Research Square

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Cervical detection of STIC-associated TUBB6 methylation in high-grade serous ovarian carcinoma compared with benign ovarian tumors

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Research Article

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Abstract

Background:

High-grade serous ovarian carcinoma (HGSOC) is the most lethal subtype of ovarian cancer and is increasingly recognized to originate from serous tubal intraepithelial carcinoma (STIC) in the fallopian tube epithelium. DNA methylation alterations occur early in serous tumorigenesis and may provide potential biomarkers for disease detection. However, whether STIC-associated methylation signals can be detected using minimally invasive cervical specimens remains unclear. This study investigated the detectability of STIC-related methylation markers in cervical scrapings from patients with HGSOC.

Methods:

In this retrospective case–control study, cervical swab samples from 185 women were analyzed, including 83 patients with HGSOC and 102 patients with benign ovarian tumors. Candidate loci previously identified from genome-wide methylation analyses of STIC lesions (*c17orf64*, *IRX2*, and *TUBB6*) were evaluated in the pilot study using quantitative methylation-specific polymerase chain reaction. Methylation levels were compared between malignant and benign groups, and diagnostic performance was assessed using receiver operating characteristic analysis.

Results:

Among the three candidate loci, *TUBB6* methylation was consistently detectable in cervical scrapings and was significantly elevated in patients with HGSOC compared with benign controls. TUBB6 methylation levels were independent of tumor stage, suggesting that the alteration occurs early during serous tumorigenesis. In benign samples, TUBB6 methylation increased with age, consistent with age-related epigenetic drift, whereas in malignant samples, methylation was elevated independent of age, indicating tumor-associated epigenetic remodeling. The *TUBB6*-only model yielded an area under the curve of 0.75. When combined with serum CA125, the discriminatory performance improved.

Conclusions:

TUBB6 methylation can be detected in cervical scrapings and reflects molecular alterations associated with STIC-related serous ovarian tumorigenesis. These findings demonstrate that STIC-associated methylation signals can be detected in cervical specimens and highlight the potential of cervical-based sampling for studying molecular alterations associated with HGSOC. Further validation studies are warranted to evaluate its potential role in ovarian cancer detection strategies.

Background

Ovarian cancer is the fifth most common cancer in women globally and also the most lethal gynecologic malignancy because of its nonspecific symptoms (1). Current therapeutic management for ovarian cancer is used with a multidisciplinary approach that mainly includes debulking surgery, chemotherapy, and targeted therapy (2). Twenty to forty percent of patients do not respond to this fundamental therapy and eighty percent of those patients with a good initial response to treatment eventually develop a platinum-resistant recurrence (3). Moreover, only about 20% of ovarian cancer cases are found at an early stage, which means more than 80% of ovarian cancer patients are diagnosed at an advanced stage (4).

Early detection is the key to cancer survival. Currently, transvaginal sonography (TVS), the protein biomarker cancer antigen 125 (CA125), and human epididymis protein 4 (HE4) are the three common approaches for ovarian cancer. Still, none is sufficient due to their low specificity or limited early-detection capacity (5–10). More recently, the development of genome-wide epigenomic profiling technologies has enabled researchers to discover that changes in DNA methylation patterns are among the earliest and most frequent epigenetic alterations that contribute to cancer development (11). Specific DNA methylation patterns and common gene methylation have been demonstrated in gynecologic cancers and the significance of DNA methylation in tumor growth is emerging as a potential biomarker for the early detection of ovarian cancer (12). Ours and other studies also show positive results in using cervical scraping for the identification of DNA methylation biomarkers in ovarian cancer (13–16). Markers for earlier precursor lesions are needed.

High-grade serous ovarian carcinoma (HGSOC) is clinically the most common histotype of ovarian cancer and is responsible for 70–80% of ovarian cancer deaths (17). The cell origin of high-grade serous ovarian carcinoma was thought to arise from the ovarian surface epithelium (OSE) and, more recently, in the fallopian tube epithelium (FTE) (18, 19). Current evidence has also pointed out that the pre-cancerous lesion of high-grade serous ovarian carcinoma, serous tubal intraepithelial carcinoma (STIC), was developed from fallopian tube epithelium (20). STIC has been widely recognized as a precursor lesion of HGSOC. Otherwise, transcriptional and DNA methylomic analyses both show high similarity between high-grade serous ovarian carcinoma and fallopian tube epithelium, as well as with SITC, supporting the hypothesis of a high-grade serous ovarian carcinoma cell of origin (21, 22). In addition, common hypermethylated regions were identified in both serous tubal intraepithelial carcinomas and high-grade serous ovarian carcinomas (23–25). Genome-wide methylation analysis of gynecologic tissue samples previously uncovered differentially methylated genes between serous tubal intraepithelial carcinoma and normal fallopian tube epithelium (26). Furthermore, hypermethylation at the three loci (*c17orf64, IRX2*, and *TUBB6*) was observed exclusively in laser-capture microdissected (LCM) fallopian tube epithelium samples from women with serous tubal intraepithelial carcinoma, but not in those from cancer-free cases. These findings suggest that methylation markers associated with STIC lesions may provide potential molecular indicators of early serous tumorigenesis.

However, most previous studies have evaluated STIC-associated methylation alterations in surgical tissue specimens. Whether such precursor-associated molecular signals can be detected using

minimally invasive clinical specimens remains largely unknown. Cervical sampling is routinely performed in gynecologic practice and may provide an accessible source of exfoliated cellular DNA from the female reproductive tract. Therefore, the present study aimed to investigate whether STIC-associated methylation markers can be detected in cervical scrapings from patients with HGSOC and to evaluate their translational potential for ovarian cancer detection. In this study, we aimed to determine whether serous tubal intraepithelial carcinoma-specific gene methylation can be detected in cervical scrapings, which may open a new door to early ovarian cancer detection.

Methods

Pilot study testing for three serous tubal intraepithelial carcinoma-related genes methylation

In this retrospective case–control study, cervical swab samples from 185 women were analyzed, including 83 patients with pathologically confirmed HGSOC and 102 patients with benign ovarian tumors. First, we collected cervical swabs from patients with high-grade serous ovarian carcinoma (HGSOC, n = 13) and benign ovarian tumors (n = 10) to assess the potential to detect serous tubal intraepithelial carcinoma-related gene methylation (*c17orf64, IRX2*, and *TUBB6*) in cervical scrapings. Quantitative methylation-specific PCR (qMSP) was performed to detect methylation in the three candidate genes among the high-grade serous ovarian carcinoma and control groups; *TUBB6* showed the most consistent methylation signal among the STIC-associated loci examined. Thus, *TUBB6* methylation was further tested in the remaining samples to evaluate clinical correlation. Finally, the performance of *TUBB6* methylation in discriminating cervical scrapings from patients with benign tumors and malignant tissues was also determined.

Clinical Samples and Specimen Processing

This study is a retrospective case-control study. It was approved by the Joint Institutional Review Board of Taipei Medical University (TMU-JIRB No. N201810036). A total of 185 cervical swab samples, including 102 from patients with benign ovarian tumors and 83 from patients with high-grade serous ovarian carcinoma (Table S1), were prospectively collected from multiple clinical centers between November 2018 and March 2020. Written informed consent was obtained from all participating women before sample collection, in accordance with the protocols approved by the ethics committee. Gynecologists collected cervical scrapings by Pap smear before surgery and the tumor tissues were further obtained by the surgeon and determined by pathological examination. Cervical samples were obtained prior to surgery to avoid contamination from surgical manipulation. All laboratory personnel involved in specimen processing were blinded to participants' clinical data.

Quantification of DNA Methylation

Genomic DNA was extracted from the Pap smear specimens using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) and stored at − 20°C. For bisulfite conversion, 800 ng of genomic DNA was treated using the EZ DNA Methylation Kit (D5008; Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. Quantitative methylation-specific PCR (qMSP) was performed to assess TUBB6 methylation levels in converted DNA samples using the LightCycler 480 System (Roche, Indianapolis, IN, USA), as previously described (27). Each sample was analyzed in duplicate. Primers for the target gene were designed using Oligo 7.0 Primer Analysis Software (Molecular Biology Insights, Inc., Colorado Springs, CO, USA). The type II collagen gene (*COL2A1*), which lacks CpG islands, was used as an internal control to normalize the input DNA quantity. Samples with a *COL2A1* Cp value greater than 36 were considered not detectable. The DNA methylation level was estimated as the methylation index (M-index) using the formula: $10000 \times 2^{[(\text{Cp of COL2A}) - (\text{Cp of } \textit{TUBB6})]}$.

Statistical Analysis

Methylation levels between benign and malignant groups were compared using the Mann-Whitney U test. Subgroup analyses were performed for patients with serous-type pathology (serous adenocarcinoma) and those with available CA125 data. Receiver operating characteristic (ROC) curves were generated to evaluate the diagnostic performance of *TUBB6* methylation alone and in combination with CA125. The area under the curve (AUC) with 95% confidence intervals (CI) was reported. Sensitivity and specificity were calculated based on the optimal cut-off point determined by the Youden index. All statistical analyses were performed using MedCalc software (version 22), and $p < 0.05$ was considered statistically significant.

Results

TUBB6 methylation can be detected in cervical swabs from patients with high-grade serous ovarian carcinoma

We conducted a pilot study and analyzed promoter methylation of the three genes (*c17orf64*, *IRX2*, and *TUBB6*) in 10 swabs from women with benign ovarian tumors and 13 swabs from patients with HGSOC using Q-MSP (Fig. 1). Methylation levels of *c17orf64* and *IRX2* showed no significant difference ($p = 0.63$ and $p = 0.32$, respectively). Only *TUBB6* methylation levels were significantly higher in the malignant (HGSOC) group compared with the benign group ($p < 0.01$). We proceed with further analysis solely on *TUBB6*.

We then expanded the sample size of cervical swabs to 185 participants (102 cases with benign tumors and 83 with malignant tumors). The demographic characteristics of the participants are shown in Supplemental Table 1 (Table S1). Patients in the malignant group were significantly older than those in the benign group ($p < 0.01$). CA125 levels were higher in the malignant group ($p < 0.01$). *TUBB6* methylation levels were significantly higher in the malignant group than in the benign group ($p < 0.01$, as shown in Fig. 2).

TUBB6 methylation can be detected in the early stages of high-grade serous ovarian carcinoma and independent of age effects

To assess the potential of *TUBB6* hypermethylation as a biomarker for early detection of high-grade serous ovarian carcinoma, we first examined its association with tumor stage in the malignant group. The data showed that *TUBB6* methylation did not differ across tumor stages (Fig. 3).

A positive correlation between methylation and age was observed in the benign group, but none in the malignant group (Fig. 4A). Furthermore, it is known that the risk of developing ovarian cancer increases with age, and those over the age of 50 have a higher risk. We then further examine the association between *TUBB6* methylation and age 50 in both groups. In swabs from the malignant group, *TUBB6* methylation showed no difference between those aged < 50 and those aged > 50 ($p = 0.78$) (Fig. 4B). Interestingly, *TUBB6* methylation was significantly higher in swabs from older women in the benign group than in those from women aged 50 or younger ($p < 0.01$). This analysis was conducted to determine whether age-related methylation drift contributes to the observed differences between benign and malignant ovarian tumors.

The add-on performance of TUBB6 methylation by CA125

To test the clinical performance, ROC curve analysis was performed to discriminate between benign and malignant groups. As shown in Fig. 5A, the AUC of ROC plots for *TUBB6* methylation in the detection of high-grade serous ovarian carcinoma was 0.75. When combined with CA125, performance improved with an AUC of 0.83 (Fig. 5B).

Discussion

For the first time, we demonstrated that specific gene methylation in serous tubal intraepithelial carcinoma can be detected in cervical scraping samples, extending tissue-based epigenomic observations into minimally invasive clinical specimens. Among the three candidate genes (*c17orf64*, *IRX2*, and *TUBB6*) previously identified through genome-wide methylation analysis of serous tubal intraepithelial carcinoma lesions, *TUBB6* showed significantly higher methylation levels in cervical swabs from patients with high-grade serous ovarian carcinoma.

A notable observation is the distinct methylation behavior of *TUBB6* in benign versus malignant conditions. In benign ovarian tumors, *TUBB6* methylation increased with age, consistent with age-related epigenetic drift. In contrast, malignant samples exhibited elevated methylation levels independent of age, indicating tumor-driven epigenetic remodeling. This divergence suggests a transition from physiological age-associated methylation drift to malignancy-dominant epigenetic reprogramming during serous tumorigenesis. Together, these findings provide a biological context for the detectability of *TUBB6* methylation in cervical samples and highlight its relevance as a lineage-associated epigenetic signature of high-grade serous ovarian carcinoma. *TUBB6* can also be detected at the early stages of high-grade serous ovarian carcinoma. Analogous to cervical carcinogenesis, in which identification and

management of precancerous cervical intraepithelial neoplasia (CIN) markedly reduced the incidence and mortality of invasive cervical cancer, early detection of serous tubal intraepithelial carcinoma could likewise mitigate the burden and downstream impact of high-grade serous ovarian carcinoma. Our findings highlight the potential of cervical-swab methylation testing to capture early molecular events, analogous to CIN detection, in cervical cancer prevention.

TUBB6 (Tubulin Beta 6 Class V) is a member of the beta tubulin superfamily and is also one major component of microtubules that contribute to many cellular processes, such as structural support, intracellular transport and DNA segregation. Studies also show *TUBB6* gene plays a role in cancer development. High expression of *TUBB6* promotes tumor growth and aggressiveness and also correlates with poorer survival outcomes in patients with certain cancers, such as glioma, colon cancer, and bladder cancer (28–30). On the other hand, hypermethylation of *TUBB6* is found in liver cancer tissues and is associated with the overall survival of patients in gastric cancer (31, 32). Genome-wide methylation analyses on gynecologic tissue samples also show evidence that DNA methylation can identify high-grade serous ovarian carcinoma, even in serous tubal intraepithelial carcinoma lesions (23–26). In our study, we demonstrated that *TUBB6* hypermethylation can be detected in cervical swabs from patients with high-grade serous ovarian carcinoma. Functional characterization of *TUBB6* in fallopian tube epithelium may further clarify the tubal-origin carcinogenesis.

Consistent with our findings, previous studies have highlighted the role of DNA methylation as a frequent molecular alteration of ovarian cancer (33). Aberrant hypermethylation of tumor suppressor genes or hypomethylation of oncogenes not only reflects cancer development but also provides opportunities for biomarker discovery or combination that may complement existing clinical tools (15). Compared with CA125 for early detection of ovarian cancer, which is limited by low sensitivity in early disease (50–62%) (34, 35), *TUBB6* methylation showed a relatively robust discriminatory capacity (AUC = 0.75, sensitivity = 81%). The diagnostic performance improved substantially when combined with CA125, achieving moderate discriminatory capacity (AUC = 0.83) and a similar sensitivity (80%). The combination of *TUBB6* methylation and CA125 improved the detection rate of high-grade serous ovarian carcinoma. Further testing of methylation markers associated with serous tubal intraepithelial carcinoma is imperative for clinical applications.

Our findings highlight the potential of detecting *TUBB6* methylation in STIC lesions by cervical swabs from women who were suspected of ovarian cancer. Since serous tubal intraepithelial carcinoma is believed to be a precursor to the majority of high-grade serous ovarian carcinoma, cervical-based methylation testing may provide a feasible translational approach for detecting molecular alterations associated with serous ovarian tumorigenesis. Our previous studies have explored DNA methylation using cervical swabs to detect ovarian and endometrial cancers (13, 14, 27). In this study, we demonstrate that *TUBB6* promoter methylation is significantly elevated in cervical swabs from patients with high-grade serous ovarian carcinoma. These findings suggest that detection of *TUBB6* methylation in cervical swabs holds promise as a molecular marker of early serous tumorigenesis originating in the fallopian tube.

Several limitations should be acknowledged. First, although the present study included a relatively large cohort of cervical samples, the findings were derived from a single study population. Independent validation in additional cohorts will be required to confirm the reproducibility of *TUBB6* methylation as a biomarker for HGSOC. Second, because this study was retrospective, complete CA125 measurements were not available for all participants. Third, while *TUBB6* demonstrated promising discriminatory capacity, reliance on a single methylation marker may not be sufficient for robust clinical application. Identification of additional complementary methylation markers will help improve diagnostic performance.

The detection of *TUBB6* methylation in cervical swabs demonstrates that serous tubal intraepithelial carcinoma-related molecular changes can be identified using cervical samples. Further research may focus on identifying additional biomarkers associated with serous tubal intraepithelial carcinoma that are detectable in cervical scrapings or blood. Combining multiple serous tubal intraepithelial carcinoma-related biomarkers may make earlier detection of high-grade serous ovarian carcinoma possible, which may substantially reduce the mortality rate of current ovarian cancer treatment. Triage of adnexal tumors using serous tubal intraepithelial carcinoma-related markers may play an essential role in clinical decision-making of a major survey.

CONCLUSIONS

TUBB6 methylation is detectable in cervical scraping samples and reflects molecular alterations associated with STIC-related serous ovarian tumorigenesis. These findings demonstrate the feasibility of detecting precursor-associated epigenetic signals using minimally invasive cervical specimens. Further studies are warranted to validate these observations and evaluate additional methylation markers to improve ovarian cancer detection strategies.

Abbreviations

AUC
Area Under The Curve
CA125
Cancer Antigen 125
CI
Confidence Interval
CIN
Cervical Intraepithelial Neoplasia
FTE
Fallopian Tube Epithelium
HE4
Human Epididymis Protein 4
HGSOC

High-Grade Serous Ovarian Carcinoma
LCM
Laser-Capture Microdissected
OSE
Ovarian Surface Epithelium
PCR
Polymerase Chain Reaction
qMSP
Quantitative Methylation-Specific PCR
ROC
Receiver Operating Characteristic
STIC
Serous Tubal Intraepithelial Carcinoma
TVS
Transvaginal Sonography

Declarations

Ethics approval and consent to participate (Human Ethics and Consent to Participate declarations)

This study involves human participants and was approved by the Institutional Review Board of Taipei Medical University (TMU-JIRB No. N201810036). Participants provided informed consent before participating in the study.

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Kuo-Min Su: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, writing - original draft. Kuo-Chang Wen: resources, funding acquisition, project administration, supervision. Lin-Yu Chen: data curation, software, funding acquisition, writing - review & editing. Hui-Chen Wang: data curation, software. Yu-Chun Weng: data curation, software. Po-Hsuan Su: data curation. Hung-Cheng Lai: conceptualization, funding acquisition, project administration, resources, supervision, writing - review & editing. All authors read and approved the final manuscript

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References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2024;74(3):229–63.
2. Lheureux S, Braunstein M, Oza AM. Epithelial ovarian cancer: Evolution of management in the era of precision medicine. Cancer J Clin. 2019;69(4):280–304.
3. Cornelison R, Llaneza DC, Landen CN. Emerging Therapeutics to Overcome Chemoresistance in Epithelial Ovarian Cancer: A Mini-Review. Int J Mol Sci. 2017;18(10).
4. Hong MK, Ding DC. Early Diagnosis of Ovarian Cancer: A Comprehensive Review of the Advances, Challenges, and Future Directions. Diagnostics. 2025;15(4).
5. Barr CE, Funston G, Jeevan D, Sundar S, Mounce LTA, Crosbie EJ. The Performance of HE4 Alone and in Combination with CA125 for the Detection of Ovarian Cancer in an Enriched Primary Care Population. Cancers. 2022;14(9).
6. Van Gorp T, Cadron I, Despierre E, Daemen A, Leunen K, Amant F, et al. HE4 and CA125 as a diagnostic test in ovarian cancer: prospective validation of the Risk of Ovarian Malignancy Algorithm. Br J Cancer. 2011;104(5):863–70.
7. Bast RC Jr., Lu Z, Han CY, Lu KH, Anderson KS, Drescher CW, et al. Biomarkers and Strategies for Early Detection of Ovarian Cancer. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research. cosponsored Am Soc Prev Oncol. 2020;29(12):2504–12.
8. Zhang R, Siu MKY, Ngan HYS, Chan KKL. Molecular Biomarkers for the Early Detection of Ovarian Cancer. Int J Mol Sci. 2022;23:19.
9. Moore RG, Miller MC, Eklund EE, Lu KH, Bast RC Jr., Lambert-Messerlian G. Serum levels of the ovarian cancer biomarker HE4 are decreased in pregnancy and increase with age. Am J Obstet Gynecol. 2012;206(4):e3491–7.
10. Yu S, Lee JK, Kim JH, Park H, Lee MY, Ryu S, et al. Diagnostic performance and establishment of reference limits of HE4 in Korean healthy women. Gynecol Oncol. 2016;143(1):128–34.
11. Baylin SB, Jones PA. Epigenetic Determinants of Cancer. Cold Spring Harb Perspect Biol. 2016;8(9).

12. 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). Int J Oncol. 2024;64(6).
13. Chang CC, Wang HC, Liao YP, Chen YC, Weng YC, Yu MH, et al. The feasibility of detecting endometrial and ovarian cancer using DNA methylation biomarkers in cervical scrapings. J gynecologic Oncol. 2018;29(1):e17.
14. Wu TI, Huang RL, Su PH, Mao SP, Wu CH, Lai HC. Ovarian cancer detection by DNA methylation in cervical scrapings. Clin epigenetics. 2019;11(1):166.
15. Lien JY, Hii LA, Su PH, Chen LY, Wen KC, Lai HC, et al. Exploring potential methylation markers for ovarian cancer from cervical scraping samples. Hum Genomics. 2025;19(1):56.
16. 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. Commun Med. 2024;4(1):88.
17. Bowtell DD, Bohm S, Ahmed AA, Aspuria PJ, Bast RC Jr., Beral V, et al. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. Nat Rev Cancer. 2015;15(11):668–79.
18. Colvin EK, Howell VM. Why the dual origins of high grade serous ovarian cancer matter. Nat Commun. 2020;11(1):1200.
19. Zhang S, Dolgalev I, Zhang T, Ran H, Levine DA, Neel BG. Both fallopian tube and ovarian surface epithelium are cells-of-origin for high-grade serous ovarian carcinoma. Nat Commun. 2019;10(1):5367.
20. Ledermann JA, Matias-Guiu X, Amant F, Concin N, Davidson B, Fotopoulou C et al. ESGO-ESMO-ESP consensus conference recommendations on ovarian cancer: pathology and molecular biology and early, advanced and recurrent disease. Annals of oncology: official journal of the European Society for Medical Oncology. 2024;35(3):248–66.
21. Lo Riso P, Villa CE, Gasparoni G, Vingiani A, Luongo R, Manfredi A, et al. A cell-of-origin epigenetic tracer reveals clinically distinct subtypes of high-grade serous ovarian cancer. Genome Med. 2020;12(1):94.
22. Klinkebiel D, Zhang W, Akers SN, Odunsi K, Karpf AR. DNA Methylome Analyses Implicate Fallopian Tube Epithelia as the Origin for High-Grade Serous Ovarian Cancer. Mol cancer research: MCR. 2016;14(9):787–94.
23. Reyes HD, Devor EJ, Warrier A, Newtson AM, Mattson J, Wagner V, et al. Differential DNA methylation in high-grade serous ovarian cancer (HGSOC) is associated with tumor behavior. Sci Rep. 2019;9(1):17996.
24. Ishak CA, De Carvalho DD. DNA Methylation Profiling of Premalignant Lesions as a Path to Ovarian Cancer Early Detection. Clin cancer research: official J Am Association Cancer Res. 2020;26(23):6083–5.
25. Oliveira D, Biskup E, O'Rourke CJ, Hentze JL, Andersen JB, Hogdall C, et al. Developing a DNA Methylation Signature to Differentiate High-Grade Serous Ovarian Carcinomas from Benign Ovarian Tumors. Mol Diagn Ther. 2024;28(6):821–34.

26. Pisanic TR 2nd, Cope LM, Lin SF, Yen TT, Athamanolap P, Asaka R, et al. Methylomic Analysis of Ovarian Cancers Identifies Tumor-Specific Alterations Readily Detectable in Early Precursor Lesions. Clin cancer research: official J Am Association Cancer Res. 2018;24(24):6536–47.
27. Wen KC, Huang RL, Chen LY, Wu TI, Lu CH, Chu TY et al. Endometrial Cancer Detection Using a Cervical DNA Methylation Assay (MPap) in Women with Abnormal Uterine Bleeding: A Multicenter Hospital-Based Validation Study. Cancers. 2022;14(17).
28. Kim B, Jung M, Moon KC, Han D, Kim K, Kim H, et al. Quantitative proteomics identifies TUBB6 as a biomarker of muscle-invasion and poor prognosis in bladder cancer. Int J Cancer. 2023;152(2):320–30.
29. Li Y, Shao Z, Jiang J, Wang H, Zhang M. Transcription factor NFKB1 mediates TUBB6 to promote the proliferation and suppress apoptosis in glioma via Wnt/beta-catenin signaling pathway. Discover Oncol. 2025;16(1):444.
30. Mariani M, Zannoni GF, Sioletic S, Sieber S, Martino C, Martinelli E, et al. Gender influences the class III and V beta-tubulin ability to predict poor outcome in colorectal cancer. Clin cancer research: official J Am Association Cancer Res. 2012;18(10):2964–75.
31. Bai Y, Wei C, Zhong Y, Zhang Y, Long J, Huang S, et al. Development and Validation of a Prognostic Nomogram for Gastric Cancer Based on DNA Methylation-Driven Differentially Expressed Genes. Int J Biol Sci. 2020;16(7):1153–65.
32. Yang Y, Zhao L, Huang B, Hou G, Zhou B, Qian J, et al. A New Approach to Evaluating Aberrant DNA Methylation Profiles in Hepatocellular Carcinoma as Potential Biomarkers. Sci Rep. 2017;7:46533.
33. Hentze JL, Hogdall CK, Hogdall EV. Methylation and ovarian cancer: Can DNA methylation be of diagnostic use? Mol Clin Oncol. 2019;10(3):323–30.
34. Li Y, Wang ZC, Luo L, Mu CY, Xu J, Feng Q, et al. The clinical value of the combined detection of sEGFR, CA125 and HE4 for epithelial ovarian cancer diagnosis. Eur Rev Med Pharmacol Sci. 2020;24(2):604–10.
35. Charkhchi P, Cybulski C, Gronwald J, Wong FO, Narod SA, Akbari MR. CA125 and Ovarian Cancer: A Comprehensive Review. Cancers. 2020;12(12).

Figures

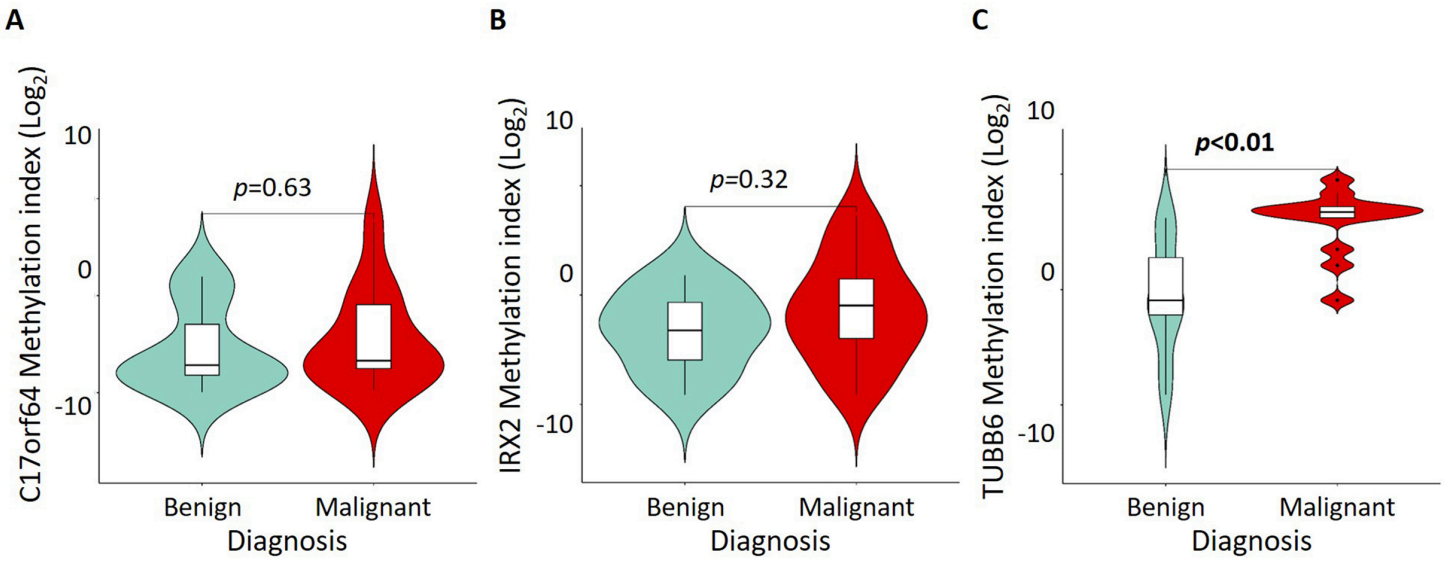


Figure 1

Violin plots showing the distribution of (A) C17orf64, (B) IRX2, (C)TUBB6 methylation index in benign (N=10, green) and malignant (N=13, red) groups.

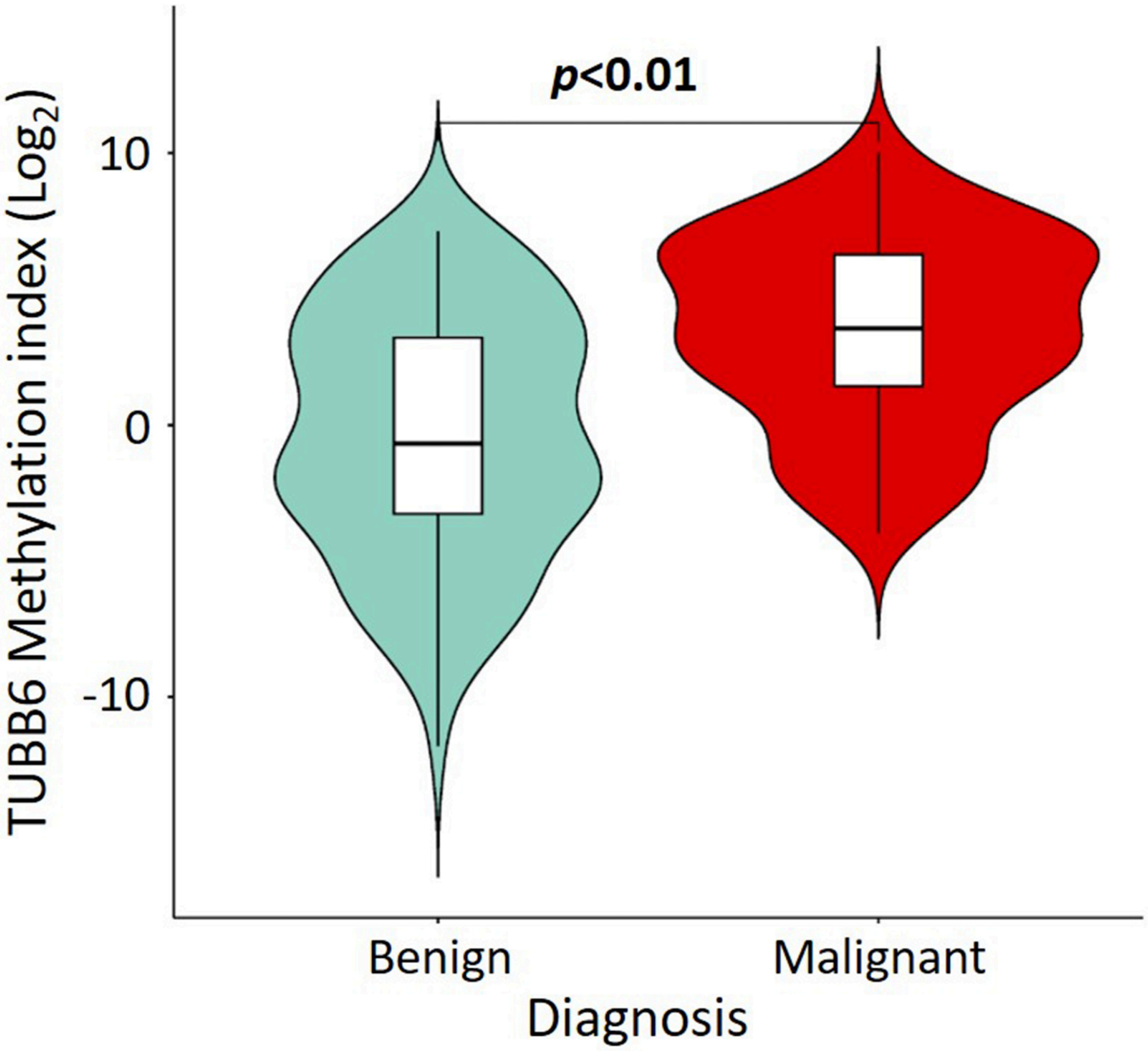


Figure 2

Violin plots showing the distribution of TUBB6 methylation level in swab samples from the benign group (N=102, green) and the malignant group (N=83, red).

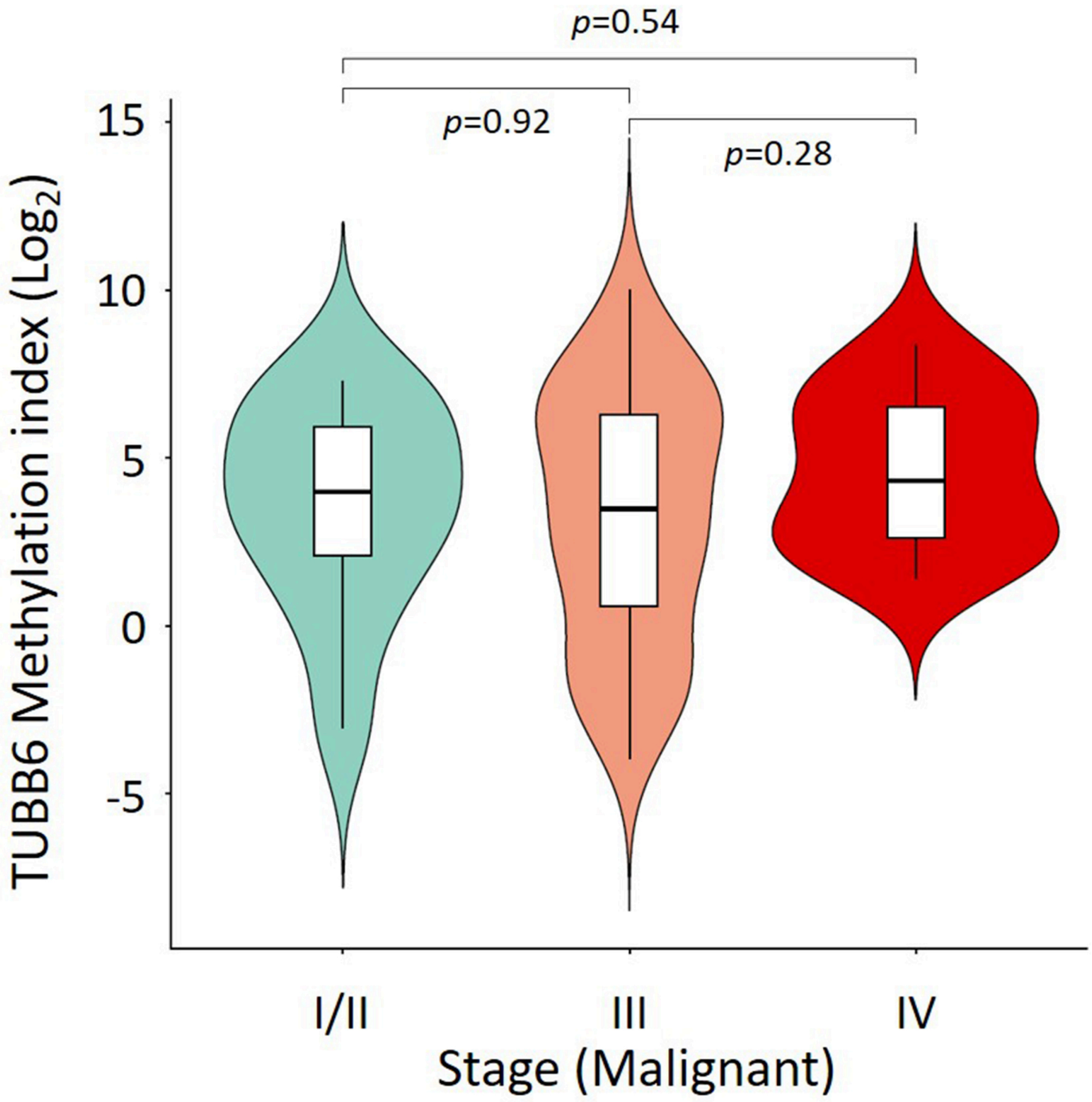


Figure 3

Violin plots showing TUBB6 methylation index in the malignant group with different stages (N=11, green; N=58, orange; N=14, red).

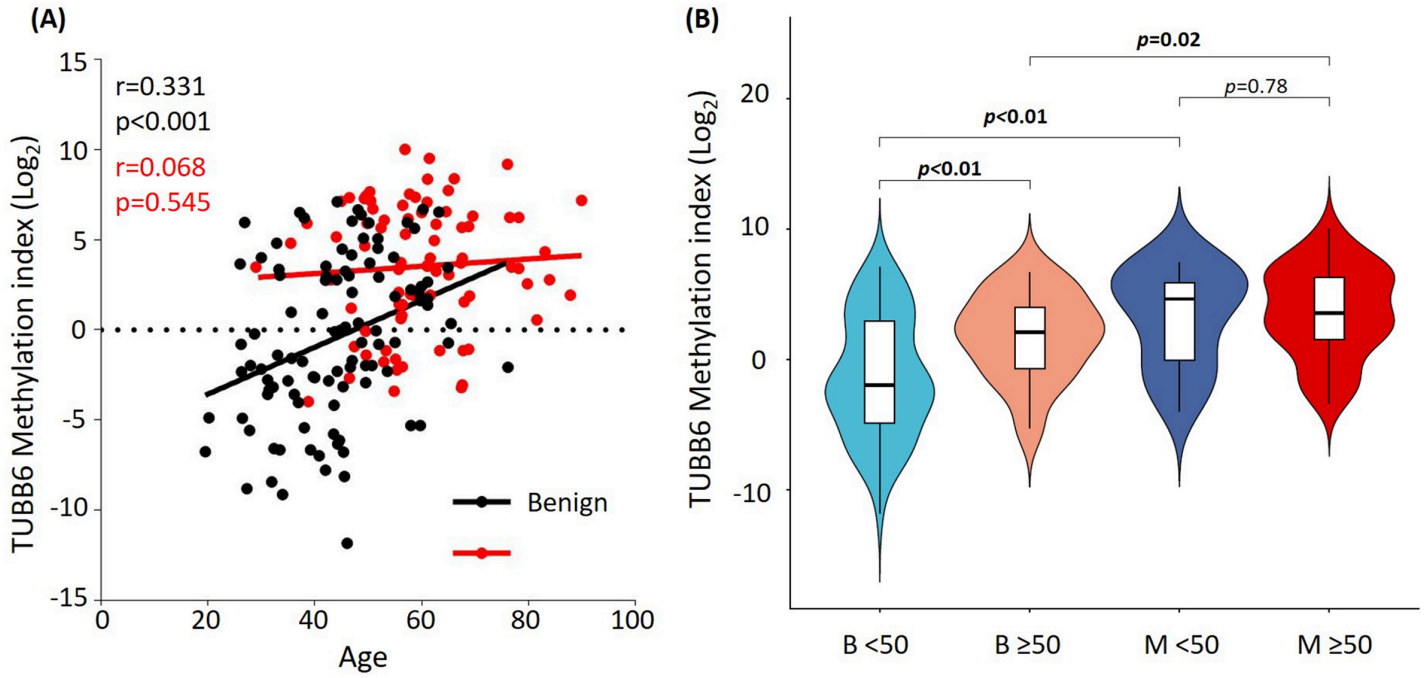


Figure 4

(A) Correlation of TUBB6 methylation with patient age in benign and malignant ovarian tumors. (B) Violin plots showing TUBB6 methylation index in benign (B) and malignant (M) groups with age below and over 50 (N=29, red; N=73, cyan-blue; N=65, green; N=17, blue).

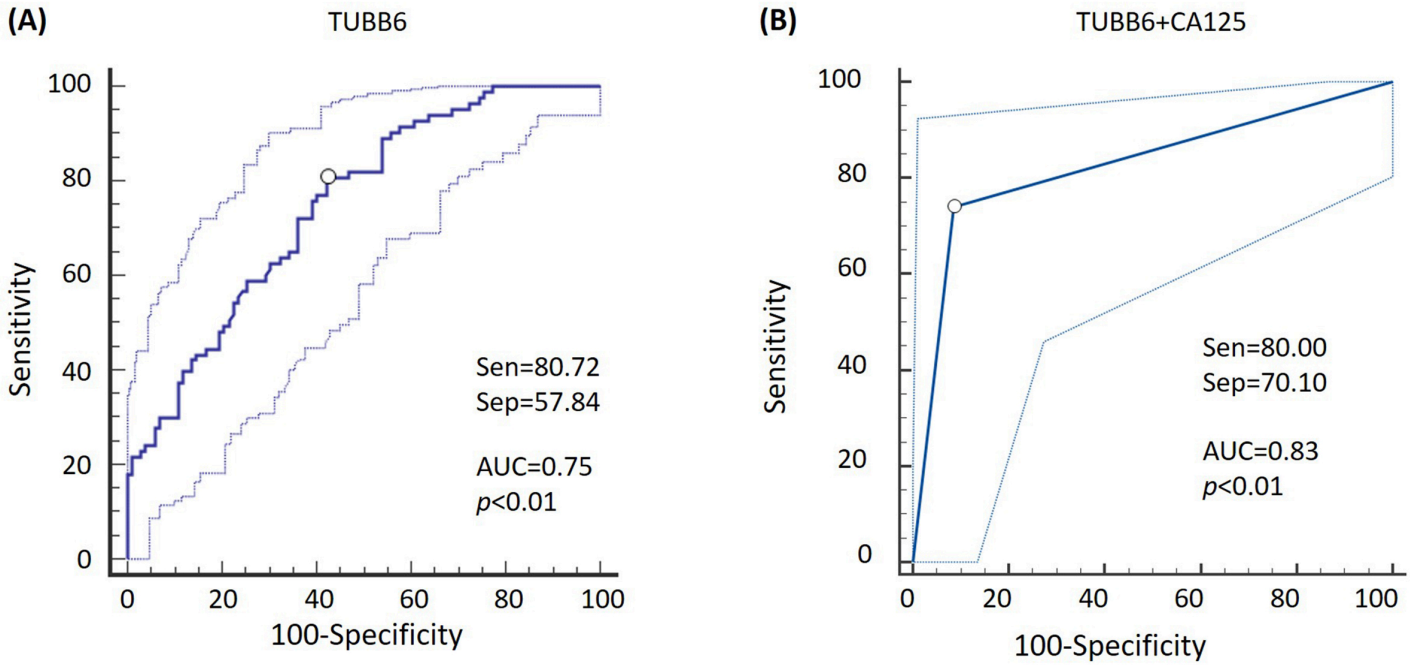


Figure 5

ROC curve for (A) TUBB6 methylation and (B) TUBB6 methylation combined with CA125 levels in discriminating malignant (N=83) from benign (N=102) groups. Solid line indicates ROC curve; dashed lines represent 95% CI.

Supplementary Files

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- TableS1JOR20260316.docx