Submitted: 05 June, 2025 Accepted: 21 July, 2025 Published: 15 October, 2025 DOI:10.22514/ejgo.2025.128

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

PRDM6 promoter methylation as a potential epigenetic biomarker in BRCA-associated ovarian cancer

Numan Comert[1], Ozge Sukruoglu Erdogan[2], Betul Celik Demirbas[2], Seda Kilic Erciyas[2], Ahmet Dinc[1], Özge Pasin[3], Hülya Yazıcı[4], Seref Bugra Tuncer[2,*]

[1]Institute of Graduate Studies in Health Sciences, Istanbul University, 34126 Istanbul, Türkiye
[2]Department of Cancer Genetics, Oncology Institute, Istanbul University, 34093 Istanbul, Türkiye
[3]Department of Biostatistics and Medical Informatics, Hamidiye Medical Faculty, Health Sciences University, 34668 Istanbul, Türkiye
[4]Department of Medical Biology, Faculty of Medicine, Istanbul Health and Technology University, 34275 Istanbul, Türkiye

***Correspondence**
seref.tuncer@istanbul.edu.tr
(Seref Bugra Tuncer)

Abstract

Background: Ovarian cancer (OC) remains one of the most lethal gynecologic malignancies, primarily due to late-stage diagnosis. Aberrant DNA methylation is critical in tumorigenesis and represents a promising avenue for biomarker development. We hypothesized that PR/SET domain 6 (*PRDM6*) methylation is associated with breast cancer gene (*BRCA*) mutation status in OC. **Methods**: Peripheral blood samples were collected from 387 patients with high-grade serous ovarian cancer, 50 individuals with benign ovarian conditions, and 100 healthy controls. DNA methylation was evaluated using methylation-sensitive restriction enzymes (MSREs) and subsequently analyzed by real-time polymerase chain reaction (PCR). Descriptive statistics were employed to summarize categorical and continuous variables. Associations between *PRDM6* methylation and clinical parameters, including *BRCA* mutation status, cancer antigen 125 (CA-125) levels, and age, were statistically analyzed. **Results**: *PRDM6* methylation was detected in 53.9% of OC patients, 60.0% of individuals with benign ovarian disease, and 37.0% of healthy controls. The methylation frequency in OC patients was significantly higher than in healthy controls ($p = 0.005$). *PRDM6* promoter methylation was detected in 64.8% of BRCA-mutated OC patients compared to 50.3% of BRCA-negative patients, indicating a significant association between *BRCA* mutation status and *PRDM6* methylation ($p = 0.016$). No significant associations were found between *PRDM6* methylation and age, menopausal status, or CA-125 levels. **Conclusions**: *PRDM6* methylation may serve as a non-invasive biomarker for early detection in high-risk populations, particularly in *BRCA* mutation carriers.

Keywords

Ovarian cancer; DNA methylation; Epigenetic biomarker; *PRDM6* methylation; *BRCA* mutation

1. Introduction

Ovarian cancer (OC) is the seventh most common cancer among women in Türkiye, with an estimated 3855 new cases diagnosed annually, representing approximately 2.6% of all female cancers, according to the Global Cancer Observatory (GLOBOCAN) 2022 data [1]. Globally and nationally, cancer remains a significant public health concern. To improve survival rates, there is an urgent need to develop new strategies focused on early detection and diagnosis [2]. OC arises from the uncontrolled proliferation of cells originating from the ovarian tissue [3].

Extensive research has revealed a variety of genetic and epigenetic alterations in both oncogenes and tumor suppressor genes in OC. In addition, loss of heterozygosity (LOH) has been observed in several chromosomal regions implicated in ovarian tumorigenesis [4]. Similar to other malignancies, the initiation and progression of OC are driven not only by somatic genetic mutations but also by epigenetic mechanisms, including DNA methylation, histone modification, and regulation of non-coding RNAs. These epigenetic processes contribute to dysregulated gene expression without altering the underlying DNA sequence [5, 6].

While earlier studies provided limited insight into the role of DNA methylation in OC, recent advances have highlighted the importance of epigenetic regulation in ovarian carcinogenesis. Promoter hypermethylation of tumor suppressor genes such as *BRCA1*, Homeobox A9 (*HOXA9*), Ras association domain family member 1 (*RASSF1A*), Secreted protein acidic and rich in cysteine (*SPARC*), and Hypermethylated In Cancer 1 (*HIC1*) has been implicated in OC development [7, 8]. Furthermore, the inactivation of DNA damage response (DDR) genes through promoter methylation has been linked to defective DNA repair mechanisms and increased genomic instability in OC. Notably, hypermethylation-induced silencing of key DDR genes has also been correlated with unfavorable clinical outcomes, thus underscoring the potential prognostic value of epigenetic dysregulation [5]. Our previous re-

search, involving monozygotic twins—one healthy and one diagnosed with OC—identified promoter hypermethylation of the *PRDM6* gene exclusively in the affected twin, suggesting a possible role for *PRDM6* in ovarian tumorigenesis [9].

PRDM6 belongs to the protein (PR) domain-containing (PRDM) gene family, which is known for its roles in transcriptional regulation and chromatin remodeling. *PRDM6* encodes a histone methyltransferase that modulates gene expression through epigenetic modifications [10]. Its oncogenic potential has been demonstrated in various malignancies. For instance, Schmidt *et al*. [11] reported that *PRDM6* localizes to the nuclei of neural epithelial stem cells and contributes to medulloblastoma by repressing chromatin accessibility and altering gene expression patterns. Although this finding was specific to brain tumors, it highlights the broader oncogenic potential of *PRDM6* through epigenetic pathways, suggesting a similar role in the pathogenesis of other cancers, including OC. Given the critical role of BRCA genes in DNA repair and *PRDM6*'s potential role in epigenetic instability, we hypothesized that alterations in *PRDM6* methylation might be particularly relevant in the context of BRCA-associated ovarian carcinogenesis. The role of *PRDM6* in OC, particularly in high-grade serous ovarian cancer (HGSOC), remains inadequately characterized. However, existing studies provide insights into its potential involvement in cancer biology through its functions in cell cycle regulation and the epithelial-to-mesenchymal transition (EMT) [10, 12, 13]. While the connection between *PRDM6* and OC remains largely unexplored, the existing body of literature emphasizes a need for more focused investigations. The exploration of *PRDM6* in the context of OC holds promise, particularly in understanding the dynamic regulatory networks that govern tumor biology and patient outcomes.

In our previous research, we conducted comprehensive genomic profiling, including healthy and affected individuals with serous OC within a family. Building on the preliminary findings, the present study investigates the methylation status of *PRDM6* in peripheral blood samples from 387 patients with serous OC, 50 individuals with benign ovarian conditions, and 100 healthy controls. We aimed to investigate whether *PRDM6* methylation is associated with *BRCA* mutations, with a particular focus on its potential role in prognosis and risk stratification [9, 14] and whether it could serve as a diagnostic biomarker.

2. Materials and methods

2.1 Study cohort

This study included a total of 537 female participants, comprising 387 patients diagnosed with high-grade serous ovarian carcinoma (HGSOC), 50 individuals with benign ovarian diseases, and 100 healthy controls. All ovarian cancer cases included in this study were histopathologically confirmed as HGSOC by board-certified gynecologic pathologists, under the diagnostic criteria established by the World Health Organization (WHO). To ensure diagnostic precision and subtype verification, histological assessment was supported by immunohistochemical staining for key markers, including p53, Antigen Kiel 67 (Ki-67), Wilms Tumor 1 (WT-1), and p16. These markers were evaluated to distinguish HGSOC from other histological subtypes and to validate the inclusion of cases in the molecular analyses. No borderline ovarian tumors were identified or included in the cohort. Somatic *BRCA* testing was not performed, as the study focused on germline mutations in patients selected according to the National Comprehensive Cancer Network (NCCN) criteria for hereditary breast and ovarian cancer (HBOC) risk.

Peripheral blood samples were collected from all participants at the Istanbul University Oncology Institute between 2021 and 2023. All individuals provided written informed consent before inclusion in the study. The study was approved by the Ethics Committee of the Istanbul Faculty of Medicine (Ethics Committee Approval: Meeting No. 2021/1122; No. 2019/1161).

2.2 DNA extraction and methylation analysis

Genomic DNA was extracted from peripheral blood lymphocytes using the Quick-DNA™ Miniprep Plus Kit (D4069, Zymo Research, Irvine, CA, USA), following the manufacturer's protocol. The combination of Methylation-Sensitive Restriction Enzyme (MSRE) and quantitative PCR (qPCR) is frequently preferred over bisulfite sequencing due to its practical advantages, including cost-efficiency, operational simplicity, and high sensitivity. Notably, MSRE + qPCR enables accurate quantification of DNA methylation from minimal input material, making it particularly well-suited for clinical and research applications where sample availability is limited [15]. DNA methylation analysis of the *PRDM6* gene was performed using the OneStep qMethyl™ Kit (D5310, Zymo Research, Irvine, CA, USA), which enables the direct quantification of DNA methylation without the need for bisulfite conversion. Real-time PCR amplification was performed using an Applied Biosystems Real-Time PCR System, with SYTO 9 fluorescent dye and gene-specific primers targeting the *PRDM6* promoter region. The methylation status was quantified by comparing threshold cycle (Ct) values between methylation-sensitive and control reactions. The sequences of the primers used for *PRDM6* methylation analysis were as follows:

• Forward primer (PRDM6METF1): 5′-GGA GTT GGT GCC TTC TCT AAC-3′

• Reverse primer (PRDM6METR1): 5′-CTC GAC TGC CTC CCA AAC-3′

All reactions were performed in duplicate, and negative controls were included to ensure the absence of contamination. *BRCA1/2* mutation analysis was conducted at the Istanbul University Cancer Genetics Laboratory using a targeted next-generation sequencing (NGS) approach on the Illumina MiSeq® platform (Benchtop Sequencer, Illumina, San Diego, CA, USA) in combination with SOPHiA DDM® software (version7.5.1, SOPHiA GENETICS, Saint-Sulpice, Switzerland) for variant calling and interpretation. Genomic DNA was isolated from peripheral blood samples using the QIAamp DNA Micro Kit (56304, Qiagen, Hilden, NRW, Germany) or GeneAll® Exgene™ Kit (1051013, GeneAll Biotechnology, Siheung, South Korea), and DNA quality was assessed by Nan-

oDrop spectrophotometry. Library preparation was performed using the BRCA MASTR Plus Dx kit (8031360, SOPHiA GENETICS, Saint-Sulpice, VD, Switzerland), following an amplicon-based enrichment strategy. The libraries were purified using AMPure XP beads and quantified prior to sequencing. For quality control, each run was spiked with 6% PhiX control. Sequencing results were analyzed using the human genome reference GRCh37/hg19. Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines, using population and functional databases including ClinVar, single-nucleotide polymorphism database (dbSNP), genome aggregation database (GnomAD), and human gene mutation database (HGMD). Pathogenic and likely pathogenic variants were confirmed by Sanger sequencing, and large genomic rearrangements were assessed using Multiplex Ligation-dependent Probe Amplification (MLPA; MRC-Holland, Amsterdam, Netherlands).

Samples exhibiting methylation levels ≤6% were classified as unmethylated, while those with values exceeding 6% were considered methylated. The 6% cutoff was selected based on the manufacturer's specifications provided in the OneStep qMethyl™ Kit manual (D5310, Zymo Research Corp., Irvine, CA, USA), which recommends this threshold as a standard reference for methylation quantification. All measurements and classifications in the current study were performed in accordance with this validated guideline [16–18].

2.3 Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize demographic and clinical characteristics. Comparisons between groups were made using Pearson's Chi-square test, Fisher's Exact test, and the Kruskal-Wallis test, as appropriate. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Descriptive statistics and cohort characteristics

Descriptive statistics were reported as counts and percentages for categorical variables (*e.g.*, *BRCA* mutation status, diagnosis, clinical stage, histological grade, histological subtype, ethnicity, and menopausal status) and as means, standard deviations, medians, minimum and maximum values for continuous variables (*e.g.*, age and CA-125 levels before and after treatment).

A total of 387 serous OC patients, 50 individuals with benign ovarian disease, and 100 healthy controls were included in the final analysis. The number of individuals included in each analysis may vary slightly due to missing data. Among individuals with benign ovarian disease, the histological subtypes were as follows: 3 hemorrhagic cysts, 22 simple anechoic cysts, 19 endometriomas, 3 cases with both endometriomas and simple cysts, one teratoma, and one dermoid cyst. Ethnically, the cohort was predominantly Turkish (68.5%), followed by individuals of Balkan (18.6%) and Eastern Anatolian (other ethnicities 9.0%) origin.

3.2 Patient demographics

The majority of patients were over 45 years old (67.2%), with a predominant late-stage diagnosis (Stage 3: 52.7%, Stage 4: 13.4%). Among OC patients, 23.8% (n = 92) were found to carry a *BRCA* mutation (either *BRCA1* or *BRCA2*), while 76.2% (n = 295) were *BRCA* mutation-negative. All individuals in the benign ovarian disease group and the healthy control group were *BRCA* mutation-negative. The cohort included individuals from Turkish (68.5%), Balkan (18.6%), and Eastern Anatolian Regions (9.0%), with other ethnicities.

3.3 *PRDM6* methylation analysis

PRDM6 methylation status was assessed in peripheral blood samples from all three study groups. A methylation threshold of 6% was applied; samples with methylation levels ≤6% were considered unmethylated, while those >6% were categorized as methylated. Consistent with Feng *et al*. [19] (2021), who demonstrated that methylation thresholds of 5–8% have clinical relevance in HGSOC, our 6% cutoff aligns with established epigenetic biomarker standards. The methylation frequency of the *PRDM6* gene was: 52.0% in OC patients (n = 375, the total number of OC patients was 387; however, 12 individuals were excluded from the study due to unavailable expression data), 60.0% in the individuals with benign ovarian disease group (n = 50), and 37.0% in healthy controls (n = 100) (Fig. 1). A significant difference in *PRDM6* promoter methylation was observed across the study groups ($p = 0.005$). While 37.0% of healthy controls exhibited *PRDM6* methylation, the frequency was markedly higher among individuals with benign ovarian disease (60.0%) and OC patients (53.9%) (Table 1). These findings suggest that *PRDM6* methylation may be associated with ovarian pathology in general, rather than being specific to malignant transformation.

3.4 Association between *BRCA* status and *PRDM6* methylation

Among OC patients with *BRCA1/2* mutations (n = 91), 64.8% (59/91) exhibited *PRDM6* promoter methylation, whereas 35.2% (32/91) had unmethylated *PRDM6* status. This indicates that *PRDM6* methylation is significantly enriched in BRCA mutation carriers, suggesting a potential link between inherited BRCA alterations and epigenetic changes in the *PRDM6* gene. The statistically significant p-value ($p = 0.016$) (Table 2) supports the hypothesis that BRCA-associated ovarian tumors may follow distinct epigenetic regulatory pathways compared to BRCA-negative cases, potentially influencing tumor biology or therapeutic responses.

3.5 Association of *PRDM6* methylation with clinical parameters

Further analyses were conducted to investigate whether *PRDM6* methylation was associated with selected clinical variables, including age at diagnosis, age at menopause, and pre- and post-treatment CA-125 levels. No statistically significant differences were observed between methylated and unmethylated groups for any of the variables examined (Table 3).

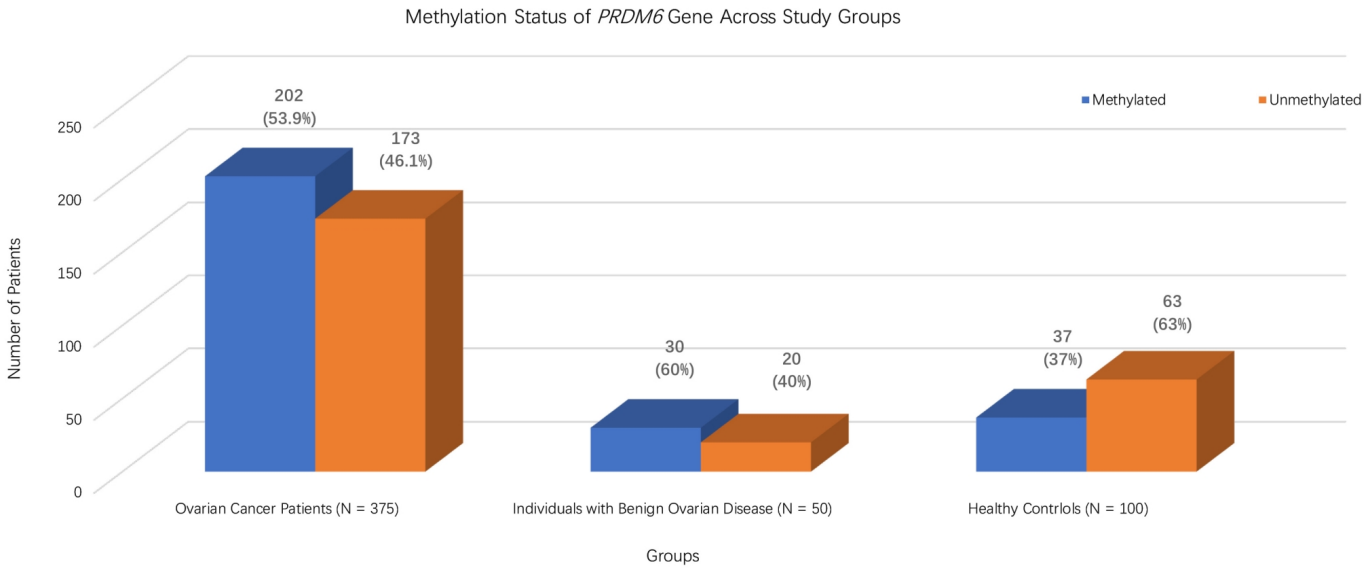

FIGURE 1. Methylation distribution of the *PRDM6* gene among study groups. *PRDM6*: PR/SET domain 6.

TABLE 1. Comparison of *PRDM6* methylation status across study groups.

	Healthy controls	Individuals with benign ovarian disease	Ovarian cancer	*p*-value
PRDM6 methylation				
Unmethylated	63 (63.0%)	20 (40.0%)	173 (46.1%)	0.005
Methylated	37 (37.0%)	30 (60.0%)	202 (53.9%)	

PRDM6: PR/SET domain 6.

TABLE 2. Relationship between *BRCA* mutation status and *PRDM6* methylation in ovarian cancer patients.

	Methylated	Unmethylated	Total	*p*-value
BRCA status				
BRCA+	59 (64.8%)	32 (35.2%)	91	0.016
BRCA−	143 (50.3%)	141 (49.7%)	284	

BRCA: Breast Cancer Gene.

4. Discussion

OC remains one of the leading causes of cancer-related mortality among women worldwide [20]. In this study, we investigated the potential of the *PRDM6* gene as a non-invasive epigenetic biomarker by evaluating its methylation status in peripheral blood DNA. We aimed to assess the diagnostic utility of *PRDM6* methylation and to contribute to the understanding of molecular mechanisms underlying ovarian tumorigenesis. Survival in OC is strongly influenced by the stage at diagnosis; earlier detection significantly improves prognosis. While CA-125 and human epididymis protein 4 (HE4) are currently the most commonly used biomarkers for diagnosis [21], they have several limitations [22], including low sensitivity in early-stage disease and lack of specificity [23–25]. One of the key challenges in OC is the lack of early, reliable biomarkers. Given that DNA hypermethylation often occurs in the early stages of tumorigenesis [26–28], the identification of methylation-based biomarkers, such as *PRDM6*, may offer a promising strategy for early detection. Our analysis revealed that *PRDM6* promoter methylation was present in 53.9% of OC patients, 60% of individuals with benign ovarian disease, and 37% of healthy controls. Notably, methylation frequency in the healthy control group was significantly lower than that observed in the OC group ($p = 0.005$), suggesting a potential association between *PRDM6* methylation and malignant transformation.

Given the role of *PRDM6* in chromatin remodeling and gene silencing through histone methylation, alterations in its methylation status may influence key regulatory pathways involved in ovarian tumorigenesis. Methylation of histones can lead to either gene activation or repression, depending on the specific site of methylation [29–31]. Since *BRCA1* and *BRCA2* are essential for DNA damage repair via homologous recombination [32, 33], aberrant *PRDM6* methylation might further disrupt genomic stability, particularly in *BRCA*-mutated tumors. Therefore, we investigated the potential association between *PRDM6* promoter methylation and *BRCA* mutation status to explore whether epigenetic regulation of *PRDM6* contributes to the distinct molecular landscape of *BRCA*-related OC. A significant association was also found between *PRDM6* methylation and *BRCA* mutation status among

TABLE 3. Evaluation of *PRDM6* methylation in relation to quantitative variables.

Variable	Methylation status	n	Mean	Median	SD	Min	Max	*p*-value
First CA-125 (individuals with benign ovarian disease)								
	Unmethylated	20	5.83	0.00	15.99	0.00	64.00	0.661
	Methylated	30	6.03	0.00	19.23	0.00	84.00	
First CA-125 (ovarian cancer)								
	Unmethylated	52	791.72	364.50	1236.28	7.97	6600.00	0.491
	Methylated	65	710.81	221.00	1406.09	2.20	10,389.50	
Last CA-125 (ovarian cancer)								
	Unmethylated	45	388.82	45.00	943.57	3.00	4284.50	0.092
	Methylated	54	269.72	19.50	1076.09	2.20	7709.00	
Diagnosis age in years (healthy control)								
	Unmethylated	63	42.24	41.00	7.41	30	65	0.584
	Methylated	37	43.08	42.00	7.46	30	65	
Diagnosis age in years (individuals with benign ovarian disease)								
	Unmethylated	20	38.35	40.00	8.71	23	57	0.079
	Methylated	30	33.47	31.50	9.05	18	50	
Diagnosis age in years (ovarian cancer)								
	Unmethylated	150	51.94	50.00	12.44	17	84	0.769
	Methylated	182	50.76	50.00	9.39	23	84	
Menopause age in years (ovarian cancer)								
	Unmethylated	86	46.73	48.00	5.31	28	56	0.507
	Methylated	113	46.17	46.00	6.15	26	60	

SD: Standard deviation; Min: Minimum; Max: Maximum; CA-125: Cancer antigen 125; PRDM6: PR/SET domain 6.

OC patients. *BRCA* mutation-positive individuals exhibited a higher frequency of *PRDM6* methylation compared to *BRCA*-negative individuals ($p = 0.016$). This suggests that *PRDM6* methylation may be more prevalent in genetically predisposed OC cases and could potentially contribute to tumor development in this subgroup. It is important to note that this study was designed to assess the germline contribution of *BRCA* mutations to epigenetic regulation, and somatic testing was not within the scope of the current analysis. However, all tumor samples underwent detailed histopathological and immunohistochemical evaluation to confirm HGSOC subtype.

Interestingly, *PRDM6* promoter methylation was also observed at a higher frequency in individuals with benign ovarian disease (60.0%) compared to healthy controls (37.0%), and was even slightly higher than in OC patients (53.9%). This finding suggests that epigenetic alterations in *PRDM6* may not be limited to malignant transformation but may also occur during benign ovarian pathologies, possibly reflecting chronic inflammation, hormonal fluctuations, or increased cellular turnover [34]. Indeed, the association between DNA methylation changes and benign ovarian conditions is an area of growing interest, particularly in efforts to improve the molecular understanding of early events in ovarian tumorigenesis [35]. Prior studies have demonstrated that specific DNA methylation patterns can help differentiate between benign and malignant ovarian lesions [36]. For example, the methylation status of the Growth Hormone Secretagogue Receptor (GHSR) gene is significantly higher in malignant ovarian tumors compared to benign lesions, underscoring the discriminatory potential of gene-specific epigenetic changes [37]. Although *PRDM6* methylation does not show a clear-cut distinction between benign and malignant tissues, the elevated levels observed in benign conditions may still reflect an active epigenetic landscape influenced by inflammation and hormonal signaling.

Furthermore, studies profiling normal ovarian tissues, benign lesions, and malignant tumors have revealed variable methylation signatures that are thought to be shaped by the immune microenvironment, particularly the presence of cytokines and inflammatory cells [38]. Chronic inflammation is a known contributor to neoplastic progression through mechanisms that promote cellular proliferation and resistance to apoptosis. These inflammatory processes may also drive DNA methylation changes in benign ovarian tissues [39]. In addition to inflammatory signaling, hormonal regulation plays a pivotal role in shaping the epigenetic profile of ovarian tissue. Hormonal cycles influence gene expression via methylation modifications, and disruptions in hormonal homeostasis may increase susceptibility to neoplastic transformation, even in benign neoplasms [39]. For instance, in benign mucinous cystadenomas, the complexity of methylation regulation is compounded by histopathological heterogeneity and microenvironmental influences such as localized inflammation [40]. A deeper understanding of the biological mechanisms linking

DNA methylation to the tissue microenvironment in benign ovarian conditions may thus open new avenues for early detection and clinical stratification. Importantly, the integration of methylation profiling using minimally invasive methods, such as liquid biopsies, holds promise for differentiating between benign and malignant ovarian lesions in real time [39]. Therefore, while *PRDM6* methylation alone may not provide sufficient diagnostic specificity, it could contribute to multimarker panels aimed at improving diagnostic accuracy and guiding patient management. The observation that *PRDM6* methylation levels were higher in individuals with benign ovarian conditions than in malignant cases suggests that its standalone diagnostic utility may be limited. However, elevated *PRDM6* methylation may serve as an additional risk indicator, particularly in *BRCA* mutation carriers.

Although *PRDM6* methylation was also detected in individuals with benign ovarian conditions, its higher frequency was observed among OC patients, particularly those with *BRCA* mutations. The increased methylation of *PRDM6* observed in *BRCA*-mutated patients can be interpreted within the framework of the "second hit" hypothesis, where the initial *BRCA1/2* mutation compromises homologous recombination repair (HRR), thereby creating genomic instability [41]. *BRCA1* and *BRCA2* play critical roles in maintaining genomic stability through HRR of DNA double-strand breaks. Defects in HRR caused by *BRCA* mutations impair HRR, leading to genomic instability [42–44]. This genomic instability may facilitate epigenetic alterations, such as the hypermethylation of genes involved in cell proliferation and differentiation, including *PRDM6* [45]. Hypermethylation of *PRDM6* could act as a secondary hit that synergizes with HRR deficiency, enhancing tumor progression by modulating gene expression profiles critical to tumor biology [46].

The increased methylation of *PRDM6* observed in *BRCA*-mutant tumors may critically influence key oncogenic pathways, particularly the wingless-related integration site (Wnt)/β-catenin signaling cascade and epithelial-to-mesenchymal transition (EMT), both of which are fundamental in cancer progression. *PRDM6* has been shown to regulate transcriptional programs associated with cell fate, and its methylation-induced silencing could disrupt standard control over the Wnt/β-catenin pathway, which is known to sustain stemness and promote chemoresistance in cancers, including breast tumors [47–49]. In *BRCA*-deficient contexts, where DNA repair is compromised, epigenetic alterations such as *PRDM6* hypermethylation may amplify β-catenin activity, thereby driving aggressive tumor phenotypes linked to enhanced proliferation and metastatic potential through EMT activation [50, 51]. Furthermore, the loss of *PRDM6* function through methylation may derepress EMT-promoting transcription factors, such as snail family transcriptional repressor 1 (SNAI1) and twist family BHLH transcription factor 1 (TWIST1), thereby facilitating cellular plasticity and invasiveness [29]. These molecular events could represent adaptive tumor strategies to overcome therapeutic pressures, supported by compensatory activation of Wnt and EMT pathways in *BRCA*-mutant malignancies [29, 52]. Collectively, this suggests a mechanistic model in which homologous recombination deficiency in *BRCA*-mutant tumors leads to *PRDM6* hypermethylation, disrupting Wnt/β-catenin signaling, and thereby enhancing EMT processes. Targeting PRDM6-mediated epigenetic dysregulation, particularly in *BRCA*-mutant tumors, may overcome the therapeutic resistance associated with Wnt/EMT activation. While our data suggest Wnt/EMT involvement, future studies should validate *PRDM6*'s direct role in these pathways in OC models. A hypothetical model summarizing these interactions—including PRDM6 hypermethylation, BRCA-associated homologous recombination deficiency, and downstream activation of Wnt/β-catenin signaling and EMT—is presented in Fig. 2.

However, no significant associations were identified between *PRDM6* methylation and other clinical parameters such as CA-125 levels, age at diagnosis, or menopausal age. The lack of association between *PRDM6* methylation and CA-125 levels suggests that this epigenetic alteration may occur independently of tumor burden, positioning it as an early event in carcinogenesis. *PRDM6* encodes a transcriptional repressor involved in chromatin remodeling and has known interactions with key epigenetic regulators, such as histone deacetylases (HDACs) and histone methyltransferases [53, 54]. It plays a role in maintaining cellular proliferative capacity and inhibiting differentiation, particularly in smooth muscle and neural tissues. While the role of *PRDM6* in developmental biology has been well described, studies on its epigenetic regulation in cancer, especially OC, remain scarce.

Our findings provide one of the first large-scale assessments of *PRDM6* methylation in this context, suggesting that its epigenetic dysregulation may contribute to ovarian tumorigenesis. In this study, DNA methylation was evaluated using MSREs and subsequently analyzed by real-time PCR. The selection of a methylation threshold, such as 6%, is a crucial step in analyzing DNA methylation, particularly in cancer studies like those on OC. By setting a threshold of 6%, researchers ensure that low levels of methylation, which may lack biological relevance, do not confound insights into the association between methylation status and tumor behavior. This is particularly important as low or negligible methylation levels may occur due to variability within both normal and cancerous cell populations [55–57]. Moreover, clinical studies have indicated that significant methylation changes (*i.e.*, above 6%) can serve as biomarkers for prognosis and response to treatment in ovarian cancer. For instance, high-grade serous OC patients exhibiting elevated levels of methylated genes have been correlated with poor prognostic outcomes and chemoresistance [19].

Despite these promising results, our study has some limitations. While peripheral blood is a minimally invasive and clinically feasible source for methylation analysis, it may not fully capture tumor-specific epigenetic alterations. The limitations of using peripheral blood to reflect tumor epigenetics involve several key factors that affect the accuracy, sensitivity, and specificity of the detected DNA methylation alterations. While peripheral blood has been explored as a non-invasive source for monitoring cancer biomarkers, including epigenetic modifications, it presents various challenges in accurately mirroring the tumor environment. In addition, detecting hypermethylation in peripheral blood represents a feasible and scientifically supported strategy, consistent with well-characterized methylation

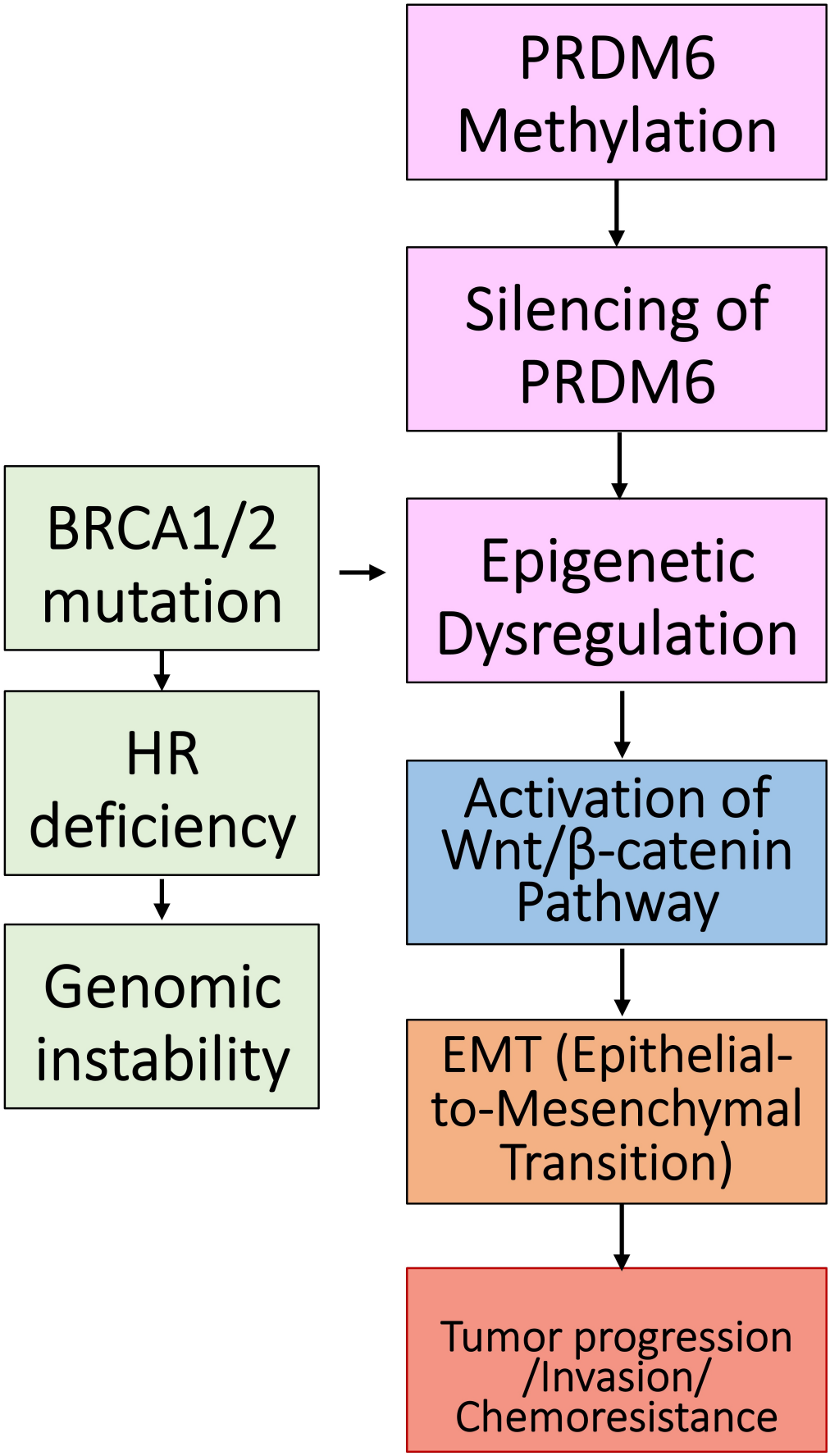

FIGURE 2. Proposed regulatory mechanism by which *PRDM6* methylation may contribute to ovarian cancer development and progression. *PRDM6*: PR/SET domain 6; *BRCA*: Breast Cancer Gene; HR: homologous recombination; Wnt: wingless-related integration site.

alterations observed in cancer, underscoring its utility as a potential biomarker for ovarian cancer. However, adopting a fixed methylation threshold (6%) may not fully capture the nuanced and heterogeneous nature of epigenetic regulation. Additionally, a key limitation of our study is the absence of an analysis exploring how *PRDM6* methylation may interact with other genetic predispositions or environmental exposures that could modulate ovarian cancer susceptibility. Lastly, although our OC cohort was relatively large, further validation in independent and prospective cohorts, along with mechanistic studies, is necessary to confirm the biological and clinical significance of *PRDM6* methylation. The extent to which *PRDM6* methylation in peripheral blood reflects epigenetic alterations in tumor tissue warrants further investigation through matched blood–tumor sample analyses in future studies.

5. Conclusions

Our findings indicate that *PRDM6* promoter methylation is significantly associated with ovarian cancer and BRCA mutation status, supporting its potential as a promising non-invasive blood-based epigenetic biomarker. This association highlights the possible role of *PRDM6* in ovarian tumorigenesis, particularly in genetically predisposed individuals. To fully establish the clinical relevance of these results, further studies are needed to validate them in larger, independent cohorts and to elucidate the molecular mechanisms by which *PRDM6* contributes to ovarian cancer development. Future research should also explore its utility in early detection and risk stratification, as well as its potential as a target for epigenetic-based therapeutic strategies.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

NC and OSE—designed the research study. SKE and BCD—provided help and advice on technical aspects of the research. AD—helped with the experiments. ÖP—analyzed the data. HY—selected the patients to be included in this study. SBT—was the principal investigator of the study.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Approvals were received for the study from the Istanbul Faculty of Medicine Ethics Committee (Ethics Committee Approval No. 2021/1122; No. 2019/1161). All individuals provided written informed consent before inclusion in the study.

ACKNOWLEDGMENT

We gratefully acknowledge the support of the Istanbul University Scientific Research Projects Coordination Unit, whose contributions played a vital role in the successful execution of this study.

FUNDING

This study was funded by the Scientific Research Projects Coordination Unit of Istanbul University (Project number: TYL-2021-38160 and TOA-2020-35780).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Filho AM, Laversanne M, Ferlay J, Colombet M, Piñeros M, Znaor A, *et al*. The GLOBOCAN 2022 cancer estimates: data sources, methods, and a snapshot of the cancer burden worldwide. International Journal of Cancer. 2025; 156: 1336–1346.

[2] Siegel RL, Kratzer TB, Giaquinto AN, Sung H, Jemal A. Cancer statistics, 2025. CA: A Cancer Journal for Clinicians. 2025; 75: 10–45.

[3] Zhou M, Pi J, Zhao Y. Integrative multi-omics analysis reveals molecular subtypes of ovarian cancer and constructs prognostic models. Journal of Immunotherapy. 2025; 48: 197–208.

[4] Marconato N, Tommasi O, Paladin D, Boscarino D, Spagnol G, Saccardi C, *et al*. Unraveling homologous recombination deficiency in ovarian cancer: a review of currently available testing platforms. Cancers. 2025; 17: 1771.

[5] He WH, Zhu HJ, Zhang SF, Shu G, Lei H, Yin G, *et al*. Promoter methylation changes in DNA damage-response genes in ovarian cancer and their correlation with prognosis. Clinical and Experimental Obstetrics & Gynecology. 2024; 51: 109.

[6] Şükrüoğlu Erdoğan Ö, Kılıç Erciyas S, Bilir A, Buğra Tunçer Ş, Akdeniz Ödemiş D, Kurul S, *et al*. Methylation changes of primary tumors, monolayer, and spheroid tissue culture environments in malignant melanoma and breast carcinoma. BioMed Research International. 2019; 2019: 1407167.

[7] Terp SK, Guldbrandsen K, Stoico MP, Mark LR, Frandsen AP, Dybkær K, *et al*. Genome-Wide cfDNA methylation profiling reveals robust hypermethylation signatures in ovarian cancer. Cancers. 2025; 17: 2026.

[8] Fu M, Deng F, Chen J, Fu L, Lei J, Xu T, *et al*. Current data and future perspectives on DNA methylation in ovarian cancer (review). International Journal of Oncology. 2024; 64: 62.

[9] Erdogan OS, Tuncer SB, Kilic S, Odemis DA, Turkcan GK, Celik B, *et al*. Genome-wide methylation profiles in monozygotic twins with discordance for ovarian carcinoma. Oncology Letters. 2020; 20: 357.

[10] Di Tullio F, Schwarz M, Zorgati H, Mzoughi S, Guccione E. The duality of PRDM proteins: epigenetic and structural perspectives. The FEBS Journal. 2022; 289: 1256–1275.

[11] Schmidt C, Cohen S, Gudenas BL, Husain S, Carlson A, Westelman S, *et al*. PRDM6 promotes medulloblastoma by repressing chromatin accessibility and altering gene expression. Scientific Reports. 2024; 14: 16074.

[12] Li P, Zheng Y, Wang YS. DEC1 is involved in TGF-β1-induced epithelial-mesenchymal transition of gastric cancer. American Journal of Cancer Research. 2024; 14: 630–642.

[13] Wang Q, Li J, Wang S, Deng Q, An Y, Xing Y, *et al*. Single-cell transcriptional profiling reveals cellular and molecular divergence in human maternal-fetal interface. Scientific Reports. 2022; 12: 10892.

[14] Tuncer SB, Erdogan OS, Erciyas SK, Saral MA, Celik B, Odemis DA, *et al*. miRNA expression profile changes in the peripheral blood of monozygotic discordant twins for epithelial ovarian carcinoma: potential

new biomarkers for early diagnosis and prognosis of ovarian carcinoma. Journal of Ovarian Research. 2020; 13: 99.

[15] Wojdacz TK, Dobrovic A. Dobrovic, methylation-sensitive high resolution melting (MS-HRM): a new approach for sensitive and high-throughput assessment of methylation. Nucleic Acids Research. 2007; 35: e41.

[16] Šestáková Š, Šálek C, Remešová H. Remesova, DNA methylation validation methods: a coherent review with practical comparison. Biological Procedures Online. 2019; 21: 19.

[17] Kurdyukov S, Bullock M. Bullock, DNA methylation analysis: choosing the right method. Biology. 2016; 5: 3.

[18] Gonc A, Sukruoglu Erdogan O, Kilic Erciyas S, Celik Demirbas B, Dinc A, Pasin O, *et al*. CYB5R4 gene methylation as a potential epigenetic marker for ovarian cancer. Clinical Medicine Insights. Oncology. 2025; 19: 11795549251340531.

[19] Feng LY, Yan BB, Huang YZ, Li L. Abnormal methylation characteristics predict chemoresistance and poor prognosis in advanced high-grade serous ovarian cancer. Clinical Epigenetics. 2021; 13: 141.

[20] Zhuang Q, Gu G, Chen J, Tang Z, Wu C, Liu J, *et al*. Global, regional, and national burden of ovarian cancer among young women during 1990–2019. European Journal of Cancer Prevention. 2025; 34: 1–10.

[21] Dochez V, Caillon H, Vaucel E, Dimet J, Winer N, Ducarme G. Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. Journal of Ovarian Research. 2019; 12: 28.

[22] Ferraro S, Braga F, Lanzoni M, Boracchi P, Biganzoli EM, Panteghini M. Serum human epididymis protein 4 *vs* carbohydrate antigen 125 for ovarian cancer diagnosis: a systematic review. Journal of Clinical Pathology. 2013; 66: 273–281.

[23] Zhang R, Siu MKY, Ngan HYS, Chan KKL. Molecular biomarkers for the early detection of ovarian cancer. International Journal of Molecular Sciences. 2022; 23: 12041.

[24] Wu Y, Wang C, Wang P, Wang C, Zhang Y, Han L. A high-performance microfluidic detection platform to conduct a novel multiple-biomarker panel for ovarian cancer screening. RSC Advances. 2021; 11: 8124–8133.

[25] Barr CE, Njoku K, Owens GL, Crosbie EJ. Urine CA125 and HE4 for the detection of ovarian cancer in symptomatic women. Cancers. 2023; 15: 1256.

[26] Lai X, Li Q, Wu F, Lin J, Chen J, Zheng H, *et al*. Epithelial-mesenchymal transition and metabolic switching in cancer: lessons from somatic cell reprogramming. Frontiers in Cell and Developmental Biology. 2020; 8: 760.

[27] Wang H, Li J, He J, Liu Y, Feng W, Zhou H, *et al*. Methyl-CpG-binding protein 2 drives the Furin/TGF-beta1/Smad axis to promote epithelial-mesenchymal transition in pancreatic cancer cells. Oncogenesis. 2020; 9: 76.

[28] Aggarwal V, Montoya CA, Donnenberg VS, Sant S. Interplay between tumor microenvironment and partial EMT as the driver of tumor progression. iScience. 2021; 24: 102113.

[29] Kim K, Ryu TY, Jung E, Han TS, Lee J, Kim SK, *et al*. Epigenetic regulation of SMAD3 by histone methyltransferase SMYD2 promotes lung cancer metastasis. Experimental & Molecular Medicine. 2023; 55: 952–964.

[30] Wanna-Udom S, Terashima M, Suphakhong K, Ishimura A, Takino T, Suzuki T. KDM2B is involved in the epigenetic regulation of TGF-β-induced epithelial-mesenchymal transition in lung and pancreatic cancer cell lines. Journal of Biological Chemistry. 2021; 296: 100213.

[31] Fog CK, Galli GG, Lund AH. PRDM proteins: important players in differentiation and disease. BioEssays. 2012; 34: 50–60.

[32] Minguillón J, Ramírez MJ, Rovirosa L, Bustamante-Madrid P, Camps-Fajol C, Ruiz de Garibay G, *et al*. CDK5RAP3, a new BRCA2 partner that regulates DNA repair, is associated with breast cancer survival. Cancers. 2022; 14: 353.

[33] Yang X, Leslie G, Doroszuk A, Schneider S, Allen J, Decker B, *et al*. Cancer risks associated with germline PALB2 pathogenic variants: an international study of 524 families. Journal of Clinical Oncology. 2020; 38: 674–685.

[34] Voros C, Varthaliti A, Mavrogianni D, Athanasiou D, Athanasiou A, Athanasiou A, *et al*. Epigenetic alterations in ovarian function and their impact on assisted reproductive technologies: a systematic review. Biomedicines. 2025; 13: 730.

[35] Oliveira DVNP, Biskup E, O'Rourke CJ, Hentze JL, Andersen JB, Høgdall C, *et al*. Developing a DNA methylation signature to differentiate high-grade serous ovarian carcinomas from benign ovarian tumors. Molecular Diagnosis & Therapy. 2024; 28: 821–834.

[36] Szafron LA, Iwanicka-Nowicka R, Sobiczewski P, Koblowska M, Dansonka-Mieszkowska A, Kupryjanczyk J, *et al*. The diversity of methylation patterns in serous borderline ovarian tumors and serous ovarian carcinomas. Cancers. 2024; 16: 3524.

[37] Wever BMM, Schaafsma M, Bleeker MCG, van den Burgt Y, van den Helder R, Lok CAR, *et al*. Molecular analysis for ovarian cancer detection in patient-friendly samples. Communications Medicine. 2024; 4: 88.

[38] Elsharkawi SM, Elkaffash D, Moez P, El-Etreby N, Sheta E, Taleb RSZ. PCDH17 gene promoter methylation status in a cohort of Egyptian women with epithelial ovarian cancer. BMC Cancer. 2023; 23: 89.

[39] Terp SK, Stoico MP, Dybkær K, Pedersen IS. Early diagnosis of ovarian cancer based on methylation profiles in peripheral blood cell-free DNA: a systematic review. Clinical Epigenetics. 2023; 15: 24.

[40] Alkan B, Ekinci S, Özcan HN, Üner M, Altunova E, Tekşam Ö, *et al*. A giant ovarian mucinous cystadenoma in a postmenarchal adolescent girl. The Turkish Journal of Pediatrics. 2025; 67: 282–287.

[41] Sahnane N, Carnevali I, Formenti G, Casarin J, Facchi S, Bombelli R, *et al*. BRCA methylation testing identifies a subset of ovarian carcinomas without germline variants that can benefit from PARP inhibitor. International Journal of Molecular Sciences. 2020; 21: 9708.

[42] Buttitta F, Di Marino P, Felicioni L, Primavera FC, Ferro B, Zampacorta C, *et al*. BRCA gene amplification in primary peritoneal high-grade serous carcinoma patient with intrinsic resistance to platinum treatment: a case report. Pathologica. 2023; 115: 107–110.

[43] Huang X, Shao D, Wu H, Zhu C, Guo D, Zhou Y, *et al*. Genomic profiling comparison of germline BRCA and Non-BRCA carriers reveals CCNE1 amplification as a risk factor for Non-BRCA carriers in patients with triple-negative breast cancer. Frontiers in Oncology. 2020; 10: 583314.

[44] Hamid AB, Frank LE, Bouley RA, Petreaca RC. Pan-cancer analysis of co-occurring mutations in RAD52 and the BRCA1-BRCA2-PALB2 axis in human cancers. PLOS ONE. 2022; 17: e0273736.

[45] Hong L, Li N, Gasque V, Mehta S, Ye L, Wu Y, *et al*. Prdm6 controls heart development by regulating neural crest cell differentiation and migration. JCI Insight. 2022; 7: e156046.

[46] Zhang H, Wang A, Xu T, Mo X, Zhang Y. Promoter DNA methylation in GWAS-identified genes as potential functional elements for blood pressure: an observational and mendelian randomization study. Frontiers in Genetics. 2021; 12: 791146.

[47] Kaur A, Lim JYS, Sepramaniam S, Patnaik S, Harmston N, Lee MA, *et al*. WNT inhibition creates a BRCA-like state in Wnt-addicted cancer. EMBO Molecular Medicine. 2021; 13: e13349.

[48] Chen C, Shi Y, Ma J, Chen Z, Zhang M, Zhao Y. Trigonelline reverses high glucose-induced proliferation, fibrosis of mesangial cells via modulation of Wnt signaling pathway. Diabetology & Metabolic Syndrome. 2022; 14: 28.

[49] McKenna JK, Wu Y, Sonkusre P, Sinclear CK, Chari R, Lebensohn AM. The ubiquitin ligase HUWE1 enhances WNT signaling by antagonizing destruction complex-mediated β-catenin degradation and through a mechanism independent of changes in β-catenin abundance. PLOS Genetics. 2025; 21: e1011677.

[50] Wang R, Liu J, Li K, Yang G, Chen S, Wu J, *et al*. An SETD1A/Wnt/beta-catenin feedback loop promotes NSCLC development. Journal of Experimental & Clinical Cancer Research. 2021; 40: 318.

[51] Shu XS, Geng H, Li L, Ying J, Ma C, Wang Y, *et al*. The epigenetic modifier PRDM5 functions as a tumor suppressor through modulating WNT/β-catenin signaling and is frequently silenced in multiple tumors. PLOS ONE. 2011; 6: e27346.

[52] Qin Q, Zhou Y, Guo J, Chen Q, Tang W, Li Y, *et al*. Conserved methylation signatures associate with the tumor immune microenvironment and immunotherapy response. Genome Medicine. 2024; 16: 47.

[53] Zou M, Mangum KD, Magin JC, Cao HH, Yarboro MT, Shelton EL, *et al*. Prdm6 drives ductus arteriosus closure by promoting ductus arteriosus smooth muscle cell identity and contractility. JCI Insight. 2023; 8: e163454.

[54] Casamassimi A, Rienzo M, Di Zazzo E, Sorrentino A, Fiore D, Proto MC, *et al*. Multifaceted role of PRDM proteins in human cancer. International

Journal of Molecular Sciences. 2020; 21: 2648.
[55] Duran-Ferrer M, Clot G, Nadeu F, Beekman R, Baumann T, Nordlund J, *et al*. The proliferative history shapes the DNA methylome of B-cell tumors and predicts clinical outcome. Nature Cancer. 2020; 1: 1066–1081.
[56] Bian S, Wang Y, Zhou Y, Wang W, Guo L, Wen L, *et al*. Integrative single-cell multiomics analyses dissect molecular signatures of intratumoral heterogeneities and differentiation states of human gastric cancer. National Science Review. 2023; 10: nwad094.
[57] Lee D, Koo B, Yang J, Kim S. Metheor: ultrafast DNA methylation heterogeneity calculation from bisulfite read alignments. PLOS Computational Biology. 2023; 19: e1010946.

How to cite this article: Numan Comert, Ozge Sukruoglu Erdogan, Betul Celik Demirbas, Seda Kilic Erciyas, Ahmet Dinc, Özge Pasin, *et al*. *PRDM6* promoter methylation as a potential epigenetic biomarker in BRCA-associated ovarian cancer. European Journal of Gynaecological Oncology. 2025; 46(10): 21-30. doi: 10.22514/ejgo.2025.128.