Original Research Article

CYB5R4 Gene Methylation as a Potential Epigenetic Marker for Ovarian Cancer

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Abstract

Background: Ovarian cancer (OC) is a significant health problem often diagnosed at an advanced stage due to the lack of early symptoms and effective screening methods. This study aimed to explore the role of *CYB5R4* gene methylation as a potential biomarker for ovarian cancer.

Methods: DNA isolation was performed in the blood samples of 387 ovarian cancer patients, 50 individuals with benign ovarian diseases, and 100 healthy controls. The *CYB5R4* gene methylation status was evaluated using the Methyl-Specific Restriction Enzymes (MSREs) technique and methylation levels were compared between the groups.

Results: Ovarian cancer patients exhibited the highest mean methylation percentage (9.45%) and median (6.23%), followed by healthy controls with a mean of 9.14% and a median value of 4.47%. Statistical analysis showed significant differences in methylation levels ($P = .041$), suggesting that *CYB5R4* methylation may be associated with ovarian cancer progression.

Conclusion: The *CYB5R4* gene methylation may serve as a potential biomarker for ovarian cancer, particularly in distinguishing between malignant and benign conditions. Further research is needed to validate these findings and explore the clinical utility of *CYB5R4* methylation in ovarian cancer management.

Keywords

Ovarian cancer, *CYB5R4* gene, DNA methylation, biomarker, early detection

Introduction

Ovarian cancer (OC) presents a significant health challenge, ranking as the eighth most prevalent gynecological malignancy and the fifth leading cause of cancer-related mortality in women.[1] Due to the lack of early symptoms and effective early detection methods, most patients are diagnosed at an advanced stage. The 5-year survival rate is around 90% for early-stage ovarian cancer, contrasting with the 20% to 40% range for late-stage disease.[2] Therefore, developing markers for early identification and diagnosis in OC patients is critical due to the severity of the disease.

An essential characteristic of cancer cells involves alterations in DNA methylation patterns, representing a key epigenetic mechanism in tumorigenesis. Differential DNA methylation patterns, specifically hypomethylation, have been observed to correlate with the cancer stage, with genome-wide DNA hypomethylation decreasing as the primary lesion progresses to metastatic cancer.[3] These findings indicate that detecting DNA hypomethylation or hypermethylation in CpG dinucleotides holds promise as a diagnostic tool for cancer. Furthermore, due to epigenetic alterations' dynamic and reversible nature, they present promising targets for advancing more efficacious therapeutic approaches in the fight against cancer. The fundamental traits of cancer cells comprise genetic instability, resistance

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to programmed cell death, and continuous activation of growth-promoting signals.[4]

The *CYB5R4* gene, located on chromosome 6q14.2, has been implicated in diverse biological processes, including cellular development, oxidative stress response, and metabolism. Its dysregulation has been associated with various cancers. For instance, hypermethylation of *CYB5R4* has been linked to late-stage breast cancer and regulation by oncogenic microRNAs, suggesting its role in tumorigenesis.[5,6] Recent research indicates that the methylation profiles of *CYB5R* isoforms, such as *CYB5R2* and *CYB5R3*, exhibit contrasting roles in cancer, with *CYB5R2* acting as a tumor suppressor and *CYB5R3* potentially promoting tumor progression.[7] Specifically, Ming et al[7] demonstrated that the transcriptional activity of *CYB5R2* affects genes associated with angiogenesis, such as *VEGFA* and *TGFBR1*, suggesting a mechanism by which epigenetic inactivation of *CYB5R2* could contribute to lymph node metastasis in cancers, including ovarian cancer. Conversely, the role of *CYB5R3* appears to be more complex. While some studies suggest that *CYB5R3* may facilitate tumor progression by promoting the extravasation and colonization of cancer cells, its exact role in ovarian cancer remains unclear.[8] This duality in function highlights the need for further investigation into how these isoforms of *CYB5R* interact within the tumor microenvironment and influence cancer progression. Fedorova noted that the evidence surrounding *CYB5R's* involvement in carcinogenesis is sparse and sometimes contradictory, indicating that while *CYB5R2* may have tumor-suppressive effects, *CYB5R3* could contribute to tumor aggressiveness.[8] The complexity of these interactions underscores the importance of understanding the molecular landscape of ovarian cancer, where both tumor-suppressive and tumor-promoting factors may coexist and influence disease outcomes. In summary, the relationship between the *CYB5R* gene and ovarian cancer is characterized by the opposing roles of its isoforms, with *CYB5R2* acting as a potential tumor suppressor and *CYB5R3* possibly facilitating tumor progression. In a comparative study of our team, which evaluated the methylation profiles in monozygotic twins discordant for serous ovarian cancer, one of the genes showing methylation changes was the *CYB5R4* gene.[9]

The investigation of epigenetic changes in cancer, particularly DNA methylation, has become increasingly significant in understanding the genetic basis of this complex disease. DNA methylation plays a pivotal role in the pathogenesis of ovarian cancer, influencing both tumor initiation and progression through epigenetic modifications. Aberrant DNA methylation patterns, particularly hypermethylation of CpG islands in promoter regions, have been implicated in the silencing of tumor suppressor genes, which is a hallmark of many cancers, including ovarian cancer.[10] In ovarian cancer, hypermethylation of tumor suppressor genes, including *BRCA1, PTEN*, and others, has been associated with poor prognosis, chemoresistance, and aggressive tumor behavior.[11] In addition, studies have shown that the downregulation of methyltransferase-like proteins, such as *METTL14*, can lead to increased cell proliferation and contribute to the aggressive nature of ovarian tumors by stabilizing oncogenic mRNAs.[12] The interplay between DNA methylation and other epigenetic mechanisms, such as histone modifications, further highlights the complexity of gene regulation in ovarian cancer.[13] Understanding these alterations could provide critical insights into cancer biology and open new avenues for therapeutic intervention.

In this context, the present study aimed to explore the methylation levels of the *CYB5R4* gene in ovarian cancer patients, individuals with benign ovarian diseases, and healthy controls. In our previous research, we conducted comprehensive genomic profiling, including healthy and affected individuals—specifically, a serous ovarian cancer patient and her healthy monozygotic twin, both of whom were *BRCA* mutation carriers.[9,14] We aimed to investigate the methylation profiles of the *CYB5R4* gene in a large cohort of serous ovarian cancer patients' peripheral blood samples since circulating tumor DNA can be used as a predictor of progression-free and overall survival in epithelial ovarian cancer patients.[15] Primarily we evaluated its potential as a diagnostic tool for distinguishing between malignant and benign ovarian conditions, while also exploring its potential role as a prognostic marker linked to tumor progression and clinical outcomes. Here in this study, the *CYB5R4* methylation was found higher in serous ovarian cancer patients' plasma samples which might suggest that *CYB5R4* methylation levels may be associated with the biological processes of cancer, and could potentially serve as a biomarker for ovarian cancer development.

Materials and Methods

The DNA was isolated from the lymphocyte samples of 387 OC patients, 50 individuals with benign ovarian diseases who presented to Istanbul University Oncology Institute, and 100 healthy controls matched to the patients in terms of age, gender, and ethnicity. Cancer genetics biologists conducted genetic counseling. The data were collected during the initial genetic counseling session, which took place when they sought genetic testing. The blood samples were drawn for the study after the written informed consent was obtained from patients and healthy controls and the study was conducted at Istanbul University Oncology Institute, Cancer Genetics Department between 2020 and 2023. The samples from patients with a diagnosis of ovarian cancer were collected before surgery and prior to initiating chemotherapy. Ethics approval of the study was obtained from the Istanbul Faculty of Medicine Ethics Committee (Ethics Committee Approval: 2021/790-2019/1161).

This study was reported in accordance with the STROBE guidelines for observational studies (Supplementary Material 1).[16] The blood samples of 387 OC patients, 50 individuals with benign ovarian diseases, and 100 healthy controls were grouped for the methylation experiments. The collected samples were preserved in liquid nitrogen tanks at −270°C and in ultra-low temperature freezers at −80°C until the commencement of the experimental procedures.

Methyl-Specific Restriction Enzymes

The DNA extraction from blood samples of the patient cohort, healthy controls, and the control group was conducted following the guidelines outlined in the "Quick-DNA™ Miniprep Plus Kit" (Zymo Research, CA, USA). The DNA concentration was assessed utilizing the Nanodrop 2000 (Thermo Fisher, USA) and the integrity of the isolated DNA was evaluated through electrophoresis on a 0.8% agarose gel. DNA methylation analysis was performed using the OneStep qMethylTM (Zymo Research, CA, USA) Kit based on the enzyme shearing method. Two different reactions of test and reference were performed using the components of the kit. While the test reaction included the Methyl-Specific Restriction Enzymes (MSREs), no enzymes were included in the reference reaction. In both reactions, DNA was amplified using the real-time PCR using SYTO 9 fluorescent dye and *CYB5R4* gene-specific primers (The *CYB5R4* gene-specific methylated (sense, 5′-GGT TTG AAG ATG CTG AAC GT-3′; antisense, 5′CCGAGAGCCTATAATACCGC3′) were designed using the Integrated DNA Technologies Oligoanalyzer tool. To determine the methylation profiles of the *CYB5R4* gene, the "Zymo Research OneStepqMethyl" kit on the Applied Biosystems Real-Time PCR (Thermo Fisher, USA) was used. Each sample was studied 3 times. Amplification was conducted by a cutting step with MSR enzymes at 37°C for 2 hours, then a denaturation step at 95°C for 10 minutes, followed by denaturation at 95°C for 10 seconds, then annealing at 54°C for 60 seconds, extension at 72°C for 60 seconds, and 72°C for 7 minutes. The PCR reactions were conducted in pairs with 10 µL of 2X preMix, 5 µL of DNA sample, 2 µL of each sense and antisense primer for *CYB5R4*, and a total volume of 20 µL with DNase/RNase-free water. The cycle threshold value (Ct) during the amplification phase differed in the test and reference reactions. The Δ^{Ct} values (target ^{CT} − reference gene ^{CT}) in the peripheral blood of patients and controls were calculated as the sum of *percentage methylation* for the *CYB5R4* gene using the $100 \times 2^{-\Delta\Delta Ct}$ formula. Qualitative and quantitative variables were analyzed and compared based on the threshold value of 6% methylation. Samples with a methylation level of 6% or below were classified as unmethylated, while those above 6% were classified as methylated.

Statistical analysis

The number and percentage are given as descriptive statistics for qualitative variables, and the mean, standard deviation, median, minimum, and maximum values are given for quantitative variables. Relationships between qualitative variables were examined with Pearson Chi-square and Fisher Exact tests. The Bonferroni correction was used for post hoc tests in the comparison of the incidence rates of multiple groups. The suitability of quantitative variables to normal distribution was examined with the Shapiro-Wilk test. The homogeneity of variance was investigated using the Levene test. Mann-Whitney U test was used to compare the medians of two independent groups. Kruskall-Wallis test was used to compare the medians of multiple independent groups. Dunn test was used as a post hoc test in multiple comparisons. The statistical significance level was taken as .05, and the Statistical Package for the Social Sciences (SPSS) (version 26, IBM Corp. in Armonk, NY) program was used in the calculations.

Results

The study included ovarian cancer patients, individuals with benign ovarian diseases, and healthy controls with qualitative variables, including diagnosis, clinical stage, histological grade, histological subtype, ethnicity, and menopausal status reported as frequencies and percentages (Table 1). Among the ovarian cancer patients, 48 (12.4%) were tested positive for *BRCA1*, while 339 (87.6%) were *BRCA1*-negative. Of the overall *BRCA* status (*BRCA1&BRCA2*), 92 ovarian cancer patients (23.8%) were *BRCA*-positive, while 295 (76.2%) were *BRCA*-negative. In contrast, all individuals with benign ovarian diseases and in the healthy control group were *BRCA*-negative. Regarding age, 77 ovarian cancer patients (19.9%) were younger than 45 years, while 260 patients (67.2%) were over 45 years old. The clinical staging of ovarian cancer patients showed that the majority were in stage 3 (204 patients, 52.7%), followed by stage 4 (52 patients, 13.4%), stage 1 (61 patients, 15.8 %), and stage 2 (42 patients, 10.9%). Histological grading revealed that most ovarian cancer patients were in stage 2 (103 patients, 26.6%), followed by stage 3 (195 patients, 50.4%) and stage 1 (57 patients, 14.7%). Menopausal status showed that 162 ovarian cancer patients (41.9%) were premenopausal, and 168 patients (43.4%) were postmenopausal period. There was no significant difference between methylated and unmethylated groups in terms of age ($P = .192$).

The findings on *CYB5R4* methylation levels in ovarian cancer patients have important clinical implications.

A statistically significant difference was observed in age distribution between the methylated and unmethylated groups. The proportion of patients over 45 years old was significantly higher in the methylated group compared with the unmethylated group ($P < .001$). When evaluated in terms of clinical stage, the proportion of patients in clinical stages 3 and 4 was significantly higher in the methylated group ($P < .001$). The proportion of grade 3 tumors was significantly higher in the methylated group, while grade 1 tumors were more common in the unmethylated group ($P < .001$). A statistically significant difference was also observed in terms of ethnicity, with a significantly higher proportion of Turkish individuals in the methylated group ($P < .001$). No statistically significant difference was observed between methylation status and menopausal status ($P = .052$).

Advanced clinical stages, particularly stage 3 ($P = .001$) and stage 4 ($P = .003$), exhibit higher methylation levels, indicating a potential link between increased methylation and disease progression. Similarly, histological grades, such as grade 3 ($P = .001$) and grade 2 ($P = .021$), are significantly associated with higher methylation. Between patients with ovarian cancer, there is a significant difference in methylation levels between age < 45 ($P = .072$) and

Table 1. The clinical and demographic features of the ovarian cancer patients and *P* values.

		Ovarian cancer patients		Methylated		Unmethylated		
		Number	%	Number	%	Number	%	*P*
Age 45	⩽45	77	19.9	20	9.5	57	44.9	<.001
	>45	260	67.2	190	90.5	70	55.1	
	Unknown	50	12.9					
Clinical Stage	Stage 1	61	15.8	15[a]	7.4	46[b]	29.3	<.001
	Stage 2	42	10.9	32[a]	15.8	10[b]	6.4	
	Stage 3	204	52.7	120[a]	59.5	84[b]	53.5	
	Stage 4	52	13.4	35[a]	17.3	17[b]	10.8	
	Unknown	28	7.2					
Histological Grade	Grade 1	57	14.7	12[a]	6.1	45[b]	28.5	<.001
	Grade 2	103	26.6	55[a]	27.9	48[b]	30.4	
	Grade 3	195	50.4	130[a]	66	65[b]	41.1	
	Unknown	32	8.3					
Ethnicity	Turkish	265	68.5	180[a]	80	85[b]	57.8	<.001
	Balkan	72	18.6	30[a]	13.3	42[b]	28.6	
	Kurdish	35	9	15[a]	6.7	20[b]	13.6	
	Unknown	15	3.9					
Histological Subtype	Serous	387	100					
Menopausal status	Premenopausal	162	41.9	112	53.3	50	41.7	.052
	Postmenopausal	168	43.4	98	46.7	70	58.3	
	Unknown	57	14.7					

[a,b]Superscript letters indicate pairwise comparisons between groups. Groups sharing the same letter do not differ significantly, while groups with different letters show a statistically significant difference in the respective proportion ($P < 0.05$).

Table 2. Comparison of *CYB5R4* gene methylation levels (%) among healthy controls, individuals with benign ovarian diseases, and ovarian cancer patients.

Groups	Median	IQR	Minimum	Maximum	*P* value
Healthy Controls	4.47474	3.000-6.500	0.979	99.309	.012
Individuals with Benign Ovarian Diseases	3.52401	2.500-4.600	0.849	29.526	
Ovarian Cancer Patients	6.22840	4.200-8.800	0.108	143.400	

age >45 ($P=.002$), ethnicity Turkish ($P=.003$) and non-Turkish-Balkan ($P=.061$) and Kurdish ($P=.055$). There is also a significant difference in methylation levels between stage 1 ($P=.045$) and stage 2 ($P=.056$) and between stage 3 ($P=.001$) and stage 4 ($P=.003$). The ethnic makeup, predominantly Turkish, also suggests genetic influences on methylation patterns. Overall, these results imply that *CYB5R4* methylation could be a valuable biomarker for prognosis and personalized treatment in ovarian cancer.

Table 2 shows the comparison of *CYB5R4* gene methylation levels among the study groups revealing notable differences. Ovarian cancer patients exhibited the highest median value (6.23%) reflecting considerable variability within this group. Healthy controls showed a slightly lower median value (4.47%), individuals with benign ovarian diseases had the lowest median value (3.52%) indicating more consistent methylation levels within this group.

The differences in methylation levels across the groups were statistically significant ($P=.041$) (Table 2), suggesting that higher *CYB5R4* methylation may be associated with the presence and progression of ovarian cancer. In our analysis, we observed differences in the median *CYB5R4* methylation levels across the 3 groups. The ovarian cancer patients had a higher median value (6.23) compared with both healthy controls (4.47) and individuals with benign ovarian diseases (3.52). This variation suggests a potential trend in *CYB5R4* methylation patterns associated with disease status, with ovarian cancer patients showing the highest median methylation levels among the groups. Statistical analysis confirmed a significant difference between groups ($P=.041$). This comparison underscores a notable increase in *CYB5R4* methylation in ovarian cancer patients compared with both benign ovarian diseases and healthy individuals, suggesting a potential association between *CYB5R4* methylation and ovarian cancer.

Figure 1 shows the distribution of methylated and unmethylated *CYB5R4* gene profiles across 3 cohorts as ovarian cancer patients (N=387), individuals with benign ovarian diseases (N=50), and healthy controls (N=100). In the ovarian cancer group, a significant proportion of samples (229/387) showed *CYB5R4* methylation, while 158 samples remained unmethylated. In contrast, individuals with benign ovarian diseases showed a much lower incidence of *CYB5R4* methylation, with only 12 methylated samples compared with 38 unmethylated samples. The healthy control group displayed similar methylation patterns to the individuals with benign ovarian diseases, with

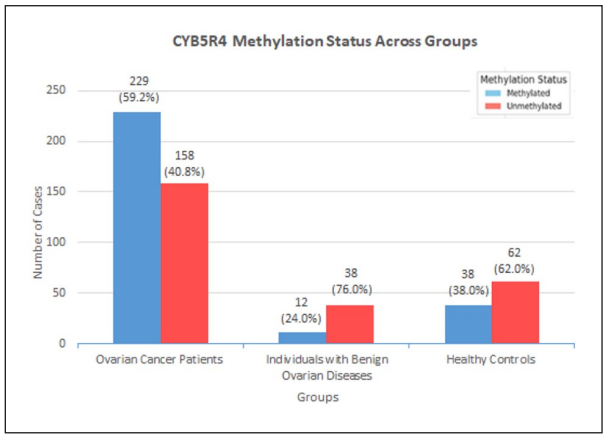

Figure.1. The methylation status of peripheral blood samples of ovarian cancer patients, individuals with benign ovarian diseases, and a healthy control group.

38 methylated and 62 unmethylated samples, indicating a potential baseline level of methylation in noncancerous tissue. These findings highlight a distinct difference in *CYB5R4* methylation profiles between those with malignant masses, benign masses, and healthy individuals' blood samples, suggesting that *CYB5R4* methylation could serve as a potential biomarker for ovarian cancer diagnosis.

Discussion

Ovarian cancer (OC) is the eighth most common cancer among women worldwide. Methylation plays a crucial role in ovarian cancer as demonstrated in various studies.[17,18] Strathdee et al[19] investigated the methylation status of 93 primary ovarian tumors at 10 loci, revealing multiple methylator phenotypes involving the tumor suppressor genes. Houshdaran et al[20] highlighted the distinct methylation profiles of different histological types of ovarian tumors, emphasizing the need for treating different histologies of ovarian cancer as different diseases. In addition, Su et al[21] found that DNA methylation of *SFRP5* was associated with malignant phenotype and chemoresistance of ovarian cancer through the Wnt signaling pathway. Feng et al[22] reviewed the correlation between aberrant methylation levels of genes and poor prognosis in ovarian cancer, shedding light on the prognostic implications of methylation alterations. Furthermore, Li et al[12] provided insights into the downregulation of methyltransferase-like 14 in ovarian carcinomas, emphasizing the complexity of methylation alterations in ovarian cancer. Based on these studies, we investigated the methylation profiles of the *CYB5R4* gene to reveal easier disease management in ovarian cancer and to examine DNA methylation changes that are considered to be useful, especially in early diagnosis in our study. In addition, its potential as a biomarker was investigated in large patient cohorts.

In this study, we focused on the *CYB5R4* gene, investigating its methylation profile in ovarian cancer patients compared with individuals with benign ovarian masses and healthy individuals. Our results demonstrated a statistically significant increase in *CYB5R4* methylation in ovarian cancer patients (P=.041), suggesting its potential as a biomarker for distinguishing between malignant and benign conditions. Unlike previous studies that focused on tumor tissues, we used blood samples, enabling a noninvasive approach that reflects circulating tumor DNA (ctDNA). This aligns with existing literature, which supports the reliability of blood-based methylation analyses as proxies for tumor-specific alterations.

In addition, ethnic variability in methylation patterns was explored, revealing higher methylation rates among Turkish patients compared with Balkan and Kurdish groups. Although not statistically significant across all groups, these findings hint at potential genetic or environmental influences on methylation patterns.

The findings of this study suggest that *CYB5R4* gene methylation may serve as a significant biomarker for ovarian cancer (OC), particularly in distinguishing between malignant and benign conditions. The detected median methylation percentage of 6.22% in ovarian cancer patients compared with the level of 4.47% in healthy controls, indicates a notable difference that could have clinical implications. This aligns with previous research that highlights the role of DNA methylation in cancer progression and its potential as a diagnostic tool. For instance, hypermethylation of tumor suppressor genes has been linked to poor prognosis in OC, emphasizing the importance of epigenetic changes in tumor biology.[22] Furthermore, studies have shown that aberrant methylation patterns can be associated with various pathways involved in cancer development, including those related to DNA repair and apoptosis.[22,23]

We found a significant increase in methylation observed in ovarian cancer patients compared with individuals with benign ovarian masses and healthy control individuals, which may indicate its involvement in tumorigenesis, warranting further investigation into its role in cancer biology. The methylation status of genes, including *CYB5R4* might play a significant role in cancer biology, influencing both diagnostic and therapeutic strategies.

Moreover, the methodology used in this study, utilizing Methyl Specific Restriction Enzymes (MSREs), is a well-established technique for assessing methylation status and has been effectively used in other studies to evaluate multiple tumor suppressor genes in ovarian cancer.[24] The ability to detect methylation in blood samples also presents a noninvasive approach for early diagnosis, which is crucial given that ovarian cancer is often diagnosed at advanced stages due to the lack of early symptoms.[25]

Despite the promising findings, this study has several limitations. First, the sample size for individuals with benign ovarian diseases (n=50) is relatively small compared with the sample size in ovarian cancer patients (n=387) and healthy controls (n=100), which may limit the statistical power and generalizability of the results. In addition, the study relied on blood samples for DNA analysis, which might not fully capture the heterogeneity of methylation patterns present in tumor tissues. But the use of blood samples in this study was chosen for their non-invasive nature, practicality, and feasibility for large-scale patient cohorts. Blood-based analyses allow for the detection of circulating tumor DNA (ctDNA), which reflects the genetic and epigenetic landscape of tumors without

requiring invasive procedures.[26] Previous studies indicate that methylation patterns observed in blood samples often mirror those found in tumor tissues, including, supporting their reliability as a proxy for tumor-specific alterations.[27] In the case of *CYB5R4*, hypermethylation is a well-documented epigenetic alteration in cancers, including ovarian cancer, where it contributes to tumorigenesis by silencing tumor suppressor genes.[28] Thus, detecting hypermethylation in blood samples is a practical and scientifically validated approach, aligning with established cancer methylation patterns and highlighting its potential as a biomarker for ovarian cancer. The use of a single threshold for methylation classification (6%) might oversimplify the complexity of methylation patterns and their biological significance. Another limitation of our study is that we did not assess potential interactions between *CYB5R4* methylation and other genetic or environmental factors that could influence ovarian cancer risk.

Conclusions

In conclusion, although the results of this study are promising, further research is warranted to validate the clinical utility of *CYB5R4* methylation as a biomarker for ovarian cancer management. Future studies should aim to explore the relationship between *CYB5R4* methylation and clinical outcomes, as well as its potential role in guiding therapeutic decisions. The integration of methylation analysis with other molecular profiling techniques may provide a more comprehensive understanding of ovarian cancer biology and improve patient stratification for targeted therapies.

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Ethical considerations

The Istanbul Faculty of Medicine Ethics Committee approved the study (Ethics Committee Approval: 2021/790-2019/1161).

Consent to participate

Blood samples were drawn after informed consent was obtained from patients and healthy controls.

Consent for publication

Not applicable.

Author contributions

AG and OSE: Acquisition, design, and the interpretation of the research study. SKE and BCD provided support and advice on technical aspects of the research and drafted the article. AD helped with the experiments. OP analyzed the data. PS and HY selected the patients to be included in this study. SBT: Supervised the study and revised it critically for important intellectual content.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability statement

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

Supplemental material

Supplemental material for this article is available online.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71:209-249.
2. Charkhchi P, Cybulski C, Gronwald J, Wong FO, Narod SA, Akbari MR. CA125 and ovarian cancer: a comprehensive review. *Cancers (Basel)*. 2020;12:3730.
3. Weber M, Davies JJ, Wittig D, et al. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nat Genet*. 2005;37:853-862.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144:646-674.
5. Newman S, Howarth KD, Greenman CD, Bignell GR, Tavaré S, Edwards PA. The relative timing of mutations in a breast cancer genome. *PLoS ONE*. 2013;8:e64991.
6. Han N, Song YK, Burckart GJ, Ji E, Kim I-W, Oh JM. Regulation of pharmacogene expression by microrna in the cancer genome atlas (TCGA) research network. *Biomol Ther (Seoul)*. 2017;25:482-489.
7. Ming H, Lan Y, He F, et al. Cytochrome b5 reductase 2 suppresses tumor formation in nasopharyngeal carcinoma by attenuating angiogenesis. *Chin J Cancer*. 2015;34:459-467.
8. Fedorova MV, Voznesensky VI, Sosnova EA, Proskurnina EV. Activity of NAD(P)H-oxidoreductases in ovarian cancer. *Biomedicines*. 2024;12:1052.
9. Erdogan OS, Tuncer SB, Kilic S, et al. Genome-wide methylation profiles in monozygotic twins with discordance for ovarian carcinoma. *Oncol Lett*. 2020;20:357.
10. Maldonado L, Hoque MO. Epigenomics and ovarian carcinoma. *Biomark Med*. 2010;4:543-570.
11. Guruvaiah P, Chava S, Sun C-W, Singh N, Penn CA, Gupta R. ATAD2 is a driver and a therapeutic target in ovarian cancer that functions by upregulating CENPE. *Cell Death Dis*. 2023;14:456.
12. Li Y, Peng H, Jiang P, et al. Downregulation of methyltransferase-like 14 promotes ovarian cancer cell proliferation through stabilizing TROAP mRNA. *Front Oncol*. 2022;12:824258.

13. Natanzon Y, Goode EL, Cunningham JM. Epigenetics in ovarian cancer. *Semin Cancer Biol*. 2018;51:160-169.
14. Tuncer SB, Erdogan OS, Erciyas SK, 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. *J Ovarian Res*. 2020;13:99.
15. Taliento C, Morciano G, Nero C, et al. Circulating tumor DNA as a biomarker for predicting progression-free survival and overall survival in patients with epithelial ovarian cancer: a systematic review and meta-analysis. *Int J Gynecol Cancer*. 2024;34:906-918.
16. Von Elm E, Altman DG, Egger M, et al. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann Intern Med*. 2007;147:573-577.
17. Fu M, Deng F, Chen J, et al. Current data and future perspectives on DNA methylation in ovarian cancer (Review). *Int J Oncol*. 2024;64:62.
18. Gautam P, Gupta S, Sachan M. Comprehensive DNA methylation profiling by MeDIP-NGS identifies potential genes and pathways for epithelial ovarian cancer. *J Ovarian Res*. 2024;17:83.
19. Strathdee G, Appleton K, Illand M, et al. Primary ovarian carcinomas display multiple methylator phenotypes involving known tumor suppressor genes. *Am J Pathol*. 2001;158:1121-1127.
20. Houshdaran S, Hawley S, Palmer C, et al. DNA methylation profiles of ovarian epithelial carcinoma tumors and cell lines. *PLoS ONE*. 2010;5:e9359.
21. Su HY, Lai H-C, Lin Y-W, et al. Epigenetic silencing of SFRP5 is related to malignant phenotype and chemoresistance of ovarian cancer through Wnt signaling pathway. *Int J Cancer*. 2010;127:555-567.
22. Feng LY, Chen CX, Li L. Hypermethylation of tumor suppressor genes is a risk factor for poor prognosis in ovarian cancer: a meta-analysis. *Medicine (Baltimore)*. 2019;98:e14588.
23. Chmelarova M, Dvorakova E, Spacek J, Laco J, Palicka V. Importance of promoter methylation of GATA4 gene in epithelial ovarian cancer. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2013;157:294-297.
24. Ozdemir F, Altinisik J, Karateke A, Coksuer H, Buyru N. Methylation of tumor suppressor genes in ovarian cancer. *Exp Ther Med*. 2012;4:1092-1096.
25. Wang YQ, Yan Q, Zhang JR, Li SD, Yang YX, Wan XP. Epigenetic inactivation of BRCA1 through promoter hypermethylation in ovarian cancer progression. *J Obstet Gynaecol Res*. 2013;39:549-554.
26. Van Zogchel LMJ, Lak NSM, Verhagen OJHM, et al. Novel circulating hypermethylated RASSF1A ddPCR for liquid biopsies in patients with pediatric solid tumors. *JCO Precis Oncol*. 2021;5:PO.21.00130.
27. Hoan NX, Van Tong H, Giang DP, et al. SOCS3 genetic variants and promoter hypermethylation in patients with chronic hepatitis B. *Oncotarget*. 2017;8:17127-17139.
28. Olivera-Salazar R, Salcedo Cabañas G, Vega-Clemente L, et al. Pilot study by liquid biopsy in gastrointestinal stromal tumors: analysis of PDGFRA D842V mutation and hypermethylation of SEPT9 presence by digital droplet PCR. *Int J Mol Sci*. 2024;25:6783.