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Clinical Research

The Relationship between the Levels of SIAH1, BIM and p53 Proteins in association with the Disease Course in Patients with Breast Cancer

Luay T. H. Alsaleem Agha[1*]

[1]*Yeditepe University, Graduate Institute of Health Sciences, Department of Molecular Medicine, Istanbul, Turkiye.*

Abstract

Cancer is one of the most significant health issues worldwide, and breast cancer is among the most common types. Despite advancements in treatment methods, breast cancer remains difficult to treat effectively. Therefore, identifying new biomarkers for the diagnosis and treatment of breast cancer is of great interest. The SIAH1 (Seven in absentia homolog 1) protein is recognized as a tumor suppressor. Although studies have demonstrated its crucial roles in cancer processes, its potential in breast cancer diagnosis and treatment has not been fully explored. Previous research has shown that SIAH1 is linked with important tumor suppressor and apoptosis-inducing proteins such as p53 and BIM. In this thesis, we used immuno-histochemical methods to investigate the relationship between the levels of SIAH1, BIM, and p53 proteins, which play key roles in the prognosis and apoptosis processes in breast cancer patients. Our results showed that the level of SIAH1 protein was significantly reduced in the tumor tissues of breast cancer patients. In contrast, there was no significant difference in the expression level of BIM. Additionally, p53 was not detected in 75% of the breast cancer patients, but in the remaining 25%, p53 expression was significantly increased. Our study also indicated that low SIAH1 expression levels are associated with important clinical characteristics such as tumor grade, stage, and breast cancer type. These findings suggest that SIAH1 plays a role in breast cancer formation and could be a novel biomarker for the diagnosis and treatment of breast cancer.

Key Words: *Apoptosis, BIM, Breast Cancer, Immunohistochemistry, SIAH1, p53*

Corresponding author: Luay T. H. Alsaleem Agha, E-mail: loaytalal1983@gmail.com, ORCID ID: 0000-0002-9423-7687

Introduction

As breast cancer is a heterogeneous malignancy with a wide variety in genetic alteration, there is a challenge in diagnosis and treatment. Therefore, the detection of cancer biomarkers can play a necessary role in the administration of invasive breast cancer patients (1). Many clinical studies have shown that some proteins are utilizable not only for a targeted therapy, but also as a biomarker for early detection of cancer (2-5). It means that biomarkers will be important in the therapy and diagnosis of cancer. Common therapeutic strategies such as immunotherapy and chemotherapy are depend on inducing apoptosis to remove abnormal cancerous cells (6-8). Recently, resistance to these therapeutic approaches is increasing resulting in cell survival and cancer progress (9-11). Therefore, it is necessary to identify novel biomarkers in breast cancer cells. In several decades, numerous abnormal expressions of signaling molecules of apoptosis have been known in breast cancer. BIM and p53 are one of the most important proteins in breast cancer cells (12-17). On the other hand, the ubiquitin ligases proteins such as SIAH1 play a pivotal role in BIM and p53 mediated apoptosis (18, 19).

SIAH1 protein is known as a tumor suppressor protein by ubiquitination and degradation of some specific proteins. The human genomic deletion of SIAH1 (a null-SIAH1 human genome) causes some malignancy such as hepatocellular carcinoma (19-21). SIAH1 plays an important role in cell proliferation and apoptosis. Previous studies have shown that up-regulation of SIAH1 gene expression causes an increase in the expression of BIM protein through JNK signaling pathway (22). The JNK pathway initiates SIAH1-associated apoptosis not only by halting the cell cycle but also by upregulating the expression of BIM (18). According to previous researches, the inhibition of SIAH1 was associated with the increased invasion of breast cancer cells and activated ERK signaling pathway that is dependent on BIM. Indeed, the activity of the SIAH1 protein may play a key role in the regression of breast cancer by increasing the expression of the BIM protein (22).

Induction of p53 can lead to cell cycle arrest, apoptosis or senescence in response to cellular stresses. In overall, p53 transcriptional activity induces several pathways, but the molecular components mediating p53 activation determine cell fate toward cell death or survival (23-25). For example, Siah1a (isoform of SIAH1) and Pw1/Peg3 have the potential to cooperate in the p53-mediated cell death pathway whereas each of Pw1/Peg3 or Siah1a

individually has no such effect (26). The co-activation of p21 with p53 leads to G_1 phase cell cycle arrest as well as cellular senescence (27, 28). Recent studies have revealed that SIAH1 appears to be one of downstream mediators of p53-mediated apoptosis. The overexpression of p53 leads to transcriptional activation of SIAH1 family genes in different mammalian cell lines and overexpression of SIAH1 can trigger apoptosis, simulating the effects of p53 activation (29, 30). Nevertheless, there is insufficient clinical evidence to determine whether the co-activation of SIAH1 and p53 relies on BIM expression. The involvement of SIAH1, BIM, and p53 proteins in the development of breast cancer remains largely unexplored. Taken together, we hypothesized that the relationship between the levels of SIAH1, BIM, and p53 proteins may play key roles in the prognosis and apoptosis processes in breast cancer patients. Therefore, in this study, we conducted immunohistochemical method in order to investigate the potential interaction between SIAH1, BIM, and p53 proteins in 58 breast cancer tissue samples.

2. Materials and methods

2.1. Study samples and analysis method

The samples used in the present study were obtained from the archive of the department of medical pathology, faculty of medicine, Gaziantep University. Breast cancer tissues of 58 patients and their adjacent healthy breast tissue samples were included in the present study. These paraffin embedded tissue samples were subjected to protein analysis by immunohistochemical method. All the experiments were done following a protocol approved by the Ethics Committee of Gaziantep University.

2.2. Antibodies

In this study, the primary antibodies anti-p53 (MA5-14516), anti-SIAH1 (PA5-29682), and anti-BIM (PA1-84838), all from Invitrogen, Thermo Fisher Scientific, Inc., were used. Additionally, a goat anti-rabbit secondary HRP antibody (31466) from the same manufacturer was employed.

2.3. Immunohistochemical Staining

For pathological examinations, formalin fixed paraffin-embedded (FFPE) breast cancer tissue samples, pre-stained with hematoxylin eosin, were examined again. Immuno-histochemical staining was achieved by using DAKO Autostainer Universal Staining System (Autostainer Link 48 DAKO, Glostrup, Denmark). Briefly, sections with a thickness of 4μm were taken into positively charged microscope slides. The sections deparaffinized with xylol were then subjected to the dehydration process by repeated ethyl alcohol treatments.

Subsequently, antigen retrieval was performed at 96 ° C (10mM citrate buffer, pH 6) for 40 min in a thermostatic bath (PT Link). Sections were further incubated with 1/200 diluted concentrations of anti-p53 (MA5-14516; Invitrogen, Thermo Fisher Scientific, Inc.), anti-SIAH1 (PA5-29682; Invitrogen, Thermo Fisher Scientific, Inc.) and anti-BIM (PA1-84838; Invitrogen, Thermo Fisher Scientific, Inc.) antibodies for 30 minutes. An automated system was used with the streptavidinbiotin immune-peroxidase technique (K8000 Envision Flex, DAKO, Glostrup, Denmark). Immunological reactions were shown with diaminobenzidine tetrachloride (DAB) to provide a color image. Sections were counterstained with hematoxylin for background staining. Sections were further passed through increasing concentration of 51 alcohol series for dehydration and covered with balsam after clarification with xylol. Following staining, sections were assessed using two blinded methods at 4x, 10x, 20x, and 40x magnifications under a light microscope (Olympus BX51, Tokyo, Japan), in conjunction with clinical data.

At least 200 cells were counted in areas with the highest nuclear staining intensity (hot spots). Staining was graded as follows: 0 for 5% staining, 1 for 5-10% staining, 2 for 11-20% staining, and 3 for staining above 20%.

2.4. Statistical Analysis

The results of the study were evaluated statistically using SPSS and GraphPad Prism package programs. For all outcomes, $p < 0.05$ was considered statistically significant. For analysis of the resulting data Wilcoxon and One Way ANOVA tests were applied.

Results

The characteristics of 58 breast cancer patients are detailed in Table 1. The average age of these patients is 51.91 ± 13.05 years. Among them, 32.8% are under 45 years old, 51.7% are between 45 and 65 years old, and 15.5% are over 65 years old. Regarding breast cancer subtypes, 79.3% of the patients have invasive ductal carcinoma, 19% have invasive lobular carcinoma, and 1.7% have a combination of invasive ductal and lobular carcinoma.

According to the histopathological staging of the patients, 8.6% were stage 1, 36.2% were stage 2, and 34.5% were stage 3. In addition, the histopathologic stage of 20.7% of these patients is unknown. TNM is very important in the detection of breast cancer stages for the determination of appropriate diagnosis and treatment methods. The frequency of patients was determined according to TNM classification. Detailed information about the patients is shown in Table 1.

Table 1: Demographic and clinical characteristics of patients included in the study.

Demographic characteristics			**n=58**	**% n**
Age		<45 (38.47 ± 4.40)	19	32.8
		45-65 (53.66 ± 5.52)	30	51.7
		>65 (74.44 ± 6.44)	9	15.5
Diagnosis		Invasive ductal	46	79.3
		Invasive lobular	11	19.0
		Invasive ductal + lobular	1	1.7
Grade		G1	5	8.6
		G2	21	36.2
		G3	20	34.5
		Unknown*	12	20.7
TNM	T	T1	11	19.0
		T2	33	56.9
		T3	12	20.7
		T4	1	1.7
	N	N0	20	34.5
		N1	13	22.4
		N2	13	22.4
		N3	11	19.0
	M	Mx	57	100.0

*There is no staging in invasive ductal carcinoma

Immuno-histochemical determination of SIAH1 protein expression level in breast cancer patients

The expression level of SIAH1 protein was determined immunohistochemically normal and tumor tissue samples of breast cancer patients. The scoring showing the expression level of SIAH1 protein according to histopathological classification of breast cancer and clinical and demographic characteristics of patients is shown in Table 2.

Table 2: Representation of the SIAH1 protein expression levels according to patient characteristics.

Characteristic			**SIAH1-normal**	**SIAH1-tumor**	**P value**
Age	<45		4.36 ± 2.33	3.73 ± 1.82	0.175
	45-65		4.16 ± 2.03	3.96 ± 2.12	0.415
	>65		5.4 ± 2.06	4 ± 1	0.062
Diagnosis	Invasiveductal		4.3 ± 2.02	3.69 ± 1.54	**0.0271**
	Invasivelobular		5 ± 2.75	4.72 ± 2.8	0.500
	Invasive ductal+ lobular		4	4	1
Grade	G1		4.6 ± 2.4	4 ± 2.34	0.5
	G2		4.23 ± 1.57	3.19 ± 1.12	**0.002**
	G3		4.3 ± 2.4	4.15 ± 1.63	0.769
TNM	T	T1	4.63 ± 2.06	2.72 ± 1.55	**0.001**
		T2	4.51 ± 2.16	4.24 ± 1.83	0.403
		T3	3.83 ± 2.16	3.75 ± 1.65	0.999
		T4	3	3	1
	N	N0	4.4 ± 1.87	3.95 ± 1.60	0.300
		N1	5.23 ± 2.62	4.3 ± 2.59	**0.031**
		N2	4.23 ± 2.04	3.92 ± 1.49	0.562
		N3	3.45 ± 1.80	2.9 ± 1.13	0.25
	M	Mx	4.43 ± 2.15	3.89 ± 1.87	**0.014**

According to the results obtained after the analysis, it was shown that the expression level of SIAH1 protein was lower in tumor tissues compared to normal tissues in all parameters. In addition, the expression levels of SIAH1 protein in invasive ductal carcinoma was significantly lower in tumoral tissue compared to normal tissues. In addition, SIAH1 protein expression was found to be significantly lower in stage 2 patients in histopathological classification. Expression levels of SIAH1 protein was found to be significantly diminished in the tumoral tissues of breast cancer patients as compared to normal tissues (p=0.0078) (Figure 1).

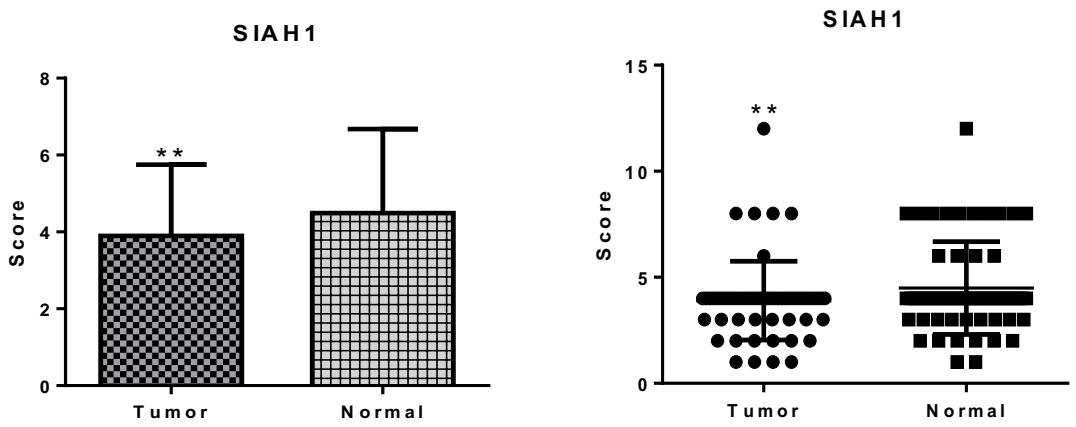

Figure 1. Expression level of SIAH1 protein in normal and tumor tissues of breast cancer patients. ** p=0.0078

In this study, the expression level of SIAH1 protein was determined in a total number of 58 patients who were diagnosed with breast cancer. In Figure 2, SIAH1 expression levels of breast cancer patients were shown according to age (<45, 45-65, >65). When SIAH1 protein levels compared with the age distribution, it was found to be diminished in tumor samples. In addition, the expression level of SIAH1 protein was found to be higher in patients over 65 years of age than in any other age groups.

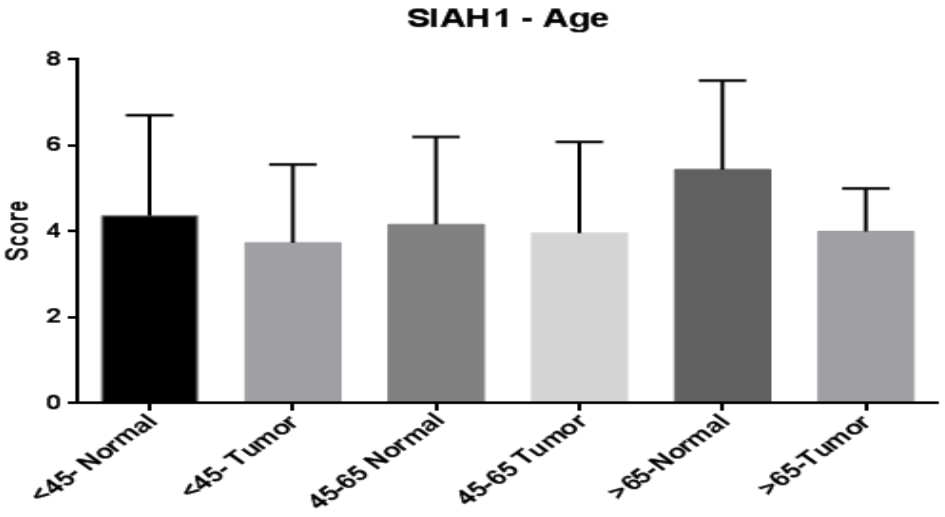

Figure 2. Expression levels of SIAH1 protein according to different age groups.

Moreover, expression level of SIAH1 protein was shown according to the invasive ductal and lobular subtypes of breast cancer. Particularly, expression level of SIAH1 protein was found to be significantly decreased in breast cancer patients with invasive ductal carcinoma. In addition, SIAH1 protein expression levels were also found to be diminished in invasive lobular carcinoma patients, yet this change was statistically insignificant (p=0.0271) (Figure 3). Statistical evaluation was performed for the invasive both ductal and lobular subtypes due the presence of only one patient.

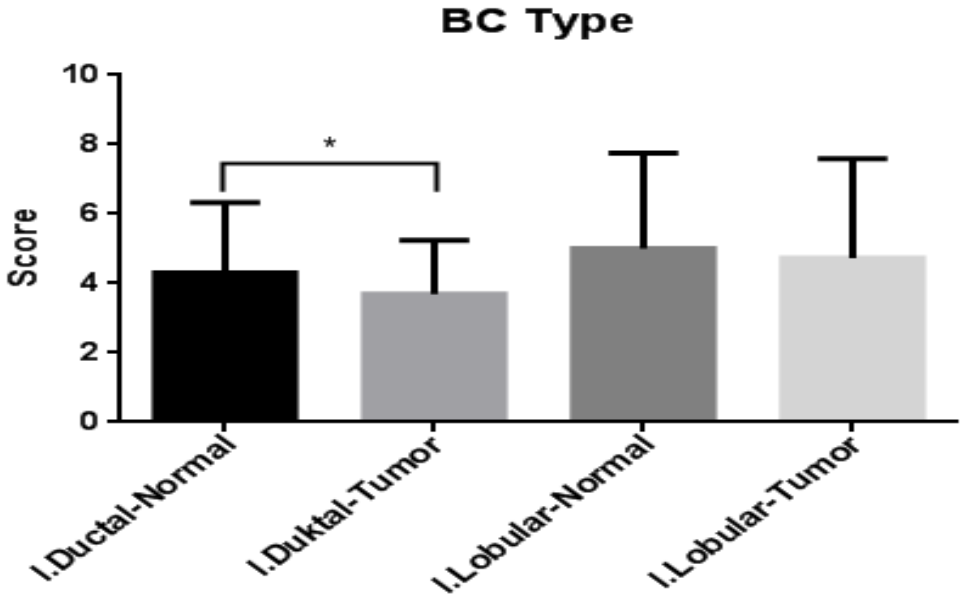

Figure 3. SIAH1 protein expression levels according to breast cancer diagnosis. * p=0.0271

Expression level of SIAH1 protein was shown according to the G1, G2 and G3 histopathological grading of breast cancer patients (Figure 4). Similar to other clinicopathological features of breast cancer patients, decreased expression level of SIAH1 was also found to be well-correlated with the histopathological grading. While decreased SIAH1 protein expression was insignificant in G1 and G3 patients, SIAH1 protein levels were found to be significantly diminished in G2 patients (p=0.002).

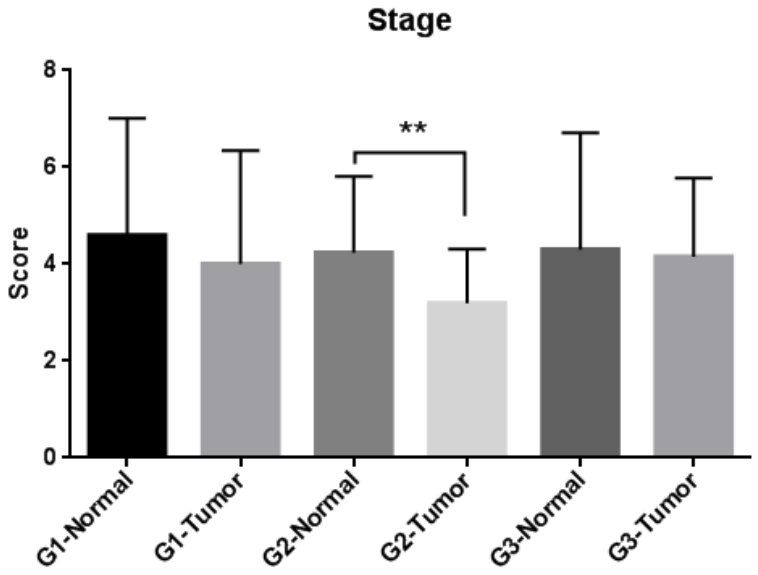

Figure 4. Expression levels of SIAH1 protein according to the histopathological grading. ** p=0.002

SIAH1 protein expression level was also assessed according to the tumor size (T), nodule (N) and metastasis (M) parameters of TNM staging of breast cancer patients (Figure 5). When the SIAH1 expression level was assessed according to the tumor size, it was found to be significantly decreased in tumor tissues compared to normal tissues in the T1 group (p=0.001). In addition, SIAH1 expression was also found to be decreased in tumor tissues compared to normal tissues in T2 and T3 groups. However, these changes were not statistically significant. Statistical analysis was not performed because there was only 1 patient in the T4 group.

Moreover, when the SIAH1 expression levelwas assessed according to the nodule, the expression level of SIAH protein in tumor tissues was significantly lower than normal tissues of patients in N1 group (p=0.0313). However, reduced expression level of SIAH1 protein was not statistically significant in the other N groups.

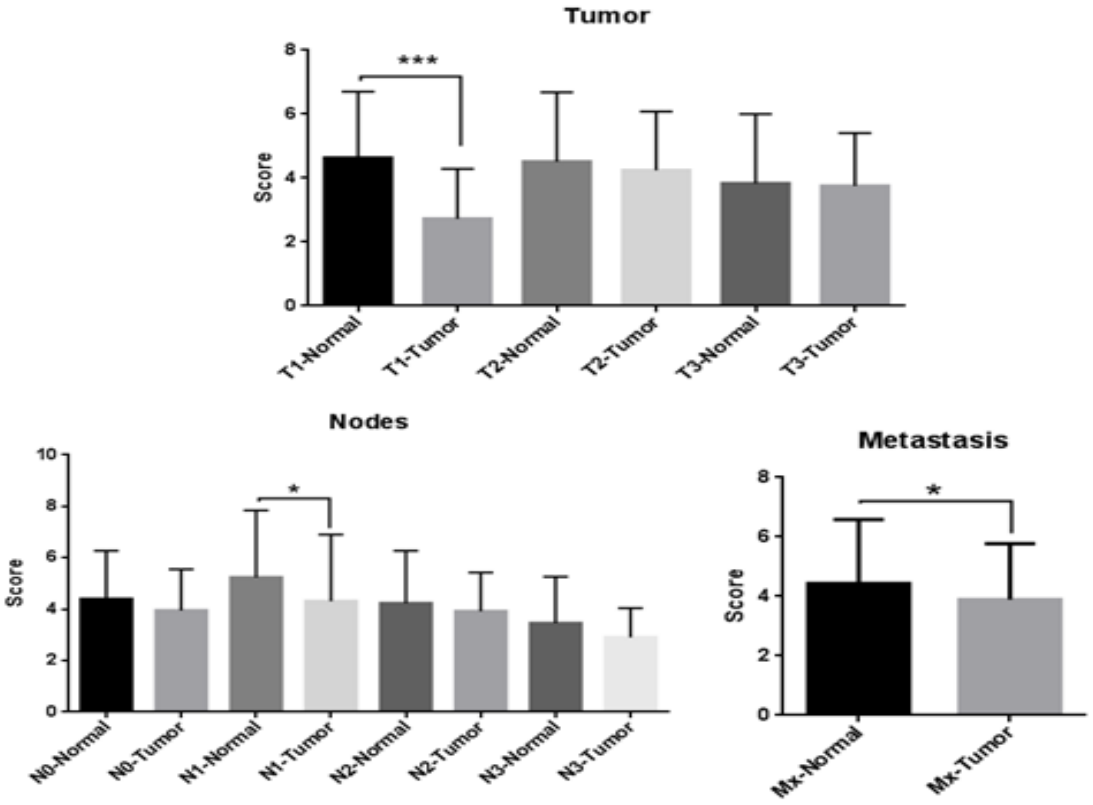

Figure 5. Expression levels of SIAH protein according to TNM staging.

Immuno-histochemical determination of BIM protein expression level in breast cancer patients

The expression level of BIM protein was determined immunohistochemically in normal and tumoral tissue samples of breast cancer patients. The scoring showing the expression level of BIM protein according to histopathological classification of breast cancer and clinical and demographic characteristics of patients is shown in Table 3. As a result, in the expression level of BIM protein, there was no statistically significant difference in tumor tissues compared to normal tissues. According to the TNM staging, the expression level of BIM protein in the N0 group was found to be significantly increased in tumor tissues compared to normal tissues.

Table 3: Representation of the BIM protein expression levels according to patient characteristics.

Characteristics			**BIM-normal**	**BIM-tumor**	**P value**
Age	<45		3.89 ± 1.04	4.21 ± 1.93	0.354
	45-65		3.53 ± 1.04	3.53 ± 1.27	0.843
	>65		3.88 ± 0.33	4.44 ± 1.94	0.500
Diagnosis	Invasive ductal		3.67 ± 0.99	3.89 ± 1.74	0.220
	Invasive lobular		3.81 ± 0.98	3.90 ± 1.30	0.999
	Invasive Ductal + lobular		4	4	1
Grade	G1		4.6 ± 1.34	4.2 ± 1.78	0.500
	G2		3.52 ± 1.03	3.85 ± 1.55	0.101
	G3		3.6 ± 0.75	3.85 ± 1.98	0.591
TNM	T	T1	3.81 ± 1.07	4.09 ± 1.70	0.625
		T2	3.66 ± 0.957	3.91 ± 1.54	0.229
		T3	3.58 ± 1.92	3.5 ± 0.79	0.999
		T4	3	4	1
	N	N0	3.5 ± 0.94	4.3 ± 1.89	**0.0156**
		N1	4.16 ± 0.98	4 ± 1.35	0.750
		N2	3.69 ± 0.85	3.54 ± 1.56	0.812
		N3	3.36 ± 0.81	3.27 ± 1.42	0.999
	M	Mx	3.71 ± 0.97	3.89 ± 1.64	0.178

There was no significant difference in BIM protein expression between normal and tumor tissues in breast cancer patients ($p>0.05$) (Figure 6).

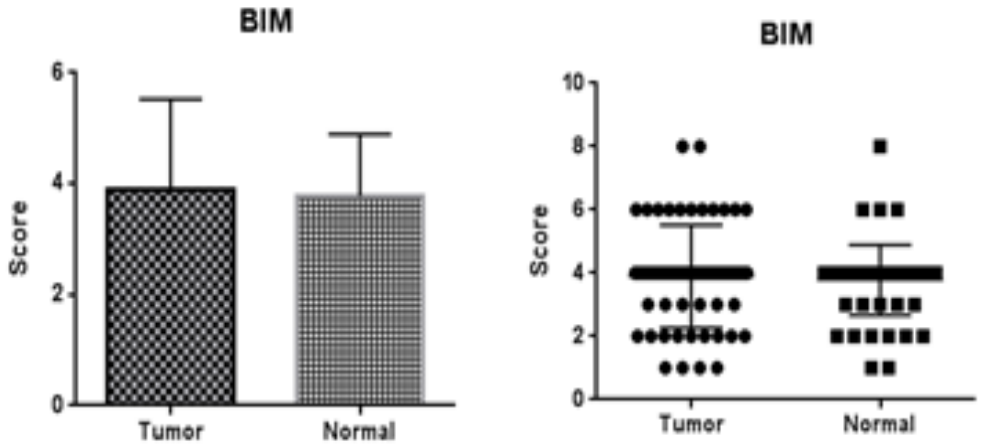

Figure 6. Expression levels of BIM protein in normal and tumor tissues of breast cancer patients.

The expression level of BIM protein was shown according to the age distribution of breast cancer patients (Figure 7). According to the age distribution, BIM protein was found to be differentially expressed in tumor tissues of breast cancer patients as compared to normal tissues. Particularly, expression level of BIM protein was found to be elevated in tumor tissues of breast cancer patients as compared to normal adjacent tissues. Nevertheless, these findings were not statistically significant.

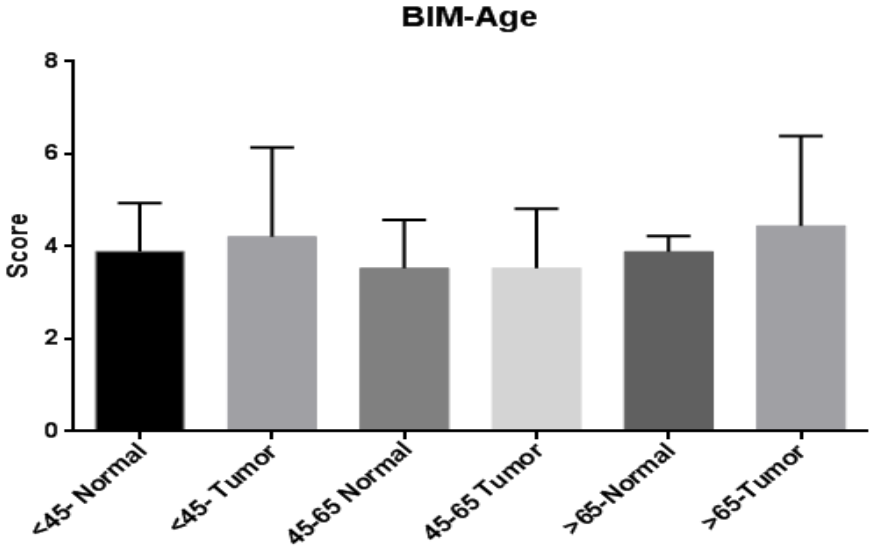

Figure 7. Expression level of BIM protein according to the age distribution.

Moreover, expression level of BIM protein was shown according to the invasive ductal and lobular subtypes of breast cancer as presented in Figure 8 In particular, expression level of BIM protein was found to be increased in tumor tissues of breast cancer patients with invasive ductal and lobular carcinoma. However, these changes were found to be statistically insignificant ($p>0.05$).

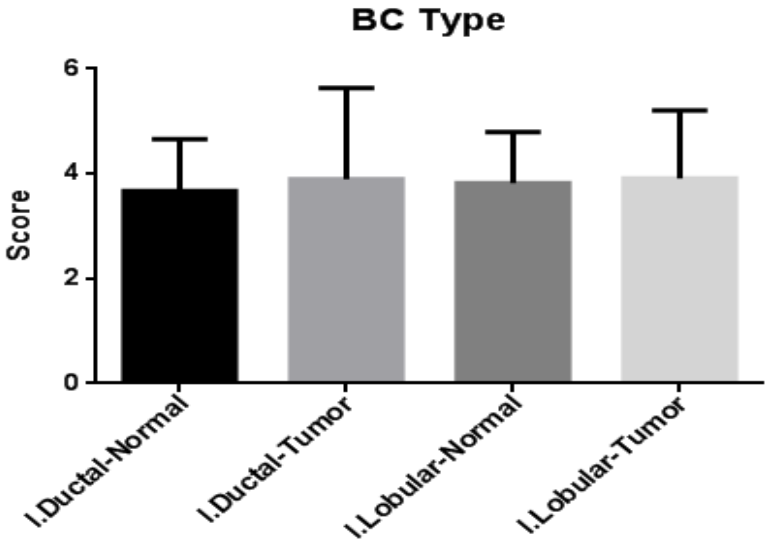

Figure 8. Expression level of BIM protein according to breast cancer diagnosis.

Expression level of BIM protein was shown according to the G1, G2 and G3 histopathological grading of breast cancer patients (Figure 9). Expression level of BIM protein was found to be increased in all tumor grade groups. However, these changes were not statistically significant ($p>0.05$).

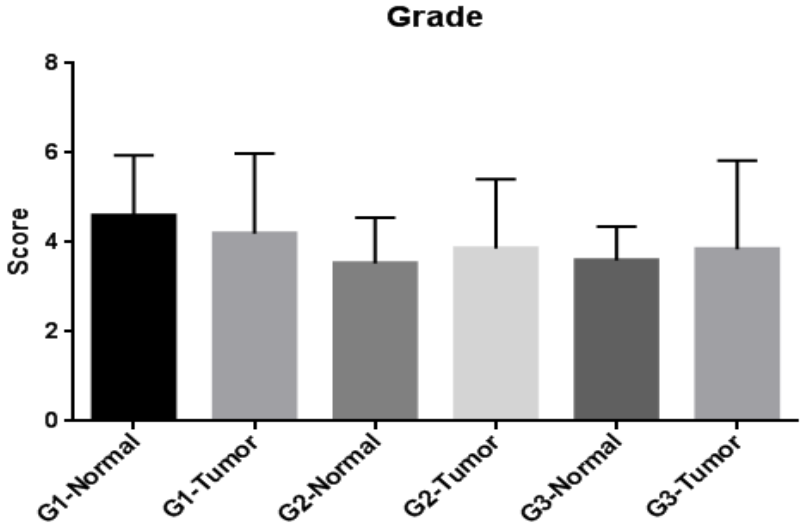

Figure 9. Expression level of BIM protein according to tumor histopathological grade.

The expression level of the BIM protein according to TNM staging in breast cancer tissues is shown in Figure 10. According to the tumor size, there was no statistically significant alteration in BIM protein expression in all T groups. In addition, according to the nodule, the expression level of BIM protein was found to be significantly increased in tumor tissues compared to normal tissues in the N0 group(p=0.0156). However, there is no significant change in other groups of nodules, such as N1, N2 and N3.

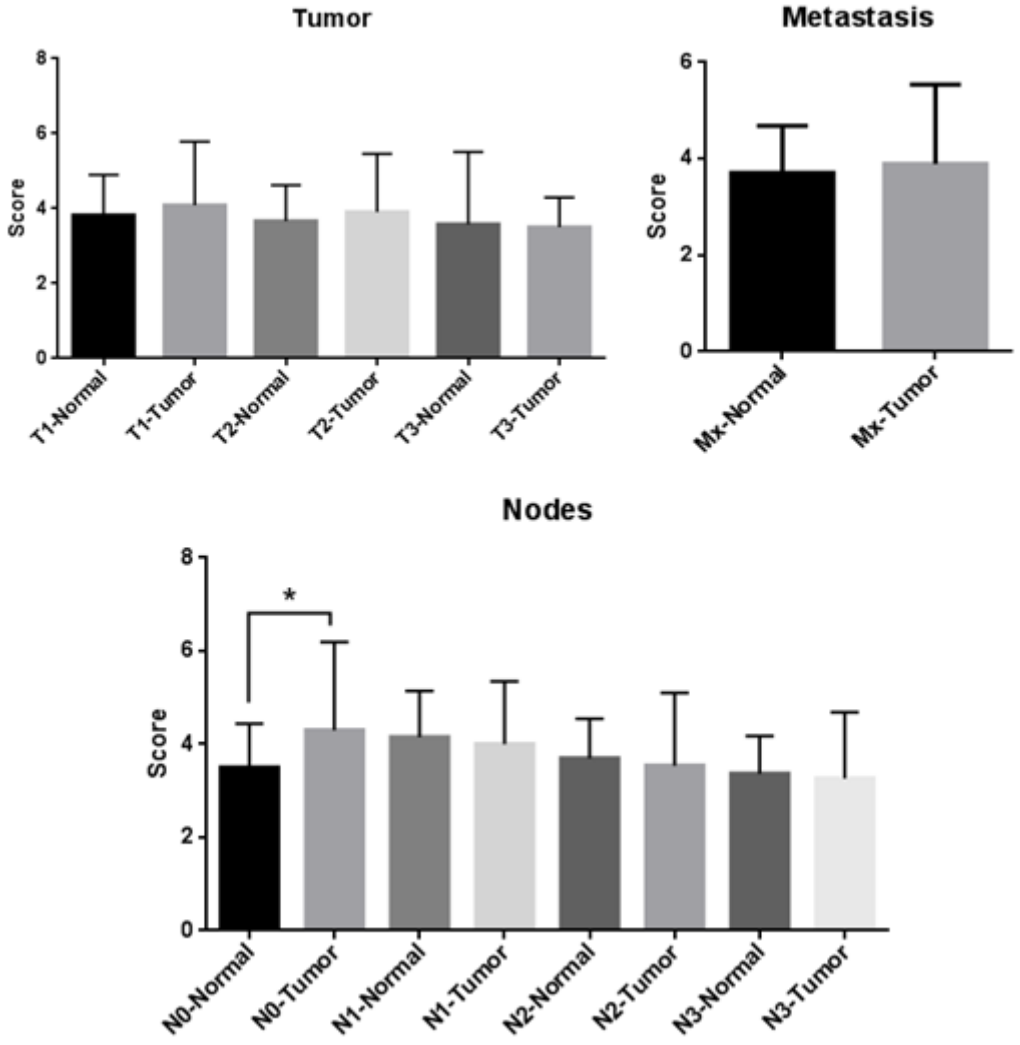

Figure 10. Expression level of BIM protein according to TNM staging.

Immuno-histochemical determination of p53 protein expression level in breast cancer patients

The expression level of p53 protein was determined immunohistochemically in normal and tumor tissues samples of breast cancer patients. The scoring showing the expression level of p53 protein according to histopathological classification of breast cancer and clinical and demographic characteristics of patients is shown in Table 4. As a result of analysis, the expression level of p53 protein, which is a well-known tumor suppressor protein, was found to be significantly increased in tumor tissues compared to normal breast tissues ($p<0.0001$). Additionally, the expression level of p53 protein was found to be increased in tumor tissues of breast cancer patients according to the age distribution, type of breast cancer, histopathological grading and TNM staging.

Table 4: Representation of the p53 protein expression levels according to the patient characteristics.

Characteristic			**P53-normal**	**P53-tumor**	**P value**
Age	<45		0	24.73 ± 35.49	**0.0156**
	45-65		0	11.37 ± 26.14	**0.0313**
	>65		0	5.5 ± 16.6	0.999
Diagnosis	Invasive ductal		0	18.89 ± 31.49	**0.0001**
	Invasive lobular		0	0	1
	Invasive Ductal+lobular		0	0	1
Grade	G1		0	0	1
	G2		0	9 ± 21.98	0.125
	G3		0	33.5 ± 37.31	**0.002**
TNM	T	T1	0	7.27 ± 16.78	0.500
		T2	0	16.56 ± 31.68	**0.0078**
		T3	0	20 ± 32.47	0.125
		T4	0	0	1
	N	N0	0	15.26 ± 31.55	0.125
		N1	0	14.61 ± 28.17	0.125
		N2	0	19.23 ± 31.21	0.0625
		N3	0	10.9 ± 24.67	0.500
	M	Mx	0	15.17 ± 28.97	**<0.0001**

The expression of p53 protein was detected to be lost in 43 breast cancer patients. In addition, p53 protein positivity was detected in 14 patients. Approximately 25 % of the patients included in this study were p53 positive whereas approximately 75% of the patients were p53 negative. In addition, the statistical analysis revealed that the expression level of p53 protein was significantly increased in tumorous breast tissues compared with normal tissues in patients with breast cancer ($p<0.0001$) (Figure 11).

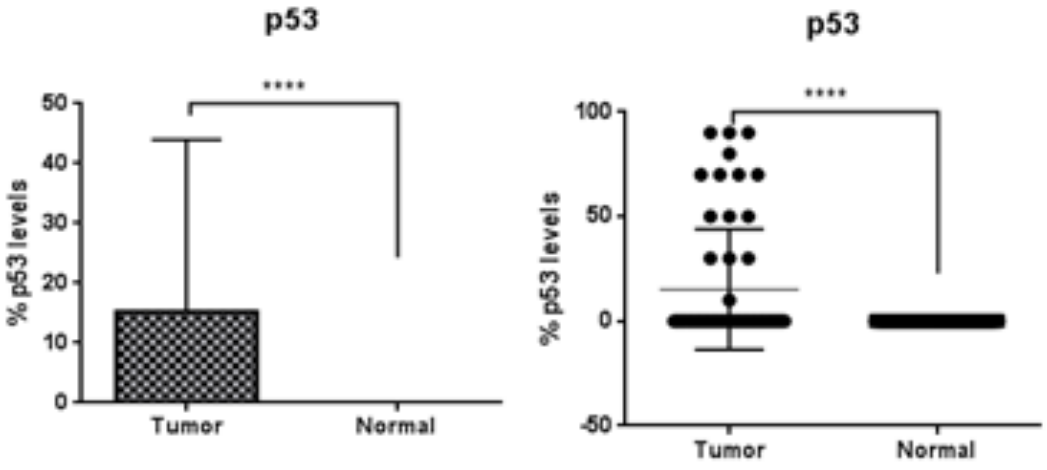

Figure 11. Expression level of p53 protein in normal and tumor tissues of breast cancer patients.

The expression level of p53 protein was shown according to the age distribution of breast cancer patients (Figure 12). According to the age distribution, expression level of p53 protein was found to be significantly increased in tumorous tissues of patients <45 years of age and 45-65 years of age groups. In contrast, no statistically significant increase was observed in patients above 65 years of age. In addition, p53 protein expression was found to be decreased with the increasing age.

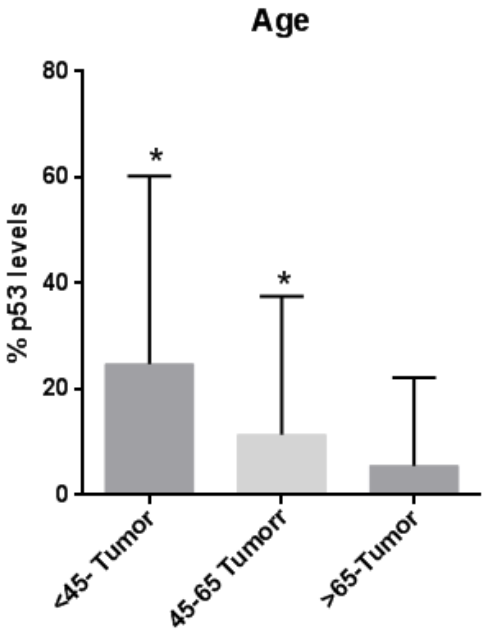

Figure 12. Expression level of p53 protein according to age distribution. *p<0.05

Expression level of p53 was found to be significantly increased in the tumorous tissues of breast cancer patients with invasive ductal carcinoma (p=0.0001) (Figure 13). However, in invasive lobular carcinoma, p53 protein was not detected in normal and tumorous breast tissues.

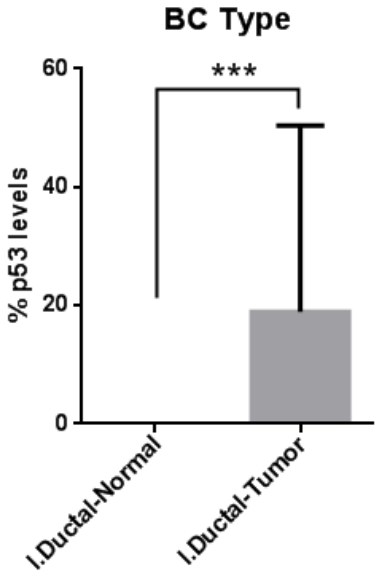

Figure 13. Expression level of p53 protein in invasive ductal carcinoma breast cancer subtype. *** p=0.0001

Expression level of p53 protein was shown according to the G1, G2 and G3 histopathological grading of breast cancer patients (Figure 14). In the G1 group, p53 protein was not detected in the normal tissues of the patients as well as in the tumor tissues. Conversely, expression levels of p53 protein were found to be significantly increased in tumor tissues of patients in groups G2 and G3. Histopathological grading showed the highest p53 expression in the G3 group, but no p53 protein in the G1 group.

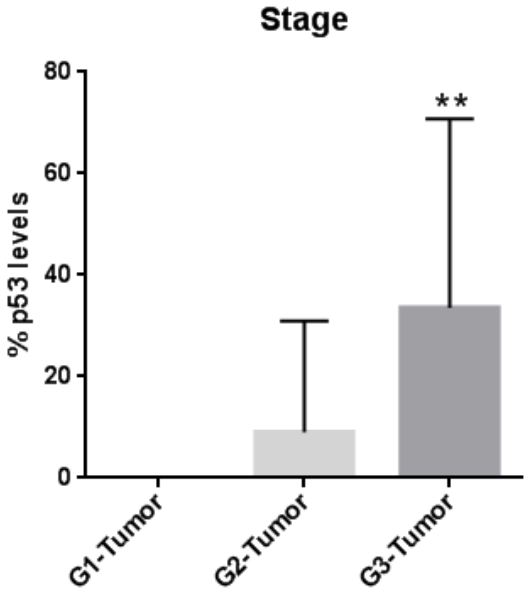

Figure 14. Expression level of p53 protein according to histopathological grading.

The expression level of p53 protein according to TNM staging in breast cancer patients is shown in Figure 15. According to the tumor size, expression level of p53 protein was found to be significantly higher in tumor tissues than that of normal tissues of these patients (p=0.0078). Although expression level of p53 protein was also increased in T1 and T2 groups, this increase was not statistically significant.

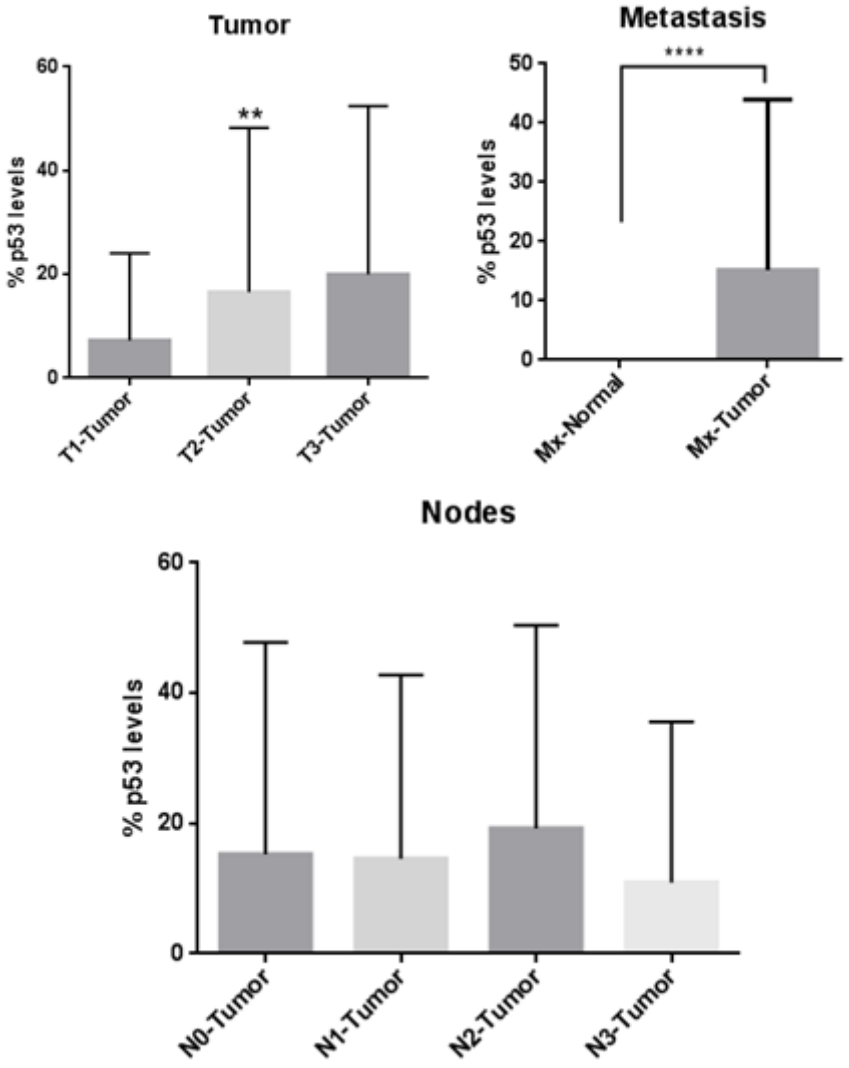

Figure 15. Expression level of p53 protein according to TNM staging. **p=0.0078, ****p<0,0001

On the other hand, in the nodule group, expression level of p53 protein was found to be high in tumor tissues compared to normal tissue. Nonetheless, these changes were not statistically significant.

Expression level of SIAH and BIM proteins according to p53 status in breast cancer tissues

Expression levels of SIAH1, BIM and p53 proteins in archival breast cancer tissues used in the study were explained in detail according to their clinical and demographic characteristics. The changes in expression levels of SIAH1 and BIM proteins according to p53 positive or negative status are shown in Figures 16 and 17, respectively.

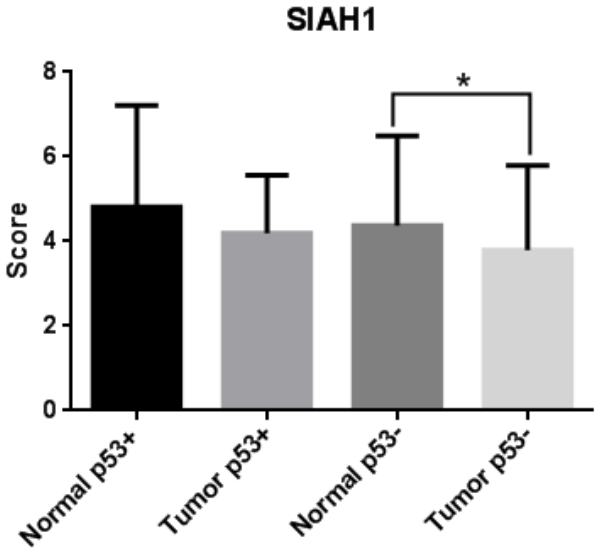

Figure 16. Expression level of SIAH1 protein in breast cancer tissues according to p53 status. *p=0.0134

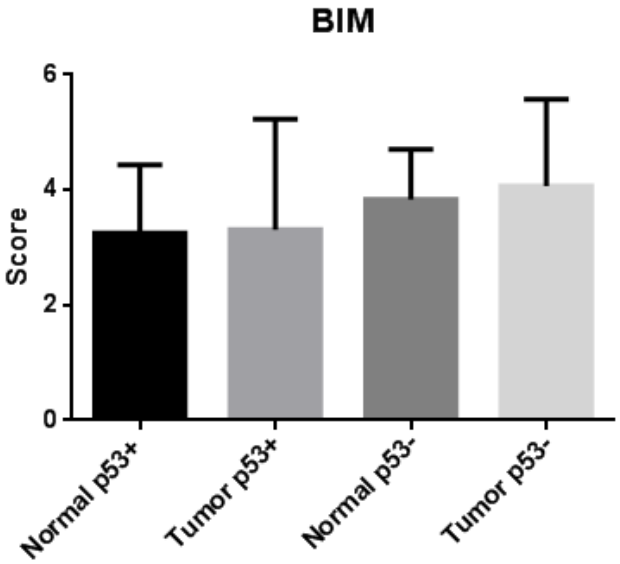

Figure 17. Expression level of BIM protein in breast cancer tissues according to p53 status.

As a result, SIAH1 protein was found to be differentially expressed according to the p53 status. In both cases, the expression of SIAH1 protein was found to be low in tumor tissues compared to normal. Also, expression level of SIAH1 protein was found to be significantly diminished in p53 negative patients (p=0.0134). In addition, SIAH1 protein expression level was found to be diminished in p53 negative patients as compared to p53 positive patients (p>0,05).

Furthermore, BIM protein was also found to be differentially expressed according to the p53 status. BIM expression level was found to be increased in both p53 positive and negative patients. Yet, these changes were found to be statistically insignificant. Also, BIM protein expression was found to be overexpressed in p53 negative patients as compared to p53 positive patients ($p>0,05$).

Discussion

Breast cancer is the leading cause of death in women all over the world, with an annual incidence of 1.3 million cases and nearly 500,000 deaths (31). Since breast cancer treatments remain ineffective worldwide, it is essential to study individual expression profiles in detail across all populations to develop effective, personalized treatment approaches (32). We conducted this study in a certain region of Turkey where consanguineous marriage is more common. At the molecular level, cancer is caused by a series of mutations and/or disorders that occur in genes that play a role in the execution and regulation of vital cellular activities such as cell proliferation, apoptosis, and differentiation. In normal cells, apoptosis is considered as a natural defensive mechanism against cancer development and the resistance of cancer cells to apoptosis is listed as one of the most important hallmarks of cancer. Thus, the basis of the currently used cancer treatment methods is to trigger apoptosis in cancer cells. However, individual expression profiles impact on such type of therapeutic strategies. Clinical studies have shown that the SIAH1 gene is frequently deleted or decreased in breast cancer patients (33). SIAH1 plays an important role in the regulation of biologically significant target proteins including proteins involved in the cell cycle control, apoptosis, and DNA repair by promoting the ubiquitination and proteasome degradation of them. Although SIAH1 recognize common targets, it also has specific targets. SIAH1 is well-known as one of target genes of p53. Also, it was reported that ectopic expression of SIAH1 triggered apoptosis of breast cancer cells through activation of the BIM and JNK signaling pathways (18, 19, 22, 34, 35).

Accordingly, in this thesis study, we aimed to reveal the correlation of SIAH1, BIM and p53 proteins expression, which play important roles in apoptosis, in Turkish breast cancer patients. A total of 58 patients with breast cancer were included in the study. Healthy tissues of patients were used as controls. Protein expression levels were determined by immunohistochemically staining. Our results show that SIAH1, BIM and p53 proteins are differentially expressed in breast cancer tissues. In most cases, SIAH1 protein expression levels were significantly decreased in tumor

tissues of breast cancer patients ($p = 0.0078$). In addition, p53 expression level was found to be significantly increased in some tumor tissues of breast cancer patients ($p < 0.0001$). Approximately 24% of the patients included in the study were p53 positive, and 75% of the breast cancer patients were p53 negative. while, there was no significant difference in expression level of BIM protein between normal and tumor tissues in breast cancer patients ($p > 0.05$). Therefore, non significant correlation was found between SIAH1, BIM expression and p53 proteins (Fig. 3.16 and Fig 3.17). However, SIAH1 seemed a great target for cancer therapy in Turkish population like other ethnicities.

p53 is one of the first discovered and well-known tumor suppressor protein. p53 functions to remove and suppress the proliferation of abnormal cells, thus preventing neoplastic growth. Under normal cellular conditions, the p53 signaling path is in "standby" mode (36, 37) Activation of p53 occurs under various cellular stress conditions. p53 mutation is the most common genetic change identified in human neoplasms. About 50% of human cancers are estimated to have p53 mutations. Mutant p53 proteins are almost always defective for sequence specific DNA binding and activation of the target protein (38, 39). Several studies have identified several independent p53 activation pathways that appear to be dependent on different upstream regulatory kinases. Some of them are ATM, ATR, Chk2 and p14ARF (40-42). In addition, p53 mutation in breast cancer is associated with more aggressive disease and decreased overall survival. Germline mutations of p53 are seen in a high proportion of individuals with Li-Fraumeni cancer susceptibility syndrome which causes an increased risk of breast cancer (37, 41, 43).

This indicates that the loss of p53 activity is a very important factor in breast cancer development. Therefore, expression level and molecular structure of p53 have been extensively studied in breast cancer. In early studies, mutant p53 expression was shown in breast cancer cell lines. Loss of heterozygosity in the p53 gene (LOH) has been shown to be a common event in primary breast carcinomas. Although the overall frequency of p53 mutations in breast cancer is approximately 20%, some types of disease are associated with high frequencies (15, 44). For example, increased rates of p53 mutation in germ-line BRCA1 and BRCA2 mutation carriers have been determined (45, 46). In addition, typical medullary breast carcinomas strikingly show a p53 mutation in most of the cases (47, 48). Hence, the molecular pathological analysis of the structure and expression of

the p53 pathway is of great importance in diagnosis, prognosis, evaluation and treatment of breast cancer. Accordingly, in our study, together with the BIM and SIAH1 protein p53 protein expression levels were also studied. As we briefly mentioned above, expression levels of p53 protein was found to be significantly increased in tumor tissues of breast cancer patients ($p < 0.0001$). However, while approximately 24% of the patients included in the study were p53 positive, 75% of the breast cancer patients were p53 negative. In the other words, p53 activity is lost in the majority of patients.

BIM is a pro-apoptotic member of the BCL2 family of proteins. DNA damaging agents, serum fasting, paclitaxel, and other stimuli can induce apoptosis by inducing BIM expression (49). BIM has the ability to trigger apoptosis in various cells such as hematopoietic, epithelial and neuronal cells (50). Moreover, in a study, SIAH1 expression level was reported to be associated with BIM expression level in breast cancer patients. The expression levels of SIAH1 and BIM proteins were analyzed by immunohistochemically staining method in clinical researches. They found a significant correlation between them and a lowered expression in tumor tissues of 231 breast cancer patients. Additionally, ectopic expression of SIAH1 was shown to stimulate expression of BIM expression and inhibit invasion of breast cancer cells by inducing apoptosis. In contrast, suppression of SIAH1 expression with siRNA transfections was shown to interfere with the BIM expression and increase cell invasion by suppressing apoptosis (18). Therefore, SIAH1 is thought to trigger apoptosis via BIM. However, in our study, no statistical difference in the protein expression level of BIM was detected.

Conclusion

The results of our study showed that SIAH1 has important roles in breast cancer development. However, contrary to our findings, no significant difference was observed in the expression of the BIM protein. This can be due to small number of patients in our study or specific polymorphism in this era. Therefore, more comprehensive studies including large number of cases are needed. In addition, p53 protein expression was not detected in approximately 75% of patients included in our study. SIAH1 has been reported to be triggered by p53 in previous studies. Thus, the reason for the reduced SIAH1 expression may be the loss of p53 expression.

In future research, it will be crucial to employ functional experiments like RNAi and cloning to elucidate the role of SIAH1 and its associated proteins and pathways in the formation of breast cancer. Based on our study's findings, SIAH1 may be involved in the development and progression of breast cancer. It has the potential to be a novel biomarker for diagnosis and treatment, as well as a therapeutic target for breast cancer.

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Author contributions

L. T. H. A. Agha: Planned the experiments, Methodology, Data curation, Formal analysis, Investigation, Resources, Software, Validation, Visualization, Writing – original draft.

A. Arslan: Conceptualization, Planned the experiments, Methodology, Data curation, Formal analysis, Investigation, Resources, Validation, Project administration, Supervision, Writing - review & editing.

The final manuscript has been read and approved by all authors.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethics Committee Approval

Ethical approval for this study was obtained from the Clinical Research Ethics Committee of Gaziantep University (Decision Number: 2014/276). Written informed consent was obtained from all individual participants included in the study.

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