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Original Article

The diagnostic role of A-kinase anchoring protein 12, Bcl-2 and high mobility group box Protein-1 levels in breast cancer

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ABSTRACT

Aim: Despite the improved diagnosis and treatment methods in recent years, the number of new breast cancer cases does not reduce and the mortality rates are still so high.
There are several proteins that play significant roles in carcinogenesis process and hold a great potential to be used as a marker of cancer diagnosis. A-Kinase Anchoring Protein 12 (AKAP12) regulates gene expression in cell cycle and apoptosis while B- cell Lymphoma 2 (BCL2) family proteins and inhibitors of apoptosis proteins are the major regulators of the apoptotic process. High mobility group box 1 (HMGB1) has multiple pro-tumor roles in tumor development.
In our study, we aimed to determine the usability and sensitivity of AKAP12, BCL2 and HMGB1 proteins as a marker of breast cancer diagnosis, and further investigate the relationship among these proteins.
Material and Methods: Between 2014 and 2017, a total number of 82 participants, 60 of them were diagnosed with early stage breast cancer and 22 healthy age-matched people as control group, were included in the study. Serum samples obtained from the groups were investigated in terms of AKAP12, BCL2 and HMGB1 levels by using Enzyme Linked Immuno-Sorbent Assay (ELISA) method.
Results: Comparing the patient group with control groups, AKAP12, BCL2 and HMGB1 levels were found to be significantly higher in the patient group ($p = 0.009$), ($p < 0.001$) and ($p < 0.001$), respectively.
Conclusion: The findings of our results indicate that these proteins levels may be used as markers in the diagnosis and follow-up of breast cancer.

1. Introduction

Breast cancer is the most common type of cancer among women. Despite the recent advances in the diagnosis and treatment, breast cancer is still the leading cause of death in women worldwide.[1] Some tumor markers have been previously used to evaluate the treatment response in breast cancer and to detect metastases at an early stage. However, there is still unmet need of identifying tumor markers that can be used in screening the early diagnosis. Moreover, with potential markers of determining the prognosis of the disease, the treatment intensity to the patients with breast cancer can be specified before the treatment.

AKAP12 is a regulator of protein kinase A (PKA) and protein kinase C (PKC) signal. AKAP12, which is also called as gravin, is firstly described as an autoantigen in patients with myasthenia gravis,[2] and regulates gene expression in cell cycle and apoptosis.[3] Furthermore, AKAP12-induced caspase-3 inhibits the growth of tumor cells via stimulating apoptosis through up-regulation of Bax and down-regulation of BCL2[3]. In addition, AKAP12 stimulates to stop cell cycle by reducing cyclin D1 expression and increasing the levels of Cip1/p21 and Kipl/p27 proteins that are the control point of the cell cycle.

Previously, epigenetic suppression of AKAP12 in different cancer types has been shown and is related to the process of tumor formation.[4] The decrease in expression of AKAP12 in various cancers has been previously shown, including stomach, prostate and

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ovarian cancer.[5] Choi et al.[6] also reported that AKAP12 re-expression repairs cell growth through inducing apoptosis in gastric cancer cells.[6] In another study, a decrease in AKAP12 expression in patients with acute leukemia has been shown along with the survival is adversely affected in patients with increased AKAP12 level.[7]

BCL2 was firstly detected in human B cell lymphoma cells and plays a role in apoptosis process. Based on their biological activities and the presence of certain areas of BCL2 homology (BH), BCL2 family proteins can generally classified with three types: anti-apoptotic, proapoptotic and effector.[8,9] While the proapoptotic members of this protein family, including Bax, Bad, Bid, Bak and Bcl-xs stimulate apoptosis, the anti-apoptotic members such as BCL2 and BCL-xl (BCL2L1) proteins prevent apoptosis.[10] Especially, BCL2 and BCL-xl levels were reported to be high in many different types of cancer.[11–13] Moreover, in the initial stages of breast cancer, BCL2 has been shown to be an independent and powerful prognostic marker.[14] A recent study by Kim et al. has further reported that BCL2-positive breast cancer patients have a better prognosis in terms of relapse-free survival and overall survival.[15]

HMGB1, which is the most important member of the HMGB protein family, is a non-histone nuclear protein with many different functions in the cell and is associated with cancer progression, angiogenesis, invasion and metastasis development.[16] HMGB proteins were firstly discovered in the calf thymus in the 1970s and the origin of the name was based on their rapid acts in gel electrophoresis.[17] Although HMGB2 and HMGB3 expression, the other two members of HMGB family, is limited, HMGB1 expression is quite abundant. HMGB1 plays a key role in maintaining the nucleosome structure in the nucleus as a DNA-binding protein[18] and in regulating gene expression as a transcription factor.[19,20] HMGB1 acts as an extracellular signaling molecule during inflammation, cell migration, cell differentiation and metastasis along with its nuclear role.[21]

Based on several previous studies, intracellular HMGB1 acts as an antitumor protein because of its ability to maintain genome stability and autophagy activity during tumor growth while extracellular HMGB1 acts as a protector protein because of cytokine, chemokine and growth factor activity.[22] Several studies have also reported that HMGB1 plays a critical role in a variety of malignancies, including breast cancer,[23] gastric cancer[24] and hepatocellular carcinoma,[25] lung cancer.[26]

For the newly developed diagnostic methods or screening programs, the purpose of methodological studies is to determine how the methods used to differentiate between healthy and patients are accurate, reliable and valid in terms of correct diagnosis. Testing validity and reliability beyond sensitivity and specificity are also very important for a newly developed test. Although validity and reliability look like two similar concepts at a glance, they in fact have complementary elements because the high validity of a test does not increase its reliability, but its high reliability may increase its validity. Therefore, both the validity and reliability of a good test should be high enough.[27]

2. Materials and methods

For this study, the approval was obtained from Gaziantep University Medical Faculty Ethics Committee (decision no. 2016/16, dated January 11, 2016). This study was conducted in accordance with Helsinki Declaration Rules. All patients in the study were informed about the study and their written consents were obtained.

Between 2014 and 2017, 60 patients who were diagnosed as early stage (stage I, II, III) breast cancer at Medicalpark Gaziantep Hospital Medical Oncology Clinic were consecutively included in the study. Serum samples for AKAP12, BCL2 and HMGB1 were collected before chemotherapy, radiotherapy, hormonal treatment and targeted therapy. Research parameters such as AKAP12, HMGB1 and BCL2 were studied on the ELISA device available in the Department of Medical Biochemistry, Faculty of Medicine, Gaziantep University. All concentration/absorption graph curves and results were calculated on Biotek_ELx808 (Winooski, Vermont, USA). Age, gender, and routine laboratory tests were also recorded. As a control group, 22 age- and gender-matched healthy people were included in the study. Our exclusion criteria were included patients diagnosed with breast cancer at age 18 and below, patients diagnosed with metastatic and locally advanced breast cancer, patients with secondary cancer, and patients without adequate liver and renal reserve. Only one of the breast cancer patients included in the study was male, the remaining patients and the control group were all female patients.

Statistical analysis was performed using SPSS 15.0 software. Normal distribution conformation of the variables was examined using both visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). In the Kolmogorov-Smirnov test, the normal distribution was confirmed if the p value was above 0.05. Because the AKAP12, BCL2 and HMGB1 values measured in the patient and control groups did normally distributed, the patients and the control group were compared using the Mann-Whitney U test. The diagnostic features of serum AKAP12, BCL2 and HMGB1 levels in determining the patients and control groups were investigated by Receiver Operating Characteristics (ROC) curve analysis. In the evaluation of the area under the curve, if the Type 1 error level was below 5%, the diagnostic value of the test was interpreted as statistically significant. The correlations between AKAP12, BCL2 and HMGB1 for the relationships between at least one of the variables not normally distributed or ordinal were assessed by using the correlation coefficients and the Spearman's test.

3. Results

A total number of 82 participants were included in the current study, 60 of them were patients and 22 were healthy controls. Only one of the patients was male while the entire control group was included only women. The mean age of the patients was 53.1 ± 11.3 (Ranging from 28 to 81). The laboratory values of the patients are reported as mean ± standard deviation as shown in Table 1.

When compared the patient group with control groups, there was a significant relationship between AKAP12 levels ($p = 0.009$). While AKAP12 level was 2381.8 ± 1205.5 ng/L (Ranging from 695.3 to 5889.3) in the patient group, it was 1638.1 ± 611.8 ng/L (Ranging from 718.8 to 3331.2) in the control group.

In ROC analysis, the assessment of AKAP12 in terms of the

Table 1
General characteristics of patients.

	Mean ± Standart Deviation	Median
Age (year)	53.1 ± 11.3	52
BUN (mg/dL)	13.2 ± 9.2	11
Creatinin (mg/dL)	0.84 ± 1.1	0.64
AST (U/L)	19.5 ± 9.9	18
ALT (U/L)	19.6 ± 9.8	18
LDH (U/L)	171.6 ± 44.2	162.5
Albumin (gr/dL)	3.8 ± 0.39	4
WBC ($10^3/mm^3$)	8.4 ± 2.6	8.5
Neutrophil ($10^3/mm^3$)	5.28 ± 2.01	5.18
Lymphocyte ($10^3/mm^3$)	2.40 ± 0.91	2.43
Platelet ($10^3/mm^3$)	249 ± 83	290
Hb (g/dL)	12.5 ± 1.4	13

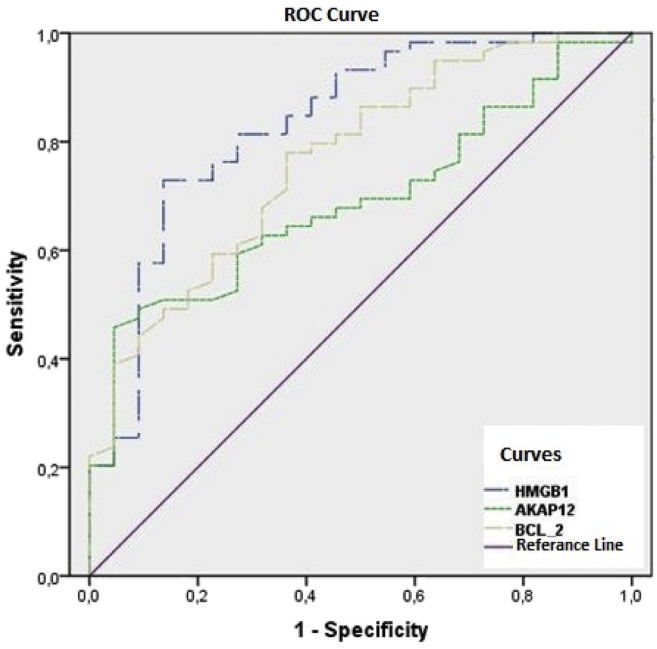

Fig. 1. ROC curves of AKAP12, BCL-2 and HMGB1.

diagnosis was statistically significant ($p = 0.009$) (Fig. 1). When AKAP12 cut-off was accepted as 1100, sensitivity was found as 88%, specificity was 18%, positive predictive value was 75%, and negative predictive value was 36%. When AKAP12 cut-off was accepted as 1200, then, sensitivity was 85%, specificity was 27%, positive predictive value was 76%, and negative predictive value was 40%. Finally, when the cut-off value of ACAP12 was 1300, the sensitivity was 80%, the specificity was 32%, the positive predictive value was 76% and the negative predictive value was 37% (Table 2).

When the patient group was compared to control group, there was a significant association between BCL2 levels ($p < 0.001$). The BCL2 level in the patient group was 478.1 ± 353.6 U/mL (ranging from 196.7 to 2554.7) while it was 286.4 ± 106.1 U/mL (ranging from 31.9 to 521.6) in the control group.

Similar to AKAP12, the diagnosis of patients with BCL2 was statistically significant ($p < 0.00$) in ROC analysis (Fig. 1). When BCL2 cut-off was considered as 230, sensitivity was 93%, specificity was 27%, positive predictive value was 78%, and negative predictive value was 60%. When BCL2 cut-off was considered 240, then, sensitivity was 90%, specificity was 36%, positive predictive value was 79% and negative predictive value was 57%. Finally when BCL2 cut-off was accepted as 250, sensitivity was 85%, specificity was 41%, positive predictive value was 80%, and negative predictive value was 50% (Table 3).

When the patient and control groups were compared in terms of HMGB1 levels, a significant association was found between HMGB1 levels ($p < 0.001$). In the patient group, the level of HMGB1 was 13.07 ± 4.74 ng/mL (ranging from 5.4 to 29.8) while in the control group it was 7.92 ± 3.66 ng/mL (ranging from 2.3 to 17.5).

The diagnosis of patients with HMGB1 was statistically significant when evaluated by ROC analysis ($p < 0.001$) (Fig. 1). Based on different cut-off values such as 7, 8 or 9, sensitivity, specificity and other diagnostic variables slightly differed. For example, in HMGB1 cut-off 7, sensitivity was 93%, specificity was 45%, positive predictive value was 82%, and negative predictive value was 71%. On the other hand, for HMGB1 cut-off 8, sensitivity was 87%, specificity was 59%, positive predictive value was 85%, and negative predictive value was 60%. Finally, for cut-off 9, sensitivity was 80%, specificity was 64%, positive predictive value was 85% and negative predictive value was 54% (Table 4).

The levels of AKAP12, BCL2 and HMGB1 in the patient and control groups are shown in the Table 5 (Table 5).

AKAP12 was significantly correlated with HMGB1 and BCL2 ($p < 0.001$) when the association of these markers was analyzed with Spearman correlation analysis. The AKAP12 correlation coefficient (Rho) with HMGB1 was 0.5, while with BCL2 was 0.445. The Rho value between HMGB1 and BCL2 was 0.519.

In Fig. 1, the best result was obtained with HMGB1 when compared to the area under the ROC curve (AUC) of 3 indicators (Fig. 1).

Table 2
Specificity, sensitivity, positive and negative predictive values based on AKAP12 limit values.

AKAP12 Cut-off Level	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
1100	88	18	75	36
1200	85	27	76	40
1300	80	32	76	37

Table 3
Specificity, sensitivity, positive and negative predictive values based on BCL2 limit values.

Bcl2 Cut-off Level	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
230	93	27	78	60
240	90	36	79	57
250	85	41	80	50

Table 4
Specificity, sensitivity, positive and negative predictive values based on HMGB1 limit values.

HMGB1 Cut-off Level	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
7	93	45	82	71
8	87	59	85	60
9	80	64	85	54

Table 5
Comparison of patient and control groups in terms of AKAP12, BCL2 and HMGB1.

	Patient (60)	Control (22)	p value
AKAP12 (ng/L)	2381.8 ± 1205.5 (Range 695.3–5889.3)	1638.1 ± 611.8 (Range 718.8–3331.2)	p = 0.009
BCL2 (U/mL)	478.1 ± 353.6 (Range 196.7–2554.7)	286.4 ± 106.1 (Range 31.9–521.6)	p < 0.001
HMGB1 (ng/mL)	13.07 ± 4.74 (Range 5.4–29.8)	7.92 ± 3.66 (Range 2.3–17.5)	p < 0.001

4. Discussion

It is very critical that the sensitivity of the diagnostic tests must be high enough because an optimum treatment can be received, the progression can be stopped and the complications can be prevented with early-diagnosis of breast cancer. In this context, we assessed AKAP12, BCL2 and HMGB1 proteins, which all play an important role in carcinogenesis process, as a potential marker in the diagnosis of breast cancer, their sensitivity for this purpose as well as investigated the interrelationships of these proteins.

AKAP12 is a recently described strong scaffold protein and a decrease in the level of AKAP12 has been shown in many types of cancers such as prostate cancer,[28] gastric cancer[6] and colon cancer,[29] suggesting that AKAP12 can be a new potential tumor suppressor. Downregulation of AKAP12 expression is due to the gene deletion or the epigenetic changes such as promoter hypermethylation.[30] Previously, loss of AKAP12A and AKAP12B expression in gastric cancer has been shown to be because of promoter hypermethylation.[6] Moreover, oncogenes like Ras and Myc have been shown to suppress the expression of AKAP12.[31]

AKAP12 is associated with protein kinases (protein kinases A and C (PKA and PKC), cyclines and F-actin.[30] It has been also shown that neoplastic formation started in AKAP12 knockout mice with prostate hyperplasia.[32] In addition, MatLyLu (MLL) regression of AKAP12 in prostate cancer cells significantly suppressed lung metastasis development; however, it did not affect the growth of the primary tumor.[28] Furthermore, AKAP12 re-expression was found to inhibit the production of vascular endothelial growth factor (VEGF) in MLL cells.[33] Therefore, it can be suggested that AKAP12 is a tumor suppressor or a metastasis suppressor.[30,32,34]

Interestingly, AKAP12 expression in some cancer cell lines and human cancers has been shown to be slightly increased, in contrast to the majority of the studies reported that AKAP12 is downregulated in cancer. This conflict may be because of the different biology of these types of cancers, or differentiated signaling pathways. Chronic myeloid leukemia, superficial bladder cancer, giant cell granuloma of the jaw and high grade follicular lymphoma are among the examples of increased AKAP12.[35–38]

Unlike in other cancer types, the role of AKAP12 in breast cancer has not been totally clarified yet. In the study of Soh et al.[39] where the role of AKAP12 on the migration of breast cancer cells was investigated, they reported that AKAP12 gene expression was higher in normal breast epithelium compared to breast cancer cell lines. They further showed that survival was longer in breast cancer patients with high AKAP12 expression. They finally concluded that AKAP12 acted as a tumor suppressor in breast cancer.[39]

Zhang et al. aimed to identify the differentially expressed genes in breast carcinoma and find the potential biomarkers for predicting the prognosis. In this study they found that AKAP12 is one of the key breast carcinoma-associated gene. They concluded that this data is useful tool to predict the progression of breast carcinoma.[40]

To the best of our knowledge, there is no study investigating AKAP12 serum levels in breast cancer patients. Based our study, we suggest that accepting the 1100 as a cut-off value that provides high sensitivity for AKAP12 in breast cancer patients can be used as a predictor (Table 2).

BCL2 is an antiapoptotic protein which belongs to the BCL2 family.[41,42] The role of BCL2 depends on its association with other proteins of the BCL2 family.[41–43] Improper expression of the BCL2 protein was firstly shown as a result of chromosomal translocation in non-Hodgkin lymphoma. Subsequent research has reported that BCL2 is also expressed in solid tumors such as breast cancer, prostate cancer, gastric cancer, lung cancer, colorectal cancer and ovarian cancer.[41,42] Although BCL2 overexpression is a poor prognostic factor in non-Hodgkin lymphoma, it is associated with low grade, slowly proliferating, estrogen receptor (ER) positive subgroup in breast cancer.[44,45] This paradoxical favorable prognostic effect could be associated with non-apoptotic functions of BCL2 in breast cancer.[46,47] In addition, an increased expression for the BCL2 protein could disrupt the balance with other members of the BCL2 family, eventually leading to expression of pro-apoptotic proteins.[48]

BCL2 family proteins (BCL2 and BAX) are also expressed in normal breast tissue.[49] BCL2 is upregulated through the effect of estrogen as a direct result of transcriptional induction.[49,50] In the study by Dawson et al.,[14] an increased expression of BCL2 was found to be a good prognostic factor in all molecular subtypes of breast cancer. However, Eom et al.[51] reported that only Luminal A subgroup was a good prognostic factor. In both studies, the researchers investigated the relationship between immunohistochemistry and BCL2 expression in tumor tissue in the molecular subtypes of breast cancer and survival. In the study by Samy et al., an increased serum level of BCL2 was found to be associated with recurrence of breast cancer.[52]

After analyzing the obtained BCL2 level with ROC analysis, we suggest that BCL2 can be used as a marker with 93% sensitivity in the diagnosis of breast cancer patients (Table 3; Fig. 1).

Recently, HMGB1 has been shown as a regulator of tumorigenesis, expansion and invasion of cancer cells.[21] HMGB1 protein can be found in many different cell types as ubiquitous nuclear proteins and typically located in the nucleus. As a nuclear cofactor, HMGB1 plays a role in the regulation of transcription. However, HMGB1 can also be found in plasma and can be released into the extracellular matrix in the cases of carcinogenesis and inflammation.[21] Following its release, HMGB1 initiates the signal transduction through the RAGE (receptor for advanced glycation end product) and TLR (Toll like receptor) receptors. Many recent studies have clearly shown that HMGB1 and RAGE receptor levels are generally higher in cancerous tissues compared to that of in normal epithelium. Colorectal, prostate and pancreatic cancers and hepatocellular cancer are among the examples of higher HMGB1 and RAGE receptor levels.[53–58] HMGB1 overexpression in these studies has been further shown to correlate with tumor invasiveness and poor prognosis.[59–61] Though the oncogenic role of HMGB1 has been well established in many cancer types,[22,62] its role in breast cancer is still poorly understood. In limited number of studies, HMGB1 has been shown to increase the growth of breast cancer cells in vitro; however, its role in breast cancer patients has not still been elucidated.[63,64]

In the current study, the diagnostic significance of HMGB1 serum levels in breast cancer was investigated and we found that HMGB1 serum levels were significantly higher in breast cancer

patients compared to healthy controls. Our findings indicate that serum HMGB1 level may be a new diagnostic marker in breast cancer. Sun et al.,[65] investigated HMGB1 levels in both tissues and serum in patients with breast cancer, patients with benign breast disease, as well as in healthy control group. Their results revealed that both HMGB1 tissue and serum levels of patients diagnosed with breast cancer were significantly higher than those in the control group and patients with benign breast disease.[65]

The most important limitation of the current study was the small sample size (total number of 82 patients). Another important limitation of our study was that we did not assess the correlation between clinical parameters such as tumor differentiation, lymph node metastasis, tumor diameter, grade, and AKAP12, BCL2 and HMGB1 levels. In addition, it would be more valuable to investigate the levels of these proteins in tumor tissue with immunohistochemistry or/and similar methods, and to correlate them with the serum levels.

Our cumulative results suggest that AKAP12, BCL2 and HMGB1 may be a diagnostic marker in breast cancer, but prospective studies with larger patient numbers are still needed.

Conflicts of interest

All authors declare they have no financial interest or conflict of interest with the present study.

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We have no financial interest or conflict of interest in association with this work.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA A Cancer J Clin*. 2018;68:394–424. https://doi.org/10.3322/caac.21492.
2. Gordon T, Grove B, Loftus JC, et al. Molecular cloning and preliminary characterization of a novel cytoplasmic antigen recognized by myasthenia gravis sera. *J Clin Investig*. 1992;90:992–999.
3. Yoon DK, Jeong CH, Jun HO, et al. AKAP12 induces apoptotic cell death in human fibrosarcoma cells by regulating CDKI-cyclin D1 and caspase-3 activity. *Cancer Lett*. 2007;254:111–118.
4. Wilhelm T, Lipka DB, Witte T, et al. Epigenetic silencing of AKAP12 in juvenile myelomonocytic leukemia. *Epigenetics*. 2016:1–10.
5. Boultwood J, Pellagatti A, Watkins F, et al. Low expression of the putative tumour suppressor gene Gravin in chronic myeloid leukaemia, myelodysplastic syndromes and acute myeloid leukaemia. *Br J Haematol*. 2004;126:508–511.
6. Choi MC, Jong HS, Kim TY, et al. AKAP12/Gravin is inactivated by epigenetic mechanism in human gastric carcinoma and shows growth suppressor activity. *Oncogene*. 2004;23:7095–7103.
7. Yıldırım M. *Akut Lösemilerde Gravin Ekspresyonunun Prognostik Önemi. Çukurova Üniversitesi Tıp Fakültesi İç Hastalıkları ABD, Uzmanlık Tezi*. 2006. Adana (Prof. Dr. Semra PAYDAŞ).
8. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol*. 2008;9:47–59.
9. Reed JC, Doctor KS, Godzik A. The domains of apoptosis: a genomics perspective. *Sci Signal*. 2004;239:re9.
10. Yıldırım M. *Mide Kanserinde P52 Ve BCL2 Ekspresyonunun Prognostik Önemi. Sağlık Bakanlığı Antalya Eğitim Ve Araştırma Hastanesi Tıbbi Onkoloji Kliniği, Yan Dal Uzmanlık Tezi*. Antalya: Doç. Dr. Mustafa YILDIZ; 2012.
11. Minn AJ, Rudin CM, Boise LH, et al. Expression of bcl-xL can confer a multidrug resistance phenotype. *Blood*. 1995;86:1903–1910.
12. Reed JC. Bcl-2-family proteins and hematologic malignancies: history and future prospects. *Blood*. 2008;111:3322–3330.
13. Yoshino T, Shiina H, Urakami S, et al. Bcl-2 expression as a predictive marker of hormone refractory prostate cancer treated with taxane-based chemotherapy. *Clin Cancer Res*. 2006;12:6116–6124.
14. Dawson SJ, Makretsov N, Blows FM, et al. BCL2 in breast cancer: a favourable prognostic marker across molecular subtypes and independent of adjuvant therapy received. *Br J Canc*. 2010;103:668–675.
15. Kim HS, Moon HG, Han W, et al. COX2 overexpression is a prognostic marker for stage III breast cancer. *Breast Canc Res Treat*. 2012;132:51–59.
16. Yıldırım M, Süren D, Demirpençe Ö, et al. High mobility group box 1 ve Kanser. *Acıbadem Üniversitesi Sağlık Bilimleri Dergisi*. 2014;5:182–186.
17. Goodwin GH, Sanders C, Johns EW. A new group of chromatin-associated proteins with a high content of acidic and basic amino acids. *Eur J Biochem*. 1973;38:14–19.
18. Thomas JO, Stott K. H1 and HMGB1: modulators of chromatin structure. *Biochem Soc Trans*. 2012;40:341–346.
19. Bustin M. Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. *Mol Cell Biol*. 1999;19:5237–5246.
20. Stros M, Muselikova-Polanska E, Pospisilova S, et al. High-affinity binding of tumor-suppressor protein p53 and HMGB1 to hemicatenated DNA loops. *Biochemistry*. 2004;43:7215–7225.
21. Tang D, Kang R, Zeh HJ, et al. High-mobility group box 1 and cancer. *Biochim Biophys Acta Gene Regul Mech*. 2010;1799:131–140.
22. Kang R, Zhang Q, Zeh HJ, et al. HMGB1 in cancer: good, bad, or both? *Clin Cancer Res*. 2013;19:4046–4057.
23. Sun S, Zhang W, Cui Z, et al. High mobility group box-1 and its clinical value in breast cancer. *OncoTargets Ther*. 2015;8:413–419.
24. Zhang J, Zhang R, Lu WW, et al. Clinical significance of hmgb1 expression in human gastric cancer. *Int J Immunopathol Pharmacol*. 2014;27:543–551.
25. Zhang L, Han J, Wu H, et al. The association of HMGB1 expression with clinicopathological significance and prognosis in hepatocellular carcinoma: a meta-analysis and literature review. *PLoS One*. 2014;9:1–8.
26. Feng A, Tu Z, Yin B. The effect of HMGB1 on the clinicopathological and prognostic features of non-small cell lung cancer. *Oncotarget*. 2016;7:20507–20519.
27. Hayran M, Hayran M. *Sağlık Araştırmaları İçin Temel İstatistik*. Ankara: Hayran Yayınevi; 2011.
28. Xia W, Unger P, Miller L, et al. The Src-suppressed C kinase substrate, SSeCKS, is a potential metastasis inhibitor in prostate cancer. *Cancer Res*. 2001;61:5644–5651.
29. Liu W, Guan M, Su B, et al. Quantitative assessment of AKAP12 promoter methylation in colorectal cancer using methylation-sensitive high resolution melting: correlation with Duke's stage. *Cancer Biol Ther*. 2010;9:862–871.
30. Gelman IH. Emerging roles for SSeCKS/Gravin/AKAP12 in the control of cell proliferation, cancer malignancy, and barriergenesis. *Genes Cancer*. 2010;1:1147–1156.
31. Lin X, Nelson PJ, Frankfort B, et al. Isolation and characterization of a novel mitogenic regulatory gene, 322, which is transcriptionally suppressed in cells transformed by src and ras. *Mol Cell Biol*. 1995;15:2754–2762.
32. Akakura S, Huang C, Nelson PJ, et al. Loss of the SSeCKS/Gravin/AKAP12 gene results in prostatic hyperplasia. *Cancer Res*. 2008;68:5096–5103.
33. Quinn DI, Henshall SM, Sutherland RL. Molecular markers of prostate cancer outcome. *Eur J Cancer*. 2005;41:858–887.
34. Su B, Zheng Q, Vaughan MM, et al. SSeCKS metastasis-suppressing activity in MatLyLu prostate cancer cells correlates with vascular endothelial growth factor inhibition. *Cancer Res*. 2006;66:5599–5607.
35. Zhu Y, Hollmen J, Raty R, et al. Investigatory and analytical approaches to differential gene expression profiling in mantle cell lymphoma. *Br J Haematol*. 2002;119:905–915.
36. Tsujimoto K, Ono T, Sato M, et al. Regulation of the expression of caspase-9 by the transcription factor activator protein-4 in glucocorticoid-induced apoptosis. *J Biol Chem*. 2005;280:27638–27644.
37. Carinci F, Piattelli A, Martinelli M, et al. Genetic profiling of central giant cell granuloma of the jaws. *J Craniofac Surg*. 2005;16:399–407.
38. Jiang BH, Agani F, Passaniti A, et al. V-SRC induces expression of hypoxia-inducible factor 1 (HIF-1) and transcription of genes encoding vascular endothelial growth factor and enolase 1: involvement of HIF-1 in tumor progression. *Cancer Res*. 1997;57:5328–5335.
39. Soh RYZ, Lim JP, Samy RP, et al. A-kinase anchor protein 12 (AKAP12) inhibits cell migration in breast cancer. *Exp Mol Pathol*. 2018;105:364–370.
40. Zhang GM, Goyal H, Song LL. Bioinformatics analysis of differentially expressed miRNA-related mRNAs and their prognostic value in breast carcinoma. *Oncol Rep*. 2018;39:2865–2872.
41. Basu A, Haldar S. The relationship between Bcl2, Bax and p53: consequences for cell cycle progression and cell death. *Mol Hum Reprod*. 1998;4:1099–1109.
42. Bouchalova K, Kharaishvili G, Bouchal J, et al. Triple negative breast cancer: BCL2 in prognosis and prediction. Review. *Curr Drug Targets*. 2014;15:1166–1175.
43. Brunelle JK, Letai A. Control of mitochondrial apoptosis by the Bcl-2 family. *J Cell Sci*. 2009;122:437–441.
44. Silvestrini R, Veneroni S, Daidone MG, et al. The Bcl-2 protein: a prognostic indicator strongly related to p53 protein in lymph node-negative breast cancer patients. *J Natl Cancer Inst*. 1994;86:499–504.
45. Lipponen P, Pietilainen T, Kosma VM, et al. Apoptosis suppressing protein bcl-2 is expressed in well-differentiated breast carcinomas with favourable prognosis. *J Pathol*. 1995;177:49–55.
46. Pietenpol JA, Papadopoulos N, Markowitz S, et al. Paradoxical inhibition of solid tumor cell growth by bcl2. *Cancer Res*. 1994;54:3714–3717.
47. O'Reilly LA, Huang DC, Strasser A. The cell death inhibitor Bcl-2 and its homologues influence control of cell cycle entry. *EMBO J*. 1996;15:6979–6990.
48. Cory S, Huang DC, Adams JM. The Bcl-2 family: roles in cell survival and oncogenesis. *Oncogene*. 2003;22:8590–8607.
49. Leung LK, Wang TT. Paradoxical regulation of Bcl-2 family proteins by 17beta-oestradiol in human breast cancer cells MCF-7. *Br J Canc*. 1999;81:387–392.
50. Wang TT, Phang JM. Effects of estrogen on apoptotic pathways in human breast

cancer cell line MCF-7. *Cancer Res*. 1995;55:2487–2489.
51. Eom YH, Kim HS, Lee A, et al. BCL2 as a subtype-specific prognostic marker for breast cancer. *J Breast Cancer*. 2016;19:252–260.
52. Samy N, Ragab HM, El Maksoud NA, et al. Prognostic significance of serum Her2/neu, BCL2, CA 15-3 and CEA in breast cancer patients: a short follow-up. *Cancer Biomark*. 2010;6:63–72.
53. Völp K, Brezniceanu ML, Bösser S, et al. Increased expression of high mobility group box 1 (HMGB1) is associated with an elevated level of the antiapoptotic c-IAP2 protein in human colon carcinomas. *Gut*. 2006;55:234–242.
54. Pardo M, García A, Thomas B, et al. The characterization of the invasion phenotype of uveal melanoma tumour cells shows the presence of MUC18 and HMG-1 metastasis markers and leads to the identification of DJ-1 as a potential serum biomarker. *Int J Cancer*. 2006;119:1014–1022.
55. Ishiguro H, Nakaigawa N, Miyoshi Y, et al. Receptor for advanced glycation end products (RAGE) and its ligand, amphoterin are over expressed and associated with prostate cancer development. *The Prostate*. 2005;64:92–100.
56. Choi YR, Kim H, Kang HJ, et al. Overexpression of high mobility group box 1 in gastrointestinal stromal tumors with KIT mutation. *Cancer Res*. 2003;63:2188–2193.
57. Hirata K, Takada M, Suzuki Y, et al. Expression of receptor for advanced glycation end products (RAGE) in human biliary cancer cells. *Hepato-Gastroenterology*. 2003;50:1205–1207.
58. Evans A, Lennard TW, Davies BR. High-mobility group protein 1(Y): metastasis-associated or metastasis-inducing? *J Surg Oncol*. 2004;88:86–99.
59. Sparvero LJ, Asafu-Adjei D, Kang R, et al. RAGE (receptor for advanced glycation endproducts), RAGE ligands, and their role in cancer and inflammation. *J Transl Med*. 2009;7:17.
60. Kostova N, Zlateva S, Ugrinova I, et al. The expression of HMGB1 protein and its receptor RAGE in human malignant tumors. *Mol Cell Biochem*. 2010;337:251–258.
61. Yang GL, Zhang LH, Bo JJ, et al. Increased expression of HMGB1 is associated with poor prognosis in human bladder cancer. *J Surg Oncol*. 2012;106:57–61.
62. Kang R, Chen R, Zhang Q, et al. HMGB1 in health and disease. *Mol Asp Med*. 2014;40C:1–116.
63. Jiao Y, Wang HC, Fan SJ. Growth suppression and radiosensitivity increase by HMGB1 in breast cancer. *Acta Pharmacol Sin*. 2007;28:1957–1967.
64. Chalmers SA, Eidelman AS, Ewer JC, et al. A role for HMGB1, HSP60 and Myd88 in growth of murine mammary carcinoma in vitro. *Cell Immunol*. 2013;282:136–145.
65. Sun S, Zhang W, Cui Z, et al. High mobility group box-1 and its clinical value in breast cancer. *OncoTargets Ther*. 2015;8:413–419.