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THE GENOMIC LANDSCAPE OF THE SWITCH/SUCROSE NON-FERMENTABLE CHROMATIN REMODELING COMPLEX IN ACUTE MYLEOID LEUKEMIA
AKUT MİYELOİD LÖSEMİDE SWITCH/SUKROZ FERMENTE EDILEMEZ KROMATİN YENİDEN ŞEKİLLENDİRME KOMPLEKSİNİN GENOMİK GÖRÜNÜMÜ

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ABSTRACT

The SWI/SNF chromatin remodeling complex is involved in the regulation of gene expression required for processes such as cell maintenance and differentiation in hematopoietic stem cells. Abnormalities in the SWI/SNF subunits involved in the homeostasis of hematologic processes contribute to the initiation or progression of hematologic malignancies, but the mechanisms underlying this phenotype are not yet fully understood. The aim of study is to comprehensively identify mutations and expression profiles in the genes forming the SWI/SNF complex using bioinformatics tools, with a focus on understanding the underlying mechanisms. Genomic sequences and expression profiles of an AML cohort (n:872) were obtained from using tools and subsequently analyzed. PolyPhen-2, SIFT, and Mutation Assessor tools were used to estimate the oncogenic/pathogenic effects of mutations identified in 9 genes encoding subunits of the complex *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PMBR1*, and *BCL7A*in AML pathogenesis. STRING analysis was performed to better understand the functional relationships of the mutant proteins in cellular processes.Furthermore, to the mutation profile, gene expression and survival profiles were also determined. A total of 17 genetic abnormalities were determined in 9 genes, including 9 missense, 6 frameshift mutations, 1 mutation in the splice region, and 1 fusion mutation. In the AML cohort, the expression levels of *ARID1A, ARID1B, SMARCA2*, and *PMBR1*were significantly higher in the patient group compared to the healthy group ($p<0.01$). Survival analysis based on low and high gene expression profiles showed no significant difference in results. In STRING analysis, our genes were found to have functional relationships with the PHF10protein, which is involved in cell cycle control. The results suggest that the

ÖZ

SWI/SNF kromatin yeniden modelleme kompleksi, hematopoietik kök hücrelerde hücre bakımı ve farklılaşma gibi süreçler için gerekli olan gen ekspresyonunun düzenlenmesinde görev alır. Hematolojik süreçlerin homeostazında yer alan SWI/SNF kompleksi alt birimlerindeki değişiklikler hematolojik malignitelerin başlamasına veya ilerlemesine katkıda bulunmaktadır, ancak bu fenotipin arkasındaki mekanizmalar tam olarak açıklanmamıştır. Çalışmada, SWI/SNF kompleksini oluşturan genlerde mutasyonların ve ekspresyon profilinin biyoinformatik araçları kullanılarak kapsamlı belirlenmesi amaçlanmıştır. AML kohortuna (n:872) ait genom dizileri ve ifade profillerine biyoinformatik araçlar aracılığı ile elde edilmiş ve analiz edilmiştir. Kompleksin alt ünitelerini kodlayan 9 gende *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PMBR1* ve *BCL7A* belirlenen mutasyonların AML patogenezinde onkojenik/patojenik etkilerinin tahmini PolyPhen-2, SIFT ve Mutation Assessor araçları kullanılmıştır. Mutasyona uğrayan proteinlerinin fonksiyenel etkilerini anlamak içinSTRING aracı ile analiz gerçekleştirilmiştir. Mutasyon profili değil aynı zamanda mutasyon varlığının gen ifadesi ve sağ kalım üzerine etkileride değerlendirilmiştir. 9 gende 9 yanlış anlam, 6 çerçeve kayması mutasyon, 1 splize bölge ve 1 füzyon mutasyonu olmak üzere toplam 17 genetik anormallik belirlenmiştir.AML kohortunda *ARID1A, ARID1B, SMARCA2 ve PMBR1*ekspresyon seviyelerin hasta grubunda sağlıklı gruba yüksek ve istatistiksel olarak anlamlıdır ($p<0.01$). Düşük ve yüksek gen ekspresyon profillerine göre yapılan sağ kalım analizi sonuçlarımızda bir farklılık görülmemiştir. STRING analizinde, hedef genlerimizin, hücre döngüsü kontrolünde görev alan PHF10ile fonksiyonel ilişkileri bulunduğu belirlenmiştir. Sonuç olarak, sonuçlarımız, *ARID1A, ARID1B, SMARCA2, SMARCA4 ve PBRM1'de* tespit ettiğimiz mutasyonlarının, SWI/SNF kromatin yeniden modelleme komplekslerinin fonksi-

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mutations identified in the *ARID1A, ARID1B, SMARCA2, SMARCA4*, and *PBRM1*may disrupt the function of SWI/SNF chromatin remodeling complexes, possibly inducing/activating different cellular pathways involving different chromatin environments during AML pathogenesis.

Keywords: AML, chromatin remodeling,gene expression, mutation, SWI/SNF complex

yonunu bozarakAML patogenezi sırasında farklı kromatin ortamlarını içeren farklı hücresel yolları indükleyebileceği/inaktive edebileceğini düşündürmektedir.

Anahtar Kelimeler: AML, gen ifadesi, kromatin remodülasyonu, mutasyon, SWI/SNF kompleksi

INTRODUCTION

The The Switch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex is a group of protein complexes responsible for regulating the organization of proteins around the cell's genetic material. This complex modulates gene expression by altering the structure of chromatin, allowing access to the genetic information of cells in various processes. Functionally, it operates by histone modification and nucleosome sliding.[1,2] The complex complex alters the structure of chromatin by modifying histone proteins around Deoxyribonucleic Acid (DNA). These histone modifications can loosen or tighten the binding of histones to DNA, thereby affecting the accessibility and expression of genes. Alternatively, it changes the physical access to DNA by shifting structures called nucleosomes, where DNA and histone proteins come together. This can influence the opening or closing of gene promoter regions, thus controlling gene expression.[3-5] In cancer, the function of the SWI/SNF complex can be disrupted or mutated. This can often contribute to the dysregulation of critical cellular processes such as cell cycle control, cell differentiation, and apoptosis, thereby contributing to cancer development.[6] Cancer genome studies have found a high prevalence of mutations in genes encoding subunits of the SWI/SNF chromatin remodeling complexes, with approximately 20-25% of all cancers having abnormalities in one or more of these genes.[6-8] Overall, the mammalian SWI/SNF complex is an evolutionarily conserved chromatin remodeling complex composed of more than 20 subunits.The SWI/SNF chromatin remodeling complex consists of genes including ARID1A (AT-rich interaction domain 1A), ARID1B (AT-rich interaction domain 1B), SMARCA2 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 2), SMARCA4 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 4), SMARCE1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1), SMARCB1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1), DPF2 (Double PHD Fingers 2), PBRM1 (Polybromo 1), and BCL7A (B-cell CLL/lymphoma 7A).[4,7,10] SWI/SNF complexes are Adenosine Triphosphate (ATP)-dependent chromatin remodeling proteins capable of displacing, removing or altering the composition of nucleosomes by ATP hydrolysis. Due to this activity, the complexes play a crucial role in normal physiological activities in cells by ensuring the appropriate expression of genes in processes such as replication, transcription, translation and post-translational modifications.[6,8-10] Hematologic malignancies represent a very heterogeneous group of diseases with different molecular and phenotypic features. Adequate SWI/SNF function is crucial for various differentiation processes, including hematopoiesis and hematopoietic stem cell maintenance.[7,11-13]

In addition, genetic abnormalities in the subunits of the SWI/SNF complex, particularly *ARID1A/1B/2, SMARCA2/4*, and *BCL7A*, are common in various lymphoid and myeloid malignancies. Most genetic abnormalities in the complex lead to a loss of function of the subunit and the acquisition of oncogenic mechanisms, suggesting a tumour suppressive role of the genes forming the complex.[2,11-13] Although more than 20 % of tumors and hematological malignancies in children and adults are characterized by a deficiency of the SWI/SNF complex, the molecular background of this phenotype is not yet fully understood.[2,10,14,15] In particular, there is increasing evidence that mutations in the subunits of the SWI/SNF complex confer resistance to various antineoplastic agents used in the treatment of hematologic malignancies.[10,12,13,16,17] Studies that elucidate the function of SWI/SNF and the outcomes of SWI/SNF abnormalities in detail are often lacking, especially in hematologic malignancies. Therefore, we aimed to establish a comprehensive genetic profile to understand the contribution of mutations or inactivation of genes encoding the subunits of the SWI/SNF complex to the pathogenesis of Acute Myeloid Leukemia (AML).

MATERIAL AND METHODS

Design of the Study Group

The AML (n:872) data set was downloaded from the cBioPortal database and the study's raw data accessible via cBioPortal. Data were downloaded on September 02, 2023.

Mutation Profile Analysis

The CBio Cancer Genomics Portal (http://cbioportal.org) is a freely accessible tool that provides mutation data from The Cancer Genome Atlas (TCGA) as a data source.[18] The AML was selected as the cancer of interest to comprehensively investigate mutations in the *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PMBR1*, and *BCL7A* in AML samples (n:872) via the web interface. Comprehensive mutation profile analyzes were then performed using the functions provided by the interface for the genes of interest, using the tools provided by the Cbio portal.

Functional/Pathogenic Effect Analysis of Identified Mutations

Scores provided by the Polymorphism Phenotyping v2 (PolyPhen-2), Sorting Intolerant from Tolerant (SIFT), and Mutation Assesor databases were used to identify the potential pathogenicity and clinical impact of muta-

tions identified in the *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2*, *PMBR1* and *BCL7A*. PolyPhen-2 is an online accessible tool that estimates the potential impact of mutations on protein stability and function by using structural and comparative evolutionary analyzes of amino acid positions for potential mutations and Single Nucleotide Polymorphisms (SNPs).[19] PolyPhen-2 assesses the likelihood that a missense mutation will cause damage to the protein based on a combination of these properties, gives the user a score, and categorizes the result as likely deleterious, possibly deleterious, benign, and unknown. The SIFT (https://sift.bii.a-star.edu.sg/) is a tool that estimates whether a change in amino acid position can impact protein function based on sequence homology and the physical properties of amino acids.[20] SIFT separates an amino acid substitution as either bearable or detrimental to protein function. Mutation Assessor (http://mutationassessor.org/r3/) estimates the functional effects of amino acid substitutions in proteins, including mutations found in cancer or polymorphisms. The assessment is based on the evolutionary preservation of the impacted amino acid in protein homologs.[21]

Identification of Differentially Expressed Genes and Survival Analysis

GEPIA (Gene Expression Profiling Interactive Analysis) (http://gepia.cancer-pku.cn/) is a database that allows users to perform differential expression analysis at the subtype level.[22] GEPIA is used to analyze the expression of genes and isoforms by comparing TCGA data. Therefore, we used this data provider to determine the differential expression of the *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PMBR1*, and *BCL7A* in AML cohorts (n:173) and healthy tissue samples. Boxplots were generated using Ribonucleic acid–sequencing (RNA-Seq) normalized expression levels, and statistical tests were automatically calculated by the GEPIA. p-values were automatically calculated, and p-values below 0.05 were considered statistically significant.TPM (Transcripts Per Million) were used to measure m-Ribonucleic Acid (RNA)expression levels. The expression data are first log2 (TPM+1) transformed for differential analysis and the log2FC is determined as median (Tumor)-median (Normal). Genes with higher [log2FC] values and lower q values than pre-set thresholds are considered differentially expressed genes.

Protein- Protein Interaction Analysis

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org) is used to determine protein-protein interactions.[23] The purpose of this database is to create a comprehensive and objective global network that includes both physical and functional interactions. The predicted interactions of ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PMBR1, and BCL7A proteins were determined by STRING, which identifies physical and functional relations between proteins.

Statistical Analysis

All statistical analyzes used in the analysis of the study data were performed using the GEPIA database. The GEPIA database uses the differential analysis method to compare gene expression in tumor and healthy study groups. The test used to calculate differential expression is the one-way Analysis of Variance (ANOVA). The analysis of overall survival was performed using Kaplan-Meier curves, with the log-rank test used to compare low and high expression groups. For all tests performed, a statistically significant value was considered to be $p < 0.05$.

RESULTS

Demographic and Clinical Characteristics of the Study Group

Detailed demographic and clinical characteristics of the dataset consisting of 872 AML patients are presented in Table 1.

Results of Mutation Profile in SWI/SNF Complex Genes in AML

In the AML cohort (n:872), mutations in the study genes were identified in 2.5% of patients, with the highest mutation frequency observed in the *ARID1A* (0.8%), while no mutations were detected in the *SMARCE1*. A total of 17 mutations were identified in 9 genes, including 9 missense, 6 frame shift and 1 splice domain mutation. Table 2 contains detailed information on the identified mutations. Figure 1-A shows the frequency of mutations in the genes of the AML cohort, and Figure 2 illustrates the localization of mutations in the domains of the proteins. The somatic mutation frequency of the *ARID1A* was detected to be 0.7%, with two of the identified mutations being putative driver mutations. The driver mutations p.S446Lfs176 and p.S949Hfs57 could lead to a shift in the reading frame, possibly resulting in premature termination of the polypeptide and the formation of a truncated protein. The p.G1234D missense mutation is located in the HIC1 binding domain, while the p.R1658Q mutation is located in the GR binding domain. The ARID1A-RPS6KA1 fusion was identified as a structural variant. Three frame-shift deletions altering the reading frame and a p.R2128P missense mutation in the DUF3518 domain were detected in *ARID1B*. The somatic mutation frequency of the *ARID1B* was detected to be 0.5%.In *SMARCA2*, the p.X119_splice mutation was found at the boundary between exon 13 and intron 14, i.e. in a splice site that is 100% conserved across all species. In addition, the p.V685L and p.L753F missense mutations on *SMARCA2* are located in the helicase ATPase binding domain. The somatic mutation frequency of the *SMARCA2* was 0.3%.The p.P653Rfs*121 frameshift mutation in the BRK domain of *SMARCA4* is a driver mutation that could lead to premature termination of the polypeptide and the formation of a truncated protein. The somatic mutation frequency of the *SMARCA4* was 0.1%. Gene amplification abnormalities were also observed in *SMARCA2*, *SMARCA4* and *SMARCB1.DPH2* had two missense mutations, p.C295S and p.G302R, in the PHD finger domain, which serves as a bridge for the components of the SWI/SNF complex. The somatic mutation frequency of the *DPH2* was 0.2%.The somatic mutation frequency of the *PBRM1* was 0.3%, with the p.K250R missense mutation identified in the Br domain and the P919Q mutation in the BAH domain.

In Silico Pathogenic/Oncogenic Feature Analysis of Identified Mutations

The PolyPhen-2, SIFT, and Mutation Assessor were used for in silico analysis to predict pathogenic/oncogenic traits. According to the analysis results of these three

programs, 5 of the 17 mutations identified in our study were classified as disease-causing. Detailed information on the mutations with oncogenic/pathogenic character can be found in Table 1. In addition, the Onco KB database classified the mutations p.S446Lfs176, p.S949Hfs57 in *ARID1A*, ARID1A-RPS6KA1 fusion mutations, *SMARCA2* p.X119_splice and *SMARCA4* p.P653Rfs*121 as oncogenic.

Survival Analysis and Expression Profile of Key Genes

The gene expression profiles of *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PMBR1* and *BCL7A* were analyzed using the AML cohort (n:173) and the matching healthy tissue (n:50) available on GEPIA. The expression levels of *ARID1A, ARID1B, SMARCA2,* and *PMBR1* were upregulated in the patients

Table 1. Demographic, clinical and genetic data of patients with AML

	Patient data n: 872(%)
Gender	
Male/Female/NA	311/251/200
Diagnosis age, years	(1-87)
Chromosomal abnormality	
t(8;21)	11 (1.2)
inv(16)	18 (2.0)
11q23	4 (0.4)
t(15,17)	15 (1.7)
Cytogenetic risk	
Favorable	109 (12.5)
Intermediate	116 (13.3)
Unfavorable	132 (15.1)
NA/other	214 (24.5)
Diagnosis type	
FAB subtype	
M0	10 (1.1)
M1	15 (1.7)
M2	13 (1.5)
M3	11(1.3)
M4	35 (4.0)
M5	32 (3.7)
M7	2(0.7)
NA/other	731 (83.8)
Overall Survival Status	
Living	282 (37.0)
Deceased	423 (55.5)
NA	57 (7.5)
Total Mutation Frequency in AML	**Case (Frequency%)**
ARID1A genetic alteration	0.8
ARID1B genetic alteration	0.5
SMARCA2 genetic alteration	**Characteristic** 0.5
SMARCA4 genetic alteration	0.3
SMARCE1 genetic alteration	0
SMARCB1 genetic alteration	0.1
DPF2 genetic alteration	0.3
PBRM1 genetic alteration	0.4
BCL7A genetic alteration	0

Abbreviations: NA: Not Applicable; M: Metastasis

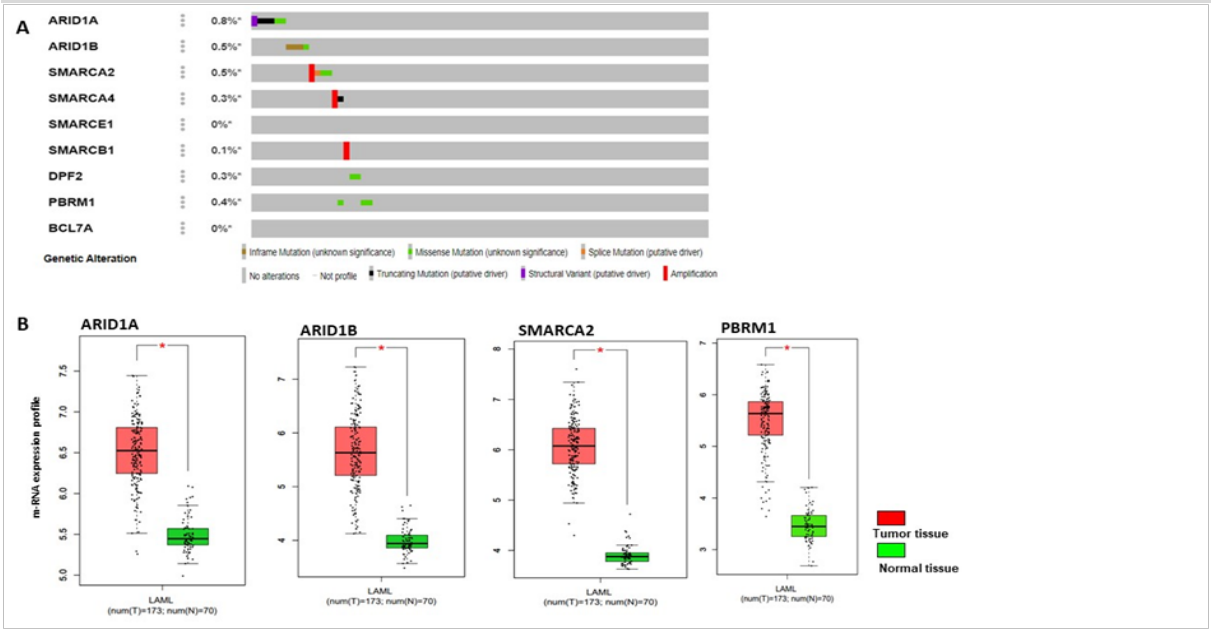

Figure 1: (A) Distribution of mutations in *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PBRM1*, and *BCL7A*genes in TCGA AML cohort from cBioPortal.Percentages of total mutations for each gene are given on the left**. (B)** GEPIA was performed to validate higher expression of thress hub genes (*ARID1A, ARID1B*, and *SMARCA2*) in AML samples compared with healthy samples. The red and green boxes represent AML and healthy tissues respectively. *represented p<0.01.

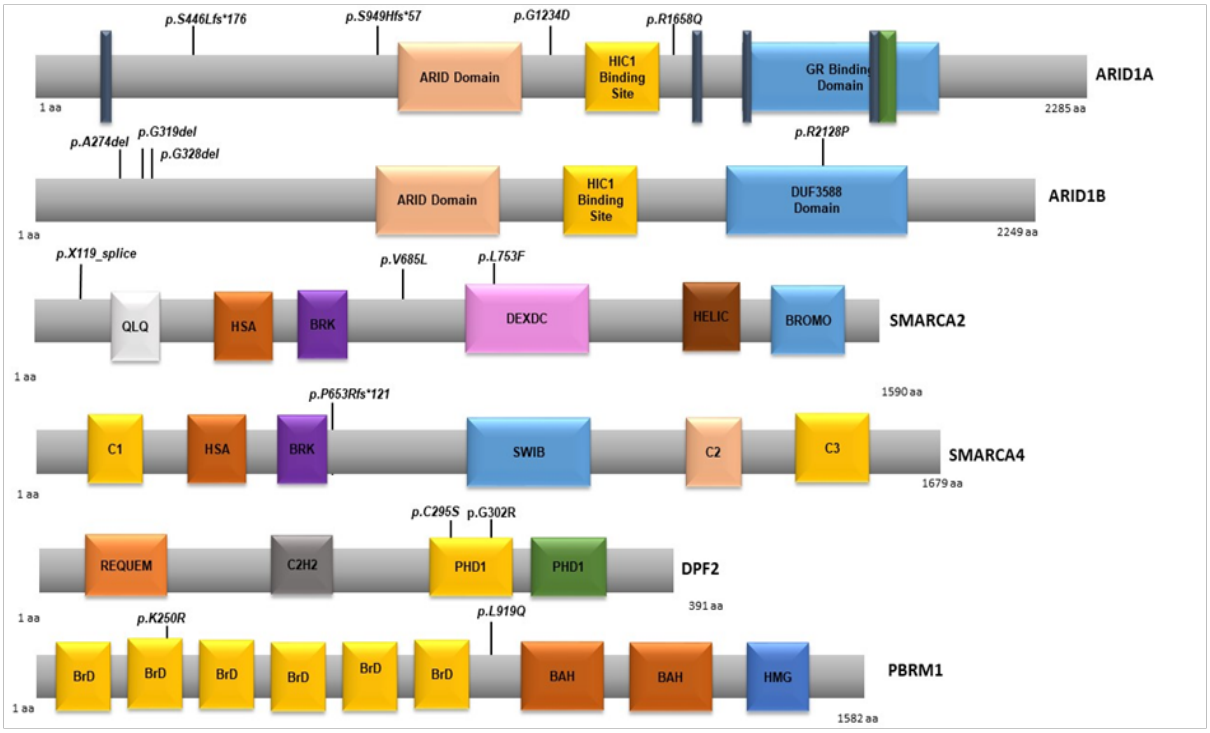

Figure 2: Schematic representation of domain architecture of the ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, and, PBRM1 proteins and mutations identfied in patients with AML. Human ARID1A is a polypeptide comprising 2285 amino acids. Human ARID1B is a polypeptide comprising 2249 amino acids. Human SMARCA2 is a polypeptide comprising 1590 amino acids. Human SMARCA4 is a polypeptide comprising 1679 amino acids. Human DPF2 is a polypeptide comprising 391 amino acids. Human PBRM1 is a polypeptide comprising 1582 amino acids.

compared to the control group (p<0.01) (Figure 2B). Our survival analysis based on low and high gene expression levels revealed that the expression levels of the genes had no significant impact on the overall survival (OS) of AML patients.

Analysis of the protein-protein interaction

STRING analysis was performed to detect the interactions of ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PMBR1 and BCL7A proteins in cellular processes. As shown in Figure 3, our target

Table 2. Detailed mutations of the *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1*, and *DPF2* in AML patient.

No	*Gene*	Nt alteration	Rs Number	Mutation Type	Localization	AA position	Type of Cancer	Pathogenic/Oncogenic Feature		
								Poly-Phen2 (score)	SIFT (score)	Mutaion Assesor (score)
M-1	*ARID1A*	c.1332_1333del	NA	Frame Shift Deletion	Exon-2	S446Lfs*176	AML with inv(16) (p13.1q22) or t(16;16) (p13.1;q22); *CBFB-MYH11*	NA	NA	NA
M-2	*ARID1A*	c.2845_2846del	NA	Frame Shift Deletion	Exon-9	S949Hfs*57	AML	NA	NA	NA
M-3	*ARID1A*	c.4973G>A	COSV61377605	Missense mutation	Exon-18	R1658Q	APL with *PML-RARA*	Probably Damaging (0.99)	Deleterious (0.00)	Low (1.70)
M-4	*ARID1A*	c.3701G>A	NA	Missense mutation	Exon-14	G1234D	AML	Possibly Damaging (0.90)	Deleterious (0.00)	Low (1.75)
M-5	*ARID1A*	-	NA	Fusion	-	ARID1A-RPS6KA1 Fusion	AML	NA	NA	NA
M-6	*ARID1B*	c.942_944del	NA	Frame Shift Deletion	Exon-1	G319del	APL with *PML-RARA*	NA	NA	NA
M-7	*ARID1B*	c.821_823del	NA	Frame Shift Deletion	Exon-1	A274del	AML with Minimal Differentiation	NA	NA	NA
M-8	*ARID1B*	c.983_985del	NA	Frame Shift Deletion	Exon-1	G328del	AML, NOS	NA	NA	NA
M-9	*ARID1B*	c.6383G>C	NA	Missense mutation	Exon-20	R2128P	APL with *PML-RARA*	Probably Damaging (1.00)	Deleterious (0.00)	Mediun (2.73)
M-10	*SMARCA2*	c.356-2A>T	NA	Splice region mutation		X119_splice	AML	NA	NA	NA
M-11	*SMARCA2*	c.2053G>T	NA	Missense mutation	Exon-14	V685L	AML	Probably Damaging (0.98)	Deleterious (0.03)	Mediun (2.00)
M-12	*SMARCA2*	c.2257C>T	COSV61812998	Missense mutation	Exon-15	L753F	AML with Mutated *NPM1*	Probably Damaging (1.00)	Deleterious (0.00)	High (3.94)
M-13	*SMARCA4*	c.1958del	NA	Frame Shift Deletion	Exon-13	P653Rfs*121	AML	NA	NA	NA
M-14	*DPF2*	c.883T>A	COSV52889333	Missense mutation	Exon-8	C295S	AML with Mutated CEPBA	Probably Damaging (1.00)	Deleterious (0.00)	High (3.74)
M-15	*DPF2*	c.904G>A	NA	Missense mutation	Exon-8	G302R	AML with Mutated NPM1	Probably Damaging (1.00)	Deleterious (0.00)	Mediun (3.29)
M-16	*PBRM1*	c.749A>G	NA	Missense mutation	Exon-8	K250R	AML	Probably Damaging (1.00)	Tolerated (0.19)	Neutral (0.66)
M-17	*PBRM1*	c.2756T>A	NA	Missense mutation	Exon-18	L919Q	AML	Benign (0.00)	Tolerated (0.42)	Low (1.25)

Abbreviations: M: Mutation; NA: Not available; Nt: Nucleotid; Rs: Register; AA: Amino acid; Inv: Inversion: t: translocation

genes SMARCA4, SMARCE1 and SMARCB1 interact with the PHF10 protein.

DISCUSSION

The SWI/SNF complex is involved in the homeostasis of hematologic processes, and mutations in the SWI/SNF subunits are thought to contribute to the development or progression of hematologic malignancies. There fore, the mutation and expression profiles of our target genes, namely *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PMBR1*, and *BCL7A*, were detected in detail using the genome sequencing data of 872 adult patients diagnosed with AML from the TCGA. Among the 17 mutations identified in a total of 9 genes in the 872 AML patients, there were 9 missense, 6 frameshift mutations, 1 mutation in the splice region and 1 fusion. *ARID1A* was found to be the most frequently mutated gene, while *SMARCE1* and *BCL7A* had no mutations. *ARID1A* is the most frequently mutated subunit in SWI/SNF complexes, with mutations occurring in approximately 8% of cancers. Mutations that render SWI/SNF subunits ineffective are found in approximately 20% of cancers, suggesting that proper function of this complex is necessary to prevent tumor formation in various tissues.[2,10,14,15,16] However, mutations of the SWI/SNF complex are rarely detected in AML compared to other hematologic cancers and solid tumors. This suggests that the complex may not exert a significant tumor suppressor function for this malignancy.[7,12,14,15,24] *ARID1A* and *SMARCA4* play a role in both myeloid and lymphoid differentiation, regulate erythropoiesis and are involved in granulocytic maturation.[7,10,25,26] *ARID1A* is located on 1p36, a chromosomal part that is frequently deleted in tumors, and it is known that nonsense and frameshift mutations in the gene lead to a loss of function.[27] The two frameshift mutations (p.S446Lfs176 and p.S949Hfs57) that we identified in the AML cohort are driver mutations and may contribute to the loss-of-function phenotype. The ARID1A-RPS6KA1 fusion variant we discovered as a structural variant encompasses the *RPS6KA1*, which encodes a member of the ribosomal S6 kinase family of serine/threonine kinases. Many RTKs are known to play an active role in cancer development due to chromosomal translocations, and this pathological condition is also referred to as overexpression for short.[28] In our AML cohort, the expression level of *ARID1A* is higher compared to the healthy group, which might be due to the structural variant ARID1A-RPS6KA1.ARID1B protein has only two identified domains: AT-Rich Interactive Domain (ARID) and Domain of Unknown Function 3518 (DUF3518). DUF3518 is approximately 260 amino acids lenght, and this domain is known to interact with the helicase subunits BRG1 and BRM in BAF complexes.[28,29] In our study, the p.R2128P mutation was determined on the DUF3518 domain, and therefore it is likely that these missense mutations interrupt the interaction between *ARID1B, BRG1*, and *BRM*. *ARID1A* and *ARID1B* have been defined as tumor suppressor genes involved in chromatin remodeling, epithelial-mesenchymal transition, and many other cellular and molecular processes.[29-31] However, their role in AML has not yet been clarified. The molecular mechanisms related to *ARID1B* mRNA expression appear to be different in different cancer tumors.[32] In our AML cohort, *ARID1B* expression levels were higher compared to the healthy group. Considering the tumor suppressor nature and the need to elucidate the molecular mechanism, this suggests that *ARID1A* and *ARID1B* may not act as tumor suppressors specifically in AML.

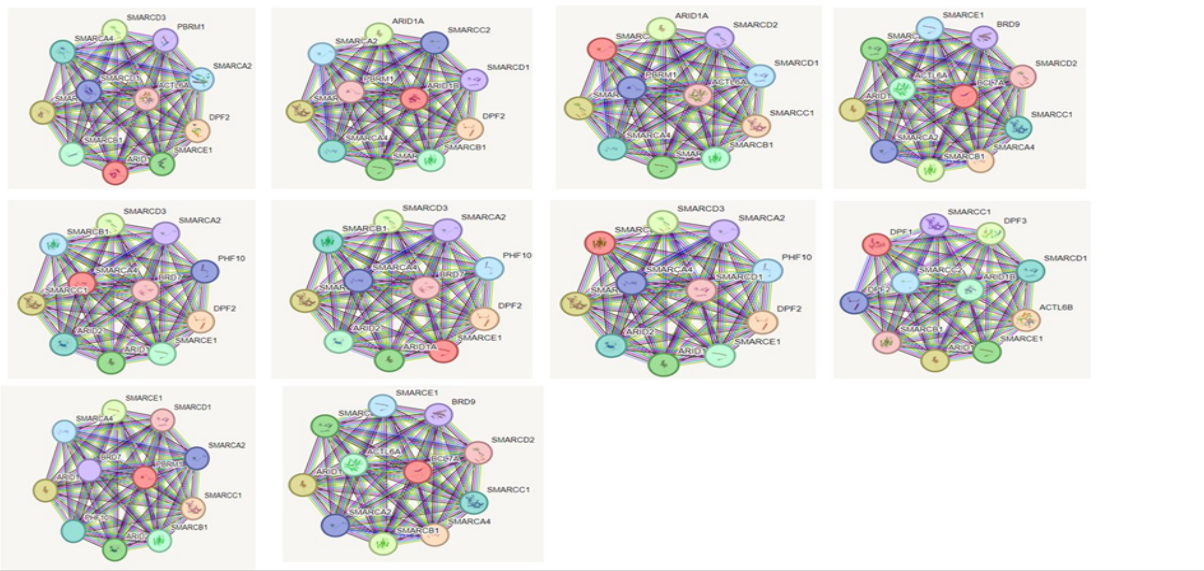

Figure 3: Schematic representation of known and predicted protein-protein interactions with the ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PMBR1, and BCL7A proteins.

SMARCA2 has been shown to process the telomerase reverse transcriptase (TERT) gene and modulate the splicing mechanism of *TERT*. Since *TERT* activation is thought to be cancer-promoting, SMARCA2 is not thought to have a tumor suppressive function.[33-34] We believe that the p.V685L and p.L753F mutations we discovered in the SNF2 ATPase domain of *SMARCA2* may have a dominant-negative effect by eliminating the

ATP-hydrolyzing motor potential of the protein, thereby abrogating its ability to reposition histones on DNA. The p.X119_splice mutation we identified on the same gene is oncogenic in nature and typically results in loss of protein function of *SMARCA2*. The *SMARCA4* encodes the BRG1 protein, which belongs to the SWI/SNF family of proteins responsible for remodeling chromatin to regulate transcription of multiple genes.[34-36] The frameshift mutation p.P653Rfs*121, which we discovered in *SMARCA4*, is a loss-of-function mutation that causes the polypeptide to break prematurely, resulting in a truncated protein. Recent studies have shown that mutations in the ATPase domain of *SMARCA4*, which we also identified, do not repress the Polycomb Repressive Complex (PRC)-1 from chromatin and result in loss of accessibility of enhancers.[33-35] *SMARCA4* also modulates the expression of CD44 and the function of MYC and can interact directly with the tumor suppressor gene BRCA1.[33-36] Upregulation of *SMARCA4* in tumor tissues is related with aggressive tumors, whereas upregulation of *SMARCA2* is associated with well-differentiated tumors, suggesting that *SMARCA4* and *SMARCA2* have opposite roles in tumor tissues.[37,38] In this study, the expression levels of *SMARCA4* did not differ from those of the control group, whereas *SMARCA2* had high expression levels in our AML cohort, suggesting that it may be a prognostic indicator for AML. In addition, the *SMARCA2* amplification identified in our study could also be a source of increased expression levels.SMARCA2 mutations can contribute to the development of new therapeutic strategies in the treatment of AML because these mutations can affect patients' response to treatment and the course of the disease.[6,7,36-38]Firstly, AML patients with SMARCA2 mutations may develop resistance to certain drugs or have reduced response to specific treatments. This can decrease the effectiveness of traditional treatment regimens and necessitate the exploration of alternative treatment options.[37,38] However, detected mutations in current study also provide an opportunity for the development of targeted therapies. For example, drugs or therapeutic agents targeting specific vulnerabilities associated with SMARCA2 mutations can be developed. This allows for the design of treatments that target the specific biological pathways affected by the mutation, potentially leading to better responses to treatment in AML patients with SMARCA2 mutations. As more information is gathered about the impact of this mutations on AML treatment, there is potential to develop more effective and personalized treatment options.

The *PBRM1* encodes BAF180, a protein that serves as a DNA target subunit of the pBAF SWI/SNF complex and contains six bromodomains. These bromodomains, particularly the one affected by the p.K250R missense mutation in our study, have the ability to recognize acetylated residues at histone tails, indicating a pathological nature that could disrupt histone-histone interactions. Bromodomains have the ability to recognize acetylation patterns and target the entire complex to specific chromatin regions.[39-41] *PBRM1* is known to be involved in DNA repair mechanisms. It facilitates DNA double-strand break repair, transcriptional silencing and maintenance of centromeric cohesion, which is critical for maintaining genomic stability.[6,40,41] The p.K250R mutation detected in our study may disrupt these interactions. Interestingly, our study showed that *PBRM1* has high mRNA expression in the AML cohort, suggesting an oncogenic role in contrast to its tumor suppressive role in other tissues. This highlights the complex role of *PBRM1* in different contexts and provides fundamental information for further research. The PBRM1 gene is a subunit of the SWI/SNF complex and can influence gene expression by regulating the structural organization of chromatin.[39-41] There fore, mutations in the PBRM1 gene are thought to potentially play a role in the prognosis of AML.

DPF2 is mutated in various cancers, including AML, lymphoma, and ALL, and the mutations frequently occur at hot-spot mutation sites, including PHD domains and the N-terminal region.[42] PHD domains are critical for reading post-translational modifications. The PHD domain is a structure that includes the ability of proteins to recognize and bind to histones. Therefore, the role of the PHD domain in the DPF2 gene in AML may influence histone modifications and consequently the ability to regulate gene expression. In our study, missense mutations (p.C295S and p.G302R) were detected in the PHD1 domain of *DPF2*. In particular, the Cys295 residue is critical for histone modifications. The ability of *DPF2* to bind to histones is necessary for the in vivo regulatory function of Hematopoietic stem/progenitor cells (HSPCs) in myeloid differentiation, and the detected mutations could affect histone modification.[42] In addition, the patient carrying this mutation also exhibits the RUNX1-RUNX1T1 translocation. Recent studies have shown that the inclusion of *DPF2* in a repressive complex containing *RUNX1* prevents the expression of RUNX1 target genes, including the myeloid-specific miR-223, and prevents myeloid distinction.[43]

In STRING protein-protein interaction analysis, the core proteins (hub proteins) SMARCA4, SMARCE1, and SMARCB1 interact with the protein PHF10, a subunit of the pBAF SWI/SNF complex that is required for its association with chromatin.[44] PHF10 protein has been reported to be required for proliferation of mouse neuroblasts and maintenance of transcriptional activation in hematopoietic progenitors and myelogenesis. Studies have shown that mice in which PHF10 protein has been knocked out die in the late stages of embryogenesis, and the surviving animals exhibit hematopoietic defects.[44]

Many studies have described the crucial functions of many SWI/SNF subunits, such as ACTL6A, ARID1A, ARID2, PBRM1, PHF10, and SMARCA2 for the maintenance of hematopoietic stem cells.[6-8,45] Specifically, SWI/SNF complexes can also interact with hematopoietic-specific transcription factors, including EKLF, RUNX1, PU.1, IKAROS, GATA1, and CEBPα.Increasing evidence has reported that mutations in SWI/SNF complex confer resistance to a variety of antineoplastic agents routinely used in the treatment of hematological malignancies, including ibrutinib, venetoclax, doxorubicin, paclitaxel, or vinblastine.[6-8,45]

CONCLUSION

Independent studies on SWI/ SNF-targeting chemotherapeutic agents, as well as on the role of SWI/ SNF mutations in drug resistance and the creation of targetable synthetic lethalities in SWI/SNF defective tumors and the mutations we demonstrated in our study, are opening new paths for improving leukemia treatment that hold a promising future.Although we have performed comprehensive molecular profiling analyses of the SWI/SNF complex, a fundamental mechanism that may be responsible for AML pathogenesis, we are aware of certain limitations of our study. This is because this study was carried out with a limited experimental design using bioinformatics tools. There fore, a wet laboratory study and a larger sample group are needed to clarify the effect of *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PMBR1*, and *BCL7A*, on AML pathogenesis. Mutations and expression differences that we have discovered in SWI/SNF subunits can often create lethal synthetic relationships with other SWI/SNF or non-SWI/SNF proteins which could be therapeutically exploitable.As a result, we examined the molecular profiles of the *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCE1, SMARCB1, DPF2, PMBR1*, and *BCL7A* genes, which we conclude would be helpful in any in vivo-in vitro clinical trials that can provide solutions in the diagnosis and treatment of AML.

Ethics Committee Approval: The data used in our study were obtained from public database TCGA, therefore, ethical approval was not required.

Informed Consent: The data used in our study were obtained from public database TCGA, therefore, informed consent was not required.

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Katılımcı Onamı: Çalışmamızda kullanılan veriler TCGA kamuya açık veri tabanından elde edildiğinden hasta onamı gerekmemiştir.

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