mutated in clear cell kidney carcinoma compared to normal kidney tissue and benign kidney tumors. It has been determined that patients with clear cell renal cell carcinoma with low BAP1 expression have a worse prognosis and poorer survival [40]. Moreover, the *BAP1* gene is mutated in about 10% of patients with clear cell renal cell carcinoma, the most common form of kidney cancer, suggesting that *BAP1* is a tumor suppressor [41].

Therefore, the *BAP1* gene and BAP1 protein expression results are consistent with findings in literature.

OGT catalyzes the addition of the O-GlcNAc group to proteins [42]. It has recently been shown that many oncogene and tumor suppressor genes can be acetylated with OGlcNAc, demonstrating the importance of O-GlcNAc glycosylation as a cancer regulator [43]. O-GlcNAc glycosylation has been shown to increase the *in vitro* invasion of breast cancer cells and *in vivo* lung metastasis [44]. It has also been recorded that with increased *OGT* expression, O-GlcNAc glycosylation increases significantly in human lung and colon cancer tissues compared to the relevant

adjacent control tissues [44]. Toivonen et al. reported that the increase in *OGT* gene expression was significantly higher in the patient group than in the control group in postmenopausal women receiving hormone therapy. In another study, it was recorded that O-GlcNAc glycosylation and in parallel to this, *OGT* expression levels, increased in premalignant and malignant skin tumors [45]. However, previous research has reported that augmentation of O-GlcNAcylation and OGT levels are associated with the malignant phenotypes of most cancers including breast [17, 46], colon [44, 47, 48], liver [49–51] and prostate [52, 53]. Similarly, in the current study, the expression level of OGT gene was found to be increased 2.85-fold in eyelid tumor tissue with BCC. Accordingly, OGT protein levels were found to be high in tumor tissue, but not statistically significant. The relationship between OGT protein expression was also examined with the IHC method. It was observed that OGT protein was expressed at a much lower level than BAP1 protein in BCC tumor areas. OGT immunolocalization was found to be weak in tumor regions with BCC. In contrast to the current study, IHC analysis of lung squamous cell carcinoma tissues in a previous study showed high O-GlcNAcylation and *OGT* expression in cancer tissues compared to neighboring non-cancerous tissues [44]. In a similar study, overexpression of *OGT* was associated with prostate cancer progression and recurrence, and O-GlcNAc over-staining as IHC was found to be an independent prognostic factor for poor survival [52, 53]. Xu et al. investigated *OGT* expression levels in colon cancer samples and adjacent normal mucosa as IHC, and reported that in colon cancer tissues, the high *OGT* expression rate was increased compared to neighboring normal tissues [54].

Zinc finger protein Yin Yang 1 (*YY1*) is a transcription factor that regulates multiple genes involved in cell death, cell cycle, cellular metabolism and inflammatory response. *YY1* is overexpressed in many types of cancer, and is associated with cell proliferation, survival, and metabolic reprogramming [55]. Recent studies have revealed that *YY1* expression is irregular in many types of cancer and is mostly associated with clinical behavior in different tumor types [56]. Accordingly, increased levels of *YY1* protein have been reported in prostate [57], breast [58], ovarian cancer [59], and colon cancer [60]. It has also been shown that *YY1*, which helps negatively regulate the expression of E cadherin in breast cancer pathogenesis, is upregulated in cervical cancer [61, 62]. *YY1* is a protein that is expressed in normal and cancer tissues and is found in all tissues, but the expression level is much higher in many cancers. Therefore, *YY1* has been recognized as a potential new prognostic biomarker and therapeutic target [16]. Arribas et al. [63] showed that both mRNA and protein levels of *YY1*

are overexpressed in thyroid cancer cells compared to neighboring normal cells. In addition, IHC analysis of cancer tissue revealed that *YY1* levels are highly expressed in papillary thyroid cancer compared to follicular thyroid cancer [64]. Similar to those studies, in the current study, a 3.06-fold increase was observed in *YY1* gene expression levels of eyelid tumor tissue with BCC. Accordingly, YY1 protein level was found to be higher in tumor tissue compared to the control samples. Finally, the relationship was examined between eyelid tumors and YY1 protein expression, and *YY1* immunolocalization was found to be moderate in tumor areas with BCC. In contrast to the current study, Zaravinos et al. showed that *YY1* expression was decreased in pediatric osteosarcomas and melanomas [65].

Limitations

The main limitation of this study was the small sample size. According to the most recent Turkish Ministry of Health statistics, 454 cases of eye tumor were reported between 2013 and 2017. The eye tumor incidence in Turkey is much lower than the incidence of other types of tumors. Eyelid tumors constitute a small proportion of the 454 cases [66] (https://hsgm.saglik.gov.tr/depo/birimler/kanserdb/istatistik/Turkiye_Kanser_Istatistikleri_2017.pdf). Therefore, it is to be expected that the number of patients was low since this study was conducted only in the Sivas region. The study was conducted on a total of 12 patients; 8 (66.7%) male and 4 (33.3%) female, who were diagnosed with eyelid tumors within a one-year period (Table 1). Within the scope of the study, BAP1, OGT and YY1 protein levels were determined using the ELISA method. Protein expression was determined with the IHC method. However, Western blot analysis could not be performed due to the limited budget of this study project.

Conclusions

It was observed that *BAP1*, *OGT* and *YY1* gene expression levels were significantly higher in patients with eyelid tumors compared to the control group. In addition, plasma BAP1 and YY1 protein levels and protein expression in tissue were found to be higher in these patients compared to the control group. In order to determine the risk factors in the formation of eyelid tumors with BCC, more comprehensive studies with more patients are needed to obtain meaningful results. As a result of obtaining more significant values, BAP1, OGT and YY1 gene and protein products, which are of great importance in various tumors,

can be recommended as biomarkers in the diagnosis of such diseases.

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Competing interests: The authors declare no competing interests.

Ethical approval: Approval for the study was granted by the Local Ethics Committee (decision no: 2014-05/18) and all procedures were applied in compliance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines. Consent forms were obtained from all patients within the scope of the study.

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