Research Article

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Decreased *GADD45A* gene expression level in MGUS

MGUS'ta azalmış GADD45A gen ekspresyonu seviyesi

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Abstract

Purpose: Monoclonal gammopathy of undetermined significance (MGUS) is a plasma cell dyscrasia. It is known that MGUS has an increased risk of progression to multiple myeloma (MM), and prepares the ground for diseases such as Waldenstrom macroglobulinemia (WM), non-Hodgkin lymphoma, and chronic lymphocytic leukemia (CLL). Our study aimed to evaluate whether some important p53 pathway genes differ in terms of expression between MGUS and healthy individuals.

Materials and methods: Bone marrow was collected from eight healthy individuals and eight individuals diagnosed with MGUS, and RNA samples were isolated. The expression levels of various genes involved in the p53 pathway were compared using an RT2-profiler PCR array. *β-Actin* housekeeping gene expression level was used for normalization. Pearson's Correlation and Receiver Operating Characteristic (ROC) analyses were conducted.

Results: Among the genes whose expression levels were examined in this study, it was determined that the expression level of only the *GADD45A* gene decreased significantly in the MGUS group compared to the control group (p=0.027). Pearson's correlation data showed that *GADD45A* gene expression was highly correlated with 12 of the other genes (*APAF1, CDK4, PCNA, BAX, CDKN2A, CASP9, CHEK2, MDM2, RB1, P53, BCL2, CHEK1*) examined in the p53 pathway (r>0.7). In addition, according to the ROC analysis, *GADD45A* was detected to have strong discrimination power between MGUS and healthy individuals (*AUC*=0.797 and p=0.015).

Conclusion: The decreased expression of the *GADD45A* gene in the MGUS group compared to the control group may be useful as a new biomarker to detect the development of MGUS.

Keywords: MGUS, p53 pathway, *GADD45A*.

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Öz

Amaç: Belirsiz öneme sahip monoklonal gamopati (MGUS), bir plazma hücre diskrazisidir. MGUS'un Multipl miyelom'a (MM) ilerleme riskini artırdığı, ayrıca Waldenstrom makroglobulinemi (WM), non-Hodgkin lenfoma ve kronik lenfositik lösemi (KLL) gibi hastalıklara zemin hazırladığı bilinmektedir. Çalışmamızda bazı önemli p53 yolağı genlerinin MGUS ve sağlıklı bireylerde ekspresyon açısından farklılık gösterip göstermediğini değerlendirmeyi amaçladık.

Gereç ve yöntem: Çalışma için 8 sağlıklı ve 8 MGUS tanılı bireyden kemik iliği toplandı ve RNA örnekleri izole edildi. p53 yolağında yer alan çeşitli genlerin (*BAX, CDKN2A, APAF1, ATM, ATR, CASP9, CDK4, CDKN1A, CHEK2, E2F1, E2F3, MCL1, MDM2, MDM4, PTEN, RB1, P53, BCL2, CHEK1, GADD45A, PCNA, PTX3*) ekspresyon seviyeleri, RT2-profiler PCR array yöntemiyle karşılaştırıldı. Normalizasyon için *β-Aktin* gen ekspresyon seviyesi kullanıldı. Pearson Korelasyon ve Receiver Operating Characteristic (ROC) analizleri yapıldı.

Bulgular: Bu çalışmada ekspresyon seviyeleri incelenen genlerden sadece *GADD45A* geninin ekspresyon seviyesinin kontrol grubuna göre MGUS'ta anlamlı olarak azaldığı belirlendi (p=0,027). Pearson korelasyon verileri, *GADD45A* gen ekspresyonunun, p53 yolağında incelenen diğer 12 gen (*APAF1, CDK4, PCNA, BAX, CDKN2A, CASP9, CHEK2, MDM2, RB1, P53, BCL2, CHEK1*) ile yüksek oranda ilişkili olduğunu gösterdi (r>0,7). Ayrıca ROC analizine göre *GADD45A*'nın MGUS ile sağlıklı bireyler arasında güçlü bir ayrım gücüne sahip olduğu saptandı (*AUC*=0,797 ve p=0,015).

Sonuç: *GADD45A* geninin MGUS grubunda kontrol grubuna göre azalmış ekspresyonu, MGUS gelişimini saptamak için yeni bir biyobelirteç olarak faydalı olabilir.

Anahtar kelimeler: MGUS, p53 yolağı, *GADD45A*.

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Introduction

Monoclonal gammopathy of undetermined significance (MGUS) is a clonal plasma cell proliferative disorder characterized by the presence of para(M) proteins in the peripheral blood or an abnormal free light chain (FLC) ratio, which progresses to multiple myeloma over time [1]. MGUS occurs as a result of acquired hyperdiploidy or a translocation [t(4;14), t(6;14), t(11;14)] involving the immunoglobulin heavy chain gene locus. Additional genetic changes, such as somatic mutations and copy number variations, are also present in subclonal populations, and the frequency of these additional mutations increases as the disease progresses to multiple myeloma [2]. The prevalence of pre-cancerous MGUS transforming into multiple myeloma is reported as approximately 1%. In a study by Akhtar et al. [3], it was suggested that myricetin is a protective supplement in cancer development from MGUS to myeloma by increasing p53 expression at both the mRNA and protein levels. *TP53* is one of the most important genes known to have a regulatory role in tumor suppression, and it achieves this by controlling genes involved in the cell cycle. Various changes that may occur in the *TP53* gene and genes related to the *TP53* pathway may be the source of various pathogeneses, especially cancers. For example, *TP53* deletion has been reported in up to 10% of MM cases [4, 5]. Therefore, the *TP53* pathway is indisputable for both MGUS and MM. Although *TP53* mutations have been associated with short survival in MM, there is limited literature on the association between the expression of this *TP53* gene and pathway-related genes in MGUS [6]. Thus, information in the literature regarding gene expressions in the *TP53* pathway needs to be enriched.

Materials and methods

Collection of samples

Bone marrow samples from eight MGUS and eight healthy controls were collected with written informed consent. Bone marrow samples collected in tubes with EDTA were obtained from İstanbul University Medicine Faculty, Department of Hematology. The healthy control group consisted of bone marrow samples obtained from transplantation donor candidates.

MGUS samples were taken in cases with plasma cell count less than 10%, abnormal protein less than 30 g/L, and without both hypercalcemia and lytic lesions. Samples of healthy controls were taken from individuals who were not relatives of MGUS patients. Healthy donors were screened for hematological diseases including MGUS. The study was approved by the İstanbul University Medical Faculty Clinical Research Ethics Committe, and was performed according to the Declaration of Helsinki.

RNA extraction and cDNA synthesis

RNA was isolated from bone marrow (BM) samples using the QiaAmp RNA Blood Mini Kit (Qiagen, USA). RNA quality and quantity were measured using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, USA). cDNA was synthesized using the Qiagen RT2 HT First Strand Kit (Qiagen, USA) with 1000 ng of total RNA.

Expression pattern determination using qRT-PCR array

The RT2 Profiler PCR array (CAPH_133446F; cat no. 330131/12 plates) was used for expression pattern analysis on the LightCycler 480 II platform. The expressions of 22 genes (*BAX, CDKN2A, APAF1, ATM, ATR, CASP9, CDK4, CDKN1A, CHEK2, E2F1, E2F3, MCL1, MDM2, MDM4, PTEN, RB1, P53, BCL2, CHEK1, GADD45A, PCNA, PTX3*) involved in the p53 pathway and known to be involved in cellular processes that are important for cancer pathogenesis were evaluated by qRT-PCR. *SYBR* Green dye was used for the qRT-PCR analysis. β-*Actin* was used for normalization, and the study was designed in duplicate.

Statistical analysis

SPSS 21 was used for statistical analysis. The Kolmogorov-Smirnov test was performed to analyze whether the gene expression levels were distributed normally. Normally distributed ones were analyzed by Student's t-test, and non-normally distributed ones were analyzed by the Mann-Whitney U test. The expression correlation analysis of genes with statistically significant expression in the MGUS group was performed using Pearson's correlation analysis. In addition, Receiver Operating Characteristic (ROC) analysis was performed using MedCalc

to determine the power of statistically significant genes to distinguish between MGUS and healthy individuals. Statistical significance was set at $p<0.05$.

Results

Eight patients with MGUS and eight healthy individuals were included in this study. In MGUS, the male/female ratio was 4/4, and the male to female ratio was 1. In the control group, male/female ratio was 3/5 (the ratio of male to female was 0.6). The MGUS and control groups had mean ages of 63.12±12.40 and 40.5±13.27, respectively. The median, minimum, and maximum ages were respectively 63.12 (40-76) and 40.5 (18-56). The mean age of the MGUS group was higher than that of the control group (t=3.523, p=0.003).

In our study, the expression levels of 22 genes selected from the p53 pathway were examined in MGUS and healthy controls. The *BAX, CDKN2A, APAF1, ATM, ATR, CASP9, CDK4, CDKN1A, CHEK2, E2F1, E2F3, MCL1, MDM2, MDM4, PTEN, RB1, P53, BCL2, CHEK1, PCNA*, and *PTX3* gene expression levels were not statistically significant. Of these, only *GADD45A* expression was significantly decreased in the MGUS group compared to that in the control group (p=0.027) (Table 1).

Table 1. Comparison of relative expressions of genes in bone marrow samples of MGUS and healthy group

Gene	Groups MGUS group (n=8) Control group (n=8)	Relative Expression Unit (Mean±SD)	*p* value
BAX	MGUS group	0.0034±0.00067	**0.352**
	Control group	0.3273±0.91941	
CDKN2A	MGUS group	0.0001±0.00005	**0.377**
	Control group	0.0005±0.00138	
APAF1	MGUS group	0.0076±0.00109	**0.841**
	Control group	0.0084±0.01071	
ATM	MGUS group	0.0020±0.00109	**0.933**
	Control group	0.0021±0.00414	
ATR	MGUS group	0.0013±0.00055	**0.788**
	Control group	0.0016±0.00342	
CASP9	MGUS group	0.0014±0.00036	**0.510**
	Control group	0.0026±0.00513	
CDK4	MGUS group	0.0026±0.00104	**0.644**
	Control group	0.0036±0.00623	
CDKN1A	MGUS group	0.0044±0.00291	**0.134**
	Control group	0.0021±0.00273	
CHEK2	MGUS group	0.0007±0.00025	**0.406**
	Control group	0.0031±0.00763	
E2F1	MGUS group	0.0011±0.00064	**0.379**
	Control group	0.0007±0.00081	
E2F3	MGUS group	0.0020±0.00041	**0.811**
	Control group	0.0021±0.00126	

Table 1. Comparison of relative expressions of genes in bone marrow samples of MGUS and healthy group (continued)

MCL1	MGUS group	0.1128±0.03413	**0.138**
	Control group	0.0781±0.05242	
MDM2	MGUS group	0.0064±0.00220	**0.472**
	Control group	0.0165±0.03737	
MDM4	MGUS group	0.0010±0.00052	**0.714**
	Control group	0.0013±0.00183	
PTEN	MGUS group	0.0178±0.00717	**0.813**
	Control group	0.0196±0.01991	
RB1	MGUS group	0.0043±0.00119	**0.385**
	Control group	0.0281±0.07244	
P53	MGUS group	0.0044±0.00182	**0.371**
	Control group	0.0482±0.12947	
BCL2	MGUS group	0.0019±0.00071	**0.352**
	Control group	0.3217±0.90720	
CHEK1	MGUS group	0.0018±0.00077	**0.409**
	Control group	0.0091±0.02359	
GADD45A	MGUS group	0.0005±0.00027	**0.027***
	Control group	0.0011±0.00063	
PCNA	MGUS group	0.0140±0.00592	**0.481**
	Control group	0.0263±0.04768	
PTX3	MGUS group	0.0035±0.00197	**0.991**

$2^{-\Delta\Delta Ct}$ analysis, $p<0.05$, ΔCt: Delta cycle threshold, MGUS: Monoclonal gammopathy of undetermined significance

Changes in gene expression in MGUS and healthy control samples were determined using a bar graph (Figure 1). A heat map plot for the MGUS and healthy control group samples was created to show the mean fold change (Figure 2). All participants included in the study had no other known diseases and were not relatives of each other. Almost all genes in control 7 were observed to have increased gene expression, clearly different from MGUS and other healthy individuals. The reason for this may be associated with genetic variants of unknown underlying causes that may affect the expression of genes in this control sample (Figure 2).

Pearson's correlation data showed that *GADD45A* expression was highly correlated with the expression of *APAF1**, *CDK4**, *PCNA**, *BAX***, *CDKN2A***, *CASP9***, *CHEK2***, *MDM2***, *RB1***, *P53***, *BCL2*** and *CHEK1*** ($r>0.7$; *$p=0.001$ and **$p=0.002$). According to the ROC analysis result, *GADD45A* had a strong discrimination power between MGUS and healthy individuals (*AUC*=0.797 and $p=0.015$) (Figure 3).

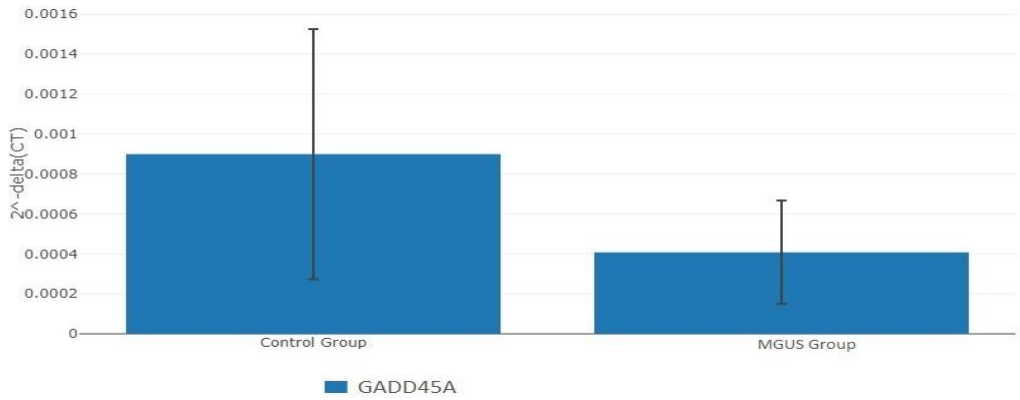

Figure 1. Boxplot representation of MGUS and control expression according to the $2^{-\Delta\Delta Ct}$ values of the *GADD45A* gene

The fold-change ($2^{-\Delta\Delta CT}$) is the normalized gene expression ($2^{-\Delta CT}$) in the MGUS sample divided by the normalized gene expression ($2^{-\Delta CT}$) in the control sample

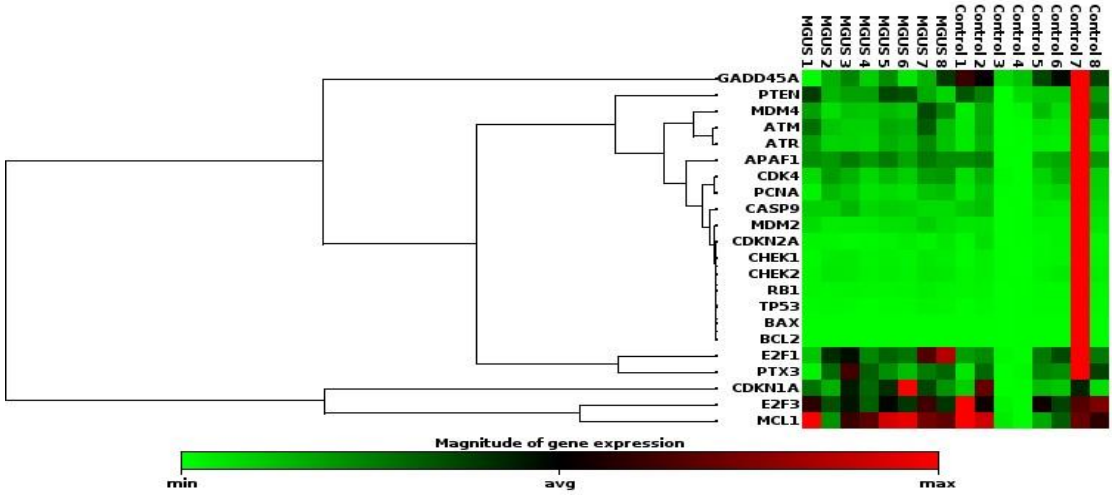

Figure 2. Evaluation of RT2 profiler PCR array data using clustergram analysis

Gene expression data are shown in a one-dimensional format (heatmap plot or clustergram) using color codes (genes). A heatmap plot (clustergram) displaying gene expression levels and grouping of genes according to their expression patterns. The RT2-PCR array expression data were compared between the two groups (MGUS and control), and the heat map displays the results in terms of up- or downregulation. The intensity of the color change represents the level of variation in gene expression. Low gene expression (ratio <1) is shown by green squares in the MGUS samples. Genes with a ratio close to one are shown as black squares. The presence of red squares indicates gene expression levels that were significantly greater than the control values (ratio >1)

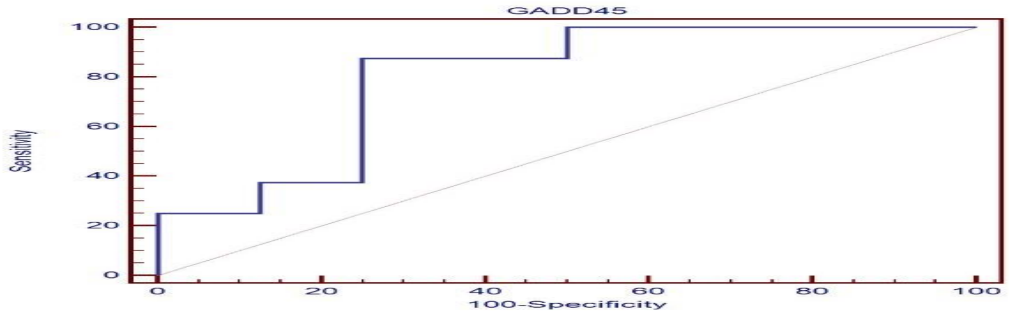

Figure 3. Strong discrimination power for *GADD45A* gene between the MGUS and healthy groups (AUC=0.797 and *p*=0.015)

Discussion

Monoclonal gammopathy of uncertain significance (MGUS), smoldering multiple myeloma (SMM), and multiple myeloma are all monoclonal gammopathies, and approximately 10% of SMM and 1% of MGUS cases progress to MM each year [7]. MGUS is a precancerous condition characterized by an asymptomatic increase in plasma cells. MGUS is observed in 3.5% of individuals over 50 years of age [8, 9]. Various pathophysiological conditions such as kidney failure, anemia, hypercalcemia, thrombocytopenia, lytic bone lesions, and bone pain occur because of the extra protein and monoclonal antibodies produced as a result of conversion from MGUS to MM, which complicates the patient's life [10]. Specific biomarkers are still needed to predict the conversion of MGUS to MM. The importance of *TP53* in tumor suppression in malignancies is well established. *TP53* dysregulation is involved in the pathogenesis of many malignancies [8]. On the other hand, the importance of other genes that regulate the cellular response to DNA damage and stress by being in the same pathway as p53 in MGUS MM transformation is not clear enough [3, 5].

The Growth Arrest and DNA Damage-inducible 45 (*GADD45*) family of proteins are important in the regulation of cellular responses to various stress factors. The *GADD45* family members (*GADD45A*, *GADD45B*, and *GADD45G*) are ubiquitously expressed in small amounts under physiological conditions in human tissues. However, their expression increases in response to various stress factors and is involved in the regulation of various cellular functions, such as cell cycle, DNA repair, and apoptosis. *GADD45* proteins lack enzymatic activity; therefore, they perform modulating functions by interacting with partner proteins [11, 12]. Although *GADD45* family members are involved in carcinogenesis in different tissues, the roles of these proteins in cancer development are not fully understood. Low expression of *GADD45* has been reported in lung [13], stomach [14], and breast [15] cancers, whereas *GADD45* overexpression has been reported in glioblastoma [16], cholangiocarcinoma [17]. In addition, *GADD45A* expression has been shown to be regulated by many transcription factors, including p53.

MGUS and smoldering MM (SMM) are benign diseases that occur before MM and are clonal plasma cell (PC) malignancies. Many oncogenes and chromosomal abnormalities found in MM PCs are also present in MGUS and SMM PCs, despite the fact that MGUS and SMM PCs do not grow rapidly. Borges et al. [18] hypothesized that MGUS/SMM PCs may be in an aging-like state, as oncogenic stress is known to induce cellular senescence. From their examination of a previously published human dataset (GSE5900), they deduced that MGUS/SMM PCs exhibit higher levels of the aging markers *CDKN1A* and *GADD45A* than healthy PCs do. *GADD45A* was identified as the most important upregulated gene in clonal PC compared to normal plasma cells (NPC) [19]. López Corral et al. [19] reported down-regulation of the *GADD45A* gene in the healthy group, in their RNAseq study which is opposite to our study findings. This situation may have been caused by the lack of confirmation by qRT-PCR in their study. Also, it may be related to the small number of samples in our study groups. But in their study, the number distribution in the comparison groups seems to be quite uneven (20 patients with MGUS, 33 with high-risk SMM, and 41 with MM were compared with only 5 healthy donors).

Plasma cells that produce an aberrant monoclonal protein, often known as the M protein, are a defining characteristic of MGUS. Even though MGUS does not cause any symptoms in itself, there is a 1% chance that it will progress into MM each year. It is unknown what causes MGUS or why certain instances of MGUS develop into MM. According to our RT2-Profiler PCR analysis of 22 genes, *GADD45A* expression was significantly decreased in the MGUS group compared to the control group (p=0.027). To address this uncertainty, it is important to identify genes that distinguish benign from malignant gammopathies. We believe that *GADD45A* may be involved in disease progression from MGUS to MM and can be used as a biomarker to distinguish benign from malignant gammopathies. Increases in *GADD45A* transcript levels have been observed under stressful growth arrest conditions and after treatment with DNA-damaging agents [20]. It is known that there is a significant correlation between *GADD45A* expression and apoptosis. Our results on *GADD45A* expression

may guide the elucidation of the complex genetic mechanism of MGUS. The decreased expression of *GADD45A* suggests that this gene may be the underlying cause of increased abnormal M protein levels, in which cells with MGUS do not achieve adequate cell cycle arrest in a p53-dependent manner. In addition, since no treatment similar to MM was applied in individuals with MGUS, we can conclude that there was no increase in the level of *GADD45A* transcription with treatment and therefore no triggering of apoptosis in cells with MGUS. The characterization of the MM progression of MGUS was explored through meta-analysis on GEO datasets by Aljabban, et al. They reported that the upregulation of *GADD45A*, a DNA-damaging protein, was discovered to encourage DNA methylation [21].

The data from the present study suggest that *GADD45* plays a relevant role in the pathogenesis of MGUS, a plasma cell neoplasm. In this respect, the role of *GADD45* in other plasma cell disorders should also be investigated. In our study, we shared the view that *GADD45A* has an important effect on the cell death mechanism in the p53 pathway. We examined the decrease in the expression of *GADD45A*, which is an aging marker, in individuals with MGUS compared to healthy controls and how the cells with MGUS progress towards multiple myeloma, where MGUS cells do not exhibit aging characteristics.

The first limitation of the current study was the small sample size of the groups, and the second was the mean age difference between the patient and control groups. However, samples from bone marrow transplant donors were collected because of the difficulty and risk associated with obtaining bone marrow samples.

In conclusion, in our study, according to the ROC analysis results, the detection of a significant difference only in the *GADD45A* gene among 22 genes suggests that it can be used as a differential biomarker, especially for diagnosis in individuals with MGUS. Although equal numbers of bone marrow samples were taken from individuals in the MGUS and control groups, the number of samples can be increased in future studies. In addition, although no difference was observed in the mRNA levels of 21 genes in our study, a difference was detected in the protein levels in MGUS patients.

In our study, it was determined that the expression level of only the *GADD45A* gene, out of 22 genes whose expression levels were examined, decreased significantly in the MGUS group compared to the control group. According to Pearson correlation data, *GADD45A* gene expression was highly correlated with 12 other genes (*APAF1, CDK4, PCNA, BAX, CDKN2A, CASP9, CHEK2, MDM2, RB1, P53, BCL2, CHEK1*) examined in the p53 pathway. Thus, these 12 genes were down-regulated in MGUS.

As a continuation of this study, it may be possible to detect changes in protein levels associated with our genes in the p53 pathway and reveal their relationship at the M protein level. Thus, the functional processes underlying the unknown mechanism of MGUS in *GADD45A* could be determined, and the possible pathogenesis of MGUS in MM could be elucidated. The detection of genetic markers that can distinguish MGUS from MM tumor cells is important in the pathophysiology of MGUS. Therefore, the idea that *GADD45A* can be used as a genetic biomarker in our study may contribute significantly to the literature.

At the same time, using genetic biomarkers can provide effective treatment protocols that will significantly halt the progression of MGUS.

Conflict of interest: The authors declare no conflicts of interest.

References

1. Landgren O. Advances in MGUS diagnosis, risk stratification, and management: introducing myeloma-defining genomic events. Hematology Am Soc Hematol Educ Program 2021;2021:662-672. https://doi.org/10.1182/hematology.2021000303
2. van de Donk NWCJ, Pawlyn C, Yong KL. Multiple myeloma. Lancet 2021;397:410-427. https://doi.org/10.1016/S0140-6736(21)00135-5
3. Akhtar S, Najafzadeh M, Isreb M, Newton L, Gopalan RC, Anderson D. An in vitro investigation into the protective and genotoxic effects of myricetin bulk and nano forms in lymphocytes of MGUS patients and healthy individuals. Toxicol Lett 2020;327:33-40. https://doi.org/10.1016/j.toxlet.2020.03.012
4. Xiong W, Wu X, Starnes S, et al. An analysis of the clinical and biologic significance of TP_{53} loss and the identification of potential novel transcriptional targets of TP_{53} in multiple myeloma. Blood 2008;112:4235-4246. https://doi.org/10.1182/blood-2007-10-119123

5. Awada H, Thapa B, Awada H, et al. A comprehensive review of the genomics of multiple myeloma: evolutionary trajectories, gene expression profiling, and emerging therapeutics. Cells 2021;10:1961. https://doi.org/10.3390/cells10081961
6. Mikulasova A, Wardell CP, Murison A, et al. The spectrum of somatic mutations in monoclonal gammopathy of undetermined significance indicates a less complex genomic landscape than that in multiple myeloma. Haematologica 2017;102:1617-1625. https://doi.org/10.3324/haematol.2017.163766
7. Geraldes C, Gonçalves AC, Cortesão E, et al. Aberrant p15, p16, p53, and DAPK gene methylation in myelomagenesis: clinical and prognostic ımplications. Clin Lymphoma Myeloma Leuk 2016;16:713-720. https://doi.org/10.1016/j.clml.2016.08.016
8. Oka S, Ono K, Nohgawa M. The effect of azacitidine therapy on the M protein of MDS patients with concomitant MGUS. Am J Hematol 2018;93:220-222. https://doi.org/10.1002/ajh.25160
9. Wang W, Shim YK, Michalek JE, et al. Serum microRNA profiles among dioxin exposed veterans with monoclonal gammopathy of undetermined significance. J Toxicol Environ Health A 2020;83:269-278. https://doi.org/10.1080/15287394.2020.1749919
10. Ho M, Patel A, Goh CY, Moscvin M, Zhang L, Bianchi G. Changing paradigms in diagnosis and treatment of monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM). Leukemia 2020;34:3111-3125. https://doi.org/10.1038/s41375-020-01051-x
11. Liebermann DA, Hoffman B. Gadd45 in stress signaling. J Mol Signal 2008;3:15. https://doi.org/10.1186/1750-2187-3-15
12. Patel K, Murray MG, Whelan KA. Roles for GADD45 in development and cancer. Adv Exp Med Biol 2022;1360:23-39. https://doi.org/10.1007/978-3-030-94804-7_2
13. Higashi H, Vallböhmer D, Warnecke Eberz U, et al. Down-regulation of Gadd45 expression is associated with tumor differentiation in non-small cell lung cancer. Anticancer Res 2006;26:2143-2147.
14. Guo W, Dong Z, Guo Y, Chen Z, Kuang G, Yang Z. Methylation-mediated repression of *GADD45A* and GADD45G expression in gastric cardia adenocarcinoma. Int J Cancer 2013;133:2043-2053. https://doi.org/10.1002/ijc.28223
15. Wang W, Huper G, Guo Y, Murphy SK, Olson JA, Marks JR. Analysis of methylation-sensitive transcriptome identifies *GADD45A* as a frequently methylated gene in breast cancer. Oncogene 2005;14:2705-2714. https://doi.org/10.1038/sj.onc.1208464
16. Reddy SP, Britto R, Vinnakota K, et al. Novel glioblastoma markers with diagnostic and prognostic value identified through transcriptome analysis. Clin Cancer Res 2008;15:2978-2987. https://doi.org/10.1158/1078-0432.CCR-07-4821
17. Myint KZ, Kongpracha P, Rattanasinganchan P, et al. Gadd45β silencing impaired viability and metastatic phenotypes in cholangiocarcinoma cells by modulating the EMT pathway. Oncol Lett 2018;15:3031-3041. https://doi.org/10.3892/ol.2017.7706
18. Borges GA, Guilatco AJ, Hachfeld CM, et al. Abstract PRO12: Pre-malignant plasma cells exhibit a senescence-like phenotype and accumulation of transposable elements. Cancer Research 2023;83. https://doi.org/10.1158/1538-7445.AGCA22-PR012
19. López Corral L, Corchete LA, Sarasquete ME, et al. Transcriptome analysis reveals molecular profiles associated with evolving steps of monoclonal gammopathies. Haematologica 2014;99:1365-1372. https://doi.org/10.3324/haematol.2013.087809
20. Salvador JM, Brown Clay JD, Fornace AJ Jr. Gadd45 in stress signaling, cell cycle control, and apoptosis. Adv Exp Med Biol 2013;793:1-19. https://doi.org/10.1007/978-1-4614-8289-5_1
21. Aljabban J, Chen D, Cottini F, et al. Characterization of monoclonal gammopathy of undetermined significance progression to multiple myeloma through meta-analysis of Geo data. Blood 2019;134:4395. https://doi.org/10.1182/blood-2019-129699

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Authors' contributions to the article

I.S., A.D.A., and G.O. contributed equally to the construction of the main idea and hypothesis of the study, developed the theory and arranged the material and method section, evaluated the data in the results section, wrote the discussion section of the article, reviewed, corrected, and approved. In addition, all authors discussed the entire study and approved the final version.