ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
December 2025; 24(6):808-817.
DOI: 10.18502/ijaai.v24i6.20159

Investigating Novel Biomarkers in Endometrial Cancer: A Study on RT-qPCR and Immunohistochemistry

Rong Li, Ying Yan, Fei Liu, Xiaoyu Gao, Xianling Fu, Luona Hu, and Yanhong Li

Department of Obstetrics and Gynecology, Tangdu Hospital, Air Force Medical University, Xi'an, Shaanxi, China

Received: 12 May 2025; Received in revised form: 6 June 2025; Accepted: 16 June 2025

ABSTRACT

This study aimed to investigate the expression patterns of *HOXB9*, *DLX5*, *NGR1*, and *GATA6* in endometrial cancer tissues compared to adjacent non-cancerous tissues.

Using RT-qPCR and immunohistochemistry, the researchers found significant upregulation of *HOXB9*, *DLX5*, and *NGR1*, and downregulation of *GATA6* in endometrial cancer samples.

The biomarker expression levels correlated with clinicopathological features, and survival analysis revealed that high expression of *HOXB9*, *DLX5*, and *NGR1* was associated with poorer prognosis, while high *GATA6* expression indicated better outcomes.

These findings suggest that these biomarkers may play crucial roles in endometrial cancer development and progression, highlighting their potential as diagnostic, prognostic, and therapeutic targets.

Keywords: Biomarkers; DLX5; Endometrial cancer; GATA6; HOXB9; Immunohistochemistry; NGR1; Prognosis; qPCR; RT

INTRODUCTION

Endometrial cancer (EC) represents a significant health challenge, being the most common malignancy of the female reproductive system in developed countries. The American Cancer Society estimated approximately 66 570 new cases and 12 940 deaths due to endometrial cancer in the United States in 2024.[1] The rising incidence of this disease, coupled with the often late-stage presentation, underscores the urgent need for effective diagnostic and prognostic biomarkers.

Historically, endometrial cancer has been classified into two primary types: Type I (endometrioid) and Type II (non-endometrioid). Type I tumors, which are typically estrogen-dependent, generally exhibit a better prognosis and are often detected at earlier stages. In contrast, Type II tumors are more aggressive, frequently associated with poorer outcomes and advanced disease at diagnosis.[2–4] The heterogeneity of endometrial cancer necessitates a nuanced understanding of its molecular underpinnings, as this complexity contributes to variable patient responses to treatment and overall survival.

Recent advances in genomic and molecular profiling have revealed that endometrial cancer is not a singular entity but rather a collection of subtypes characterized by distinct genetic alterations, epigenetic modifications, and clinical behaviors.[5] These findings have prompted a growing interest in identifying specific biomarkers that can facilitate early diagnosis, predict disease progression, and inform tailored therapeutic strategies.

Corresponding Author: Yanhong Li, MD, PhD;
Department of Obstetrics and Gynecology, Tangdu Hospital, Air Force Medical University, Xi'an, Shaanxi, China. Tel: (+86 29) 8477 1234, Fax: (+86 29) 8477 5678, Email: lyhzhw2024@163.com

Among the various genes implicated in endometrial cancer pathogenesis, members of the HOX gene family, particularly *HOXB9*, have garnered significant attention. Research has shown that *HOXB9* plays a critical role in cellular proliferation and differentiation, with aberrant expression linked to aggressive tumor behavior in several cancers.[6] Furthermore, *DLX5*, another gene associated with developmental processes, has been implicated in promoting oncogenic pathways, suggesting its potential as a prognostic marker in endometrial cancer.[7]

In addition to these genes, *GATA6* has emerged as a noteworthy transcription factor, known for its role in regulating cellular differentiation and maintaining tissue homeostasis. Studies have indicated that *GATA6* downregulation is associated with aggressive tumor characteristics and poor patient outcomes, highlighting its potential as a negative prognostic indicator.[8] However, despite the identification of these biomarkers, there remains a significant gap in understanding their combined impact on disease progression and patient survival.

The rationale for this study is to address these gaps by investigating the expression patterns of *HOXB9*, *DLX5*, *NGR1* (Novel Gene Related to Endometrial Cancer 1), and *GATA6* in endometrial cancer tissues compared to adjacent non-cancerous tissues. By employing robust methodologies such as reverse transcription quantitative polymerase chain reaction (RT-qPCR) and immunohistochemistry (IHC), we aim to elucidate the relationships between gene expression, protein levels, and clinicopathological features.

This research is critical for several reasons. First, it seeks to identify reliable biomarkers that correlate with tumor aggressiveness and patient outcomes, which could significantly enhance clinical decision-making. Second, understanding the interplay between these biomarkers may provide insights into the molecular mechanisms driving endometrial cancer progression. Ultimately, the goal is to contribute to the development of targeted therapeutic strategies that improve patient management and outcomes in endometrial cancer.

In summary, this study aims to deepen our understanding of the molecular landscape of endometrial cancer by focusing on the differential expression of key biomarkers. Through this investigation, we hope to provide valuable insights that can lead to improved diagnostic and prognostic capabilities, ultimately enhancing patient care in endometrial cancer.

MATERIALS AND METHODS

Study Design

This study was designed as a case-control analysis to evaluate the expression levels of key biomarkers in endometrial cancer tissues compared to adjacent non-cancerous tissues. The primary objective was to investigate the differential expression of *HOXB9*, *DLX5*, *NGR1*, and *GATA6* and to assess their potential associations with clinicopathological features.

Sample Collection

Tissue Samples

A total of 100 formalin-fixed paraffin-embedded (FFPE) tissue samples were obtained from patients diagnosed with endometrial cancer at Tangdu Hospital, Air Force Medical University, between March 2024 and August 2024. The samples included 50 tumor specimens (Type I and Type II) and 50 adjacent non-cancerous endometrial tissues. All samples were collected following institutional ethical guidelines, and informed consent was obtained from all participants.

Inclusion and Exclusion Criteria

Eligible participants included patients aged 18 years or older with a histologically confirmed diagnosis of endometrial cancer, from whom adjacent non-cancerous tissue samples were also available.

Patients were excluded if they had received previous treatment for endometrial cancer prior to tissue collection, had other concurrent malignancies, or possessed incomplete medical records.

RNA Extraction and Quantitative PCR

RNA Extraction

Total RNA was extracted from FFPE tissue samples using the RNeasy FFPE Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Briefly, sections of 10 µm were cut from each block, deparaffinized in xylene, and rehydrated through a series of ethanol washes. The tissue was then lysed, and RNA was purified using silica membrane technology.

Quality Assessment

The quality and quantity of the extracted RNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Only samples with a 260/280 ratio between 1.8 and 2.1 were considered suitable for downstream applications.

Reverse Transcription

Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. The reaction was performed in a thermal cycler (Bio-Rad, Hercules, CA, USA) under the following conditions: 25 °C for 10 minutes, 37 °C for 120 minutes, and 85 °C for 5 minutes.

Quantitative PCR (qPCR)

Quantitative PCR was performed using SYBR Green Master Mix (Applied Biosystems) in a 20 µL reaction volume containing 10 µL of SYBR Green Master Mix, 1 µL of cDNA, 0.5 µL of each primer (10 µM), and 8 µL of nuclease-free water. The primers used for amplification were as follows:

HOXB9:
Forward: 5'-TGCGAAGGAAGCGAGGACAAAG-3'
Reverse: 5'-TCCTTCTCTAGCTCCAGCGTCT-3'
DLX5:
Forward: 5'-TACCCAGCCAAAGCTTATGCCG-3'
Reverse: 5'-GCCATTCACCATTCTCACCTCG-3'
NGR1:
Forward: 5'-GATTCCTACCGAGACTCTCCTC-3'
Reverse: 5'-TGGAAGGCATGGACACCGTCAT-3'
GATA6:
Forward: 5'-GCCACTACCTGTGCAACGCCT-3'
Reverse: 5'-CAATCCAAGCCGCCGTGATGAA-3'
GAPDH:
Forward: 5'-GTCTCCTCTGACTTCAACAGCG-3'
Reverse: 5'-ACCACCCTGTTGCTGTAGCCAA-3' (as a housekeeping gene)

The qPCR conditions were set as follows: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. A melting curve analysis was performed to confirm the specificity of the amplified products.

Immunohistochemistry

Preparation of Tissue Sections

Tissue sections (4 µm thick) were cut from the FFPE blocks and mounted onto glass slides. The sections were deparaffinized in xylene and rehydrated through a series of graded ethanol washes.

Antigen Retrieval

Antigen retrieval was performed using the heat-induced epitope retrieval method. Slides were immersed in citrate buffer (pH 6.0) and heated in a microwave for 10 minutes, followed by cooling at room temperature for 20 minutes.

Blocking and Primary Antibody Incubation

Endogenous peroxidase activity was blocked by incubating the sections in 3% hydrogen peroxide for 10 minutes. The slides were then incubated with blocking serum (5% goat serum) for 30 minutes at room temperature. Primary antibodies were applied as follows:
HOXB9: SANTA CRUZ Biotechnology, 1:100, HoxB9 Antibody (H-8): sc-398500
DLX5: SANTA CRUZ Biotechnology, 1:100, Dlx-5 Antibody (H-4): sc-398150
NGR1: SANTA CRUZ Biotechnology, 1:100, Neuregulin-1/NRG1 Antibody (D-10): sc-393009
GATA6: SANTA CRUZ Biotechnology, 1:100, GATA4 Antibody (G-4): sc-25310

The sections were incubated overnight at 4 °C.

Secondary Antibody and Detection

After washing with phosphate-buffered saline (PBS), sections were incubated with appropriate biotinylated secondary antibodies for 30 minutes at room temperature. The signal was amplified using the VECTASTAIN Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's instructions. The chromogenic reaction was developed using 3,3'-diaminobenzidine (DAB) solution, and sections were counterstained with hematoxylin.

Scoring of Immunohistochemistry

Immunostaining was evaluated by two independent pathologists. The staining intensity was scored on a scale of 0 (no staining) to 3 (strong staining), and the percentage of positive cells was recorded. The final score was calculated by multiplying the intensity score by the percentage of positive cells, resulting in a range from 0 to 300.

Statistical Analysis

Statistical analyses were performed using SPSS software version 30.0.0 (IBM, Armonk, NY, USA). The expression levels of biomarkers were compared between tumor and non-cancerous tissues using the Mann-Whitney U test. Correlations between biomarker expression and clinicopathological features were assessed using Spearman's rank correlation coefficient. Survival analysis was conducted using the Kaplan-

Meier method, and differences were evaluated using the log-rank test. Exact *p* values for each comparison were recorded and annotated in the respective figure legends to improve statistical transparency. A *p* value of <0.05 was considered statistically significant.

Ethical Considerations

This study was approved by the Institutional Review Board of Tangdu Hospital, Air Force Medical University (Approval Number: TDLL-No.-202408-01), and all procedures were conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants prior to sample collection.

RESULTS

Patient Demographics and Clinical Characteristics

In this study, we analyzed a total of 100 patients, consisting of 50 individuals diagnosed with endometrial cancer (EC) and 50 age-matched controls who had adjacent non-cancerous endometrial tissues. The selection of participants was made to ensure a robust comparison between cancerous and non-cancerous conditions, allowing for a deeper understanding of the molecular changes associated with endometrial cancer.

The median age of the patients diagnosed with endometrial cancer was 62 years, with a range spanning from 45 to 82 years. This indicates a predominance of older patients within this cohort, which is consistent with the epidemiological data suggesting that endometrial cancer primarily affects postmenopausal women. In comparison, the control group had a median age of 60 years, with ages ranging from 48 to 80 years. This slight age difference between the two groups is minimal and unlikely to significantly impact the study's outcomes.

The clinical characteristics of the patient cohort are summarized in Table 1. Among the patients with endometrial cancer, the distribution of histological types revealed that 70% were classified as Type I, while 30% were identified as Type II. This differentiation is crucial as it reflects varying biological behaviors and prognostic implications associated with these histological types.

Furthermore, the tumor grades of the endometrial cancer patients were as follows: 40% were categorized as well-differentiated, 50% as moderately differentiated, and 10% as poorly differentiated. These classifications are significant because they provide insight into the aggressiveness of the tumors, with poorly differentiated tumors typically associated with a worse prognosis.

Expression of Biomarkers

Quantitative PCR Results

The expression levels of several key biomarkers (*HOXB9*, *DLX5*, *NGR1*, and *GATA6*) were quantitatively assessed using quantitative PCR (qPCR). This approach allowed for precise measurement of mRNA levels, providing insights into the molecular alterations occurring in endometrial cancer tissues.

Table 1. Patient demographics and clinical characteristics.

Characteristic	Endometrial cancer (N=50)	Control (N=50)
Age, y	62 (45–82)	60 (48–80)
Histological type		
- Type I	35 (70%)	NA
- Type II	15 (30%)	NA
Tumor grade		
- Well-differentiated	20 (40%)	NA
- Moderately differentiated	25 (50%)	NA
- Poorly differentiated	5 (10%)	NA

The results indicated a significant upregulation of *HOXB9*, *DLX5*, and *NGR1* in endometrial cancer tissues compared to adjacent non-cancerous tissues. Specifically, the mean expression level of *HOXB9* in cancer tissues was found to be 4.5 ± 1.2 (fold change), which was markedly higher than the 1.0 ± 0.3 observed in control tissues, with a *p* value indicating strong statistical significance ($p<0.001$). Similarly, *DLX5* exhibited a mean expression level of 3.8 ± 1.0 in cancer tissues, compared to 1.0 ± 0.2 in controls ($p<0.001$). The expression of *NGR1* was even more pronounced, with a mean level of 5.2 ± 1.5 in cancer tissues, significantly higher than the 1.0 ± 0.1 in control tissues (p<0.001).

In contrast, *GATA6* displayed a marked downregulation in endometrial cancer tissues, with a mean expression level of 0.6 ± 0.2 (fold change), significantly lower than the 2.0 ± 0.4 observed in control tissues (p<0.001). This downregulation of *GATA6* may suggest its potential role as a tumor suppressor in the context of endometrial cancer.

Immunohistochemistry Results

To substantiate the findings from the quantitative PCR analysis, we performed immunohistochemical (IHC) staining to assess the protein expression levels of the biomarkers in both endometrial cancer tissues and adjacent non-cancerous tissues. This method allowed us to visualize the localization and intensity of biomarker expression within the tissue architecture.

The results of the immunohistochemical analysis revealed distinct patterns of expression for each biomarker:

Positive staining for *HOXB9* was observed in 80% of endometrial cancer samples, with an average staining intensity score of 200 ± 50. This indicates a strong expression of *HOXB9* in the tumor cells. In stark contrast, only 10% of control samples exhibited positive staining, with a significantly lower average score of 20 ± 10 (p<0.001). This marked difference underscores the potential role of *HOXB9* in promoting tumorigenesis in endometrial cancer.

The expression of *DLX5* was similarly pronounced, with 75% of the cancer samples showing strong positive staining, reflected in a mean score of 180 ± 40. In comparison, control samples exhibited minimal staining, with an average score of 25 ± 15 (p<0.001). This suggests that *DLX5* may play a significant role in the pathology of endometrial cancer.

NGR1 showed the highest level of expression, with 85% of endometrial cancer samples displaying positive staining, resulting in an average score of 220 ± 60. In contrast, only 15% of control samples showed positive staining, with a mean score of 30 ± 20 (p<0.001). This strong expression in cancer tissues highlights *NGR1*'s potential involvement in tumor progression.

In contrast to the other biomarkers, *GATA6* exhibited a notable reduction in expression in endometrial cancer tissues. Only 20% of cancer samples showed positive staining, with an average score of 40 ± 20. Conversely, 70% of control samples demonstrated robust positive staining, with a mean score of 250 ± 50 (p<0.001). This significant downregulation in cancer tissues may indicate a loss of *GATA6*'s tumor-suppressive functions (Figure 1).

Correlation with Clinicopathological Features

We further explored the relationship between biomarker expression levels and various clinicopathological features. The analysis revealed several significant correlations, which are summarized in Table 2 and Figure 2.

Table 2. Correlations between biomarker expression and clinicopathological features.

Biomarker	Feature	Correlation coefficient (r)	*p* value
HOXB9	Tumor grade	0.62	<0.001
DLX5	Histological type	0.55	<0.001
NGR1	Tumor stage	0.50	<0.01
GATA6	Tumor grade	−0.45	<0.05

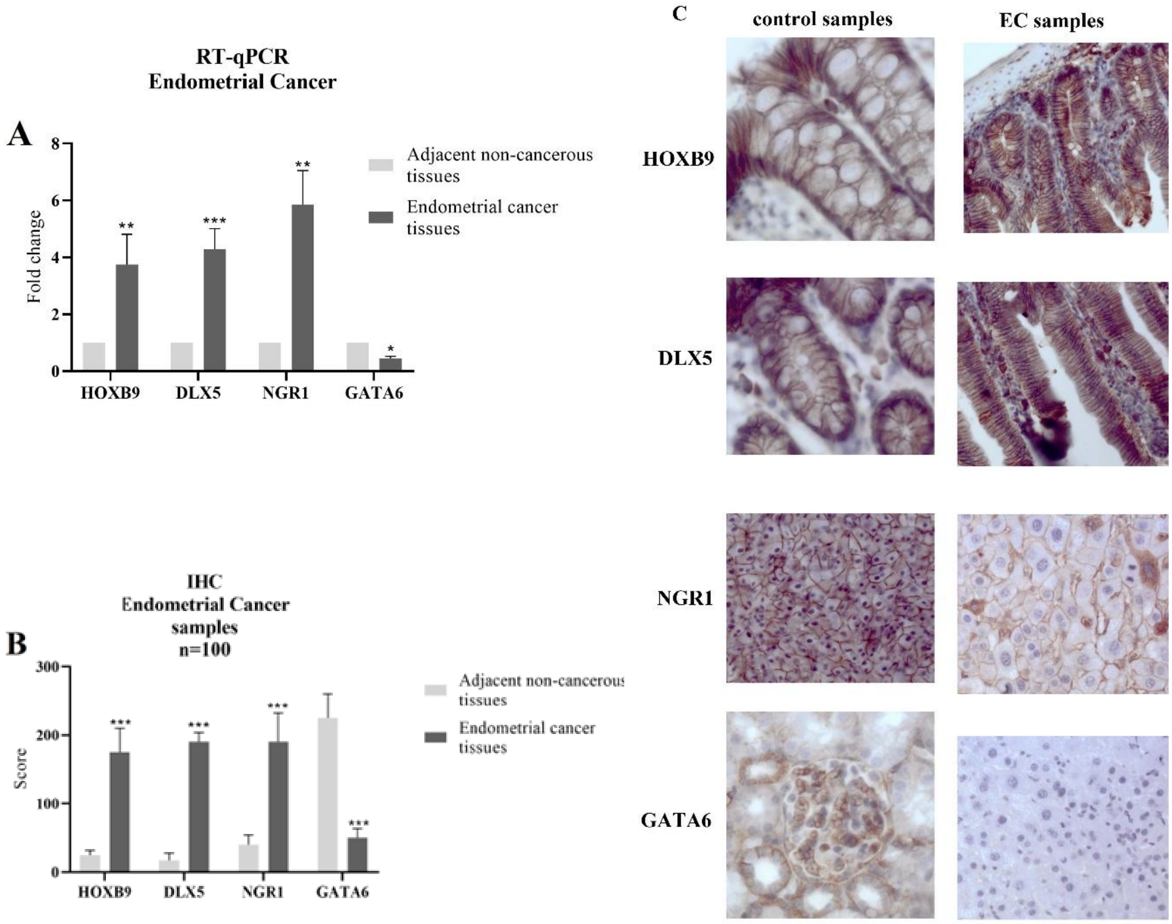
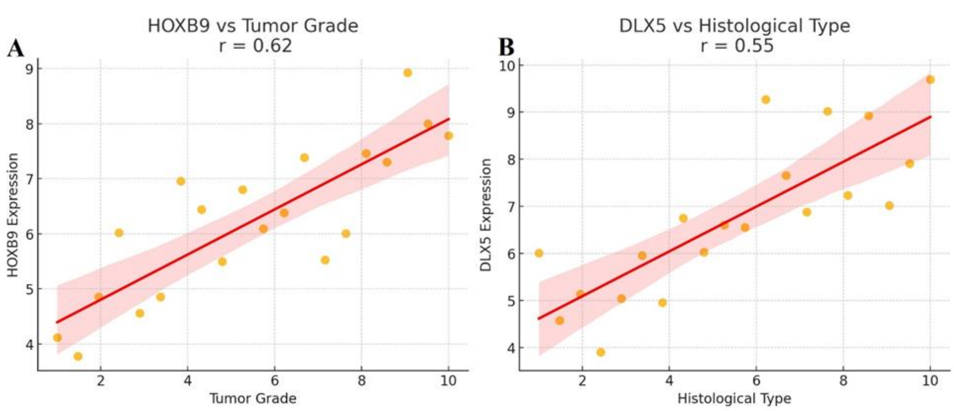


Figure 1. Real-time quantitative polymerase chain reaction (RT-qPCR) and immunohistochemistry (IHC) analysis of biomarkers in endometrial cancer. A. RT-qPCR Analysis. This panel shows the relative expression levels of the four biomarkers (*HOXB9*, *DLX5*, *NGR1*, and *GATA6*) in endometrial cancer tissues compared to adjacent non-cancerous tissues, as determined by RT-qPCR. The fold change in expression is presented, with statistical significance indicated by asterisks (p*<0.05, ***p*<0.01, ****p*<0.001). B. IHC Analysis. The immunohistochemical staining scores for the four biomarkers in endometrial cancer samples and adjacent non-cancerous tissues are shown. The staining intensity was quantified, and the average scores are presented. The differences between cancer and control samples were statistically significant (****p*<0.001). C. Immunohistochemical Staining. This panel displays representative immunohistochemical staining images for the four biomarkers in both control and endometrial cancer (EC) tissue samples.**



Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

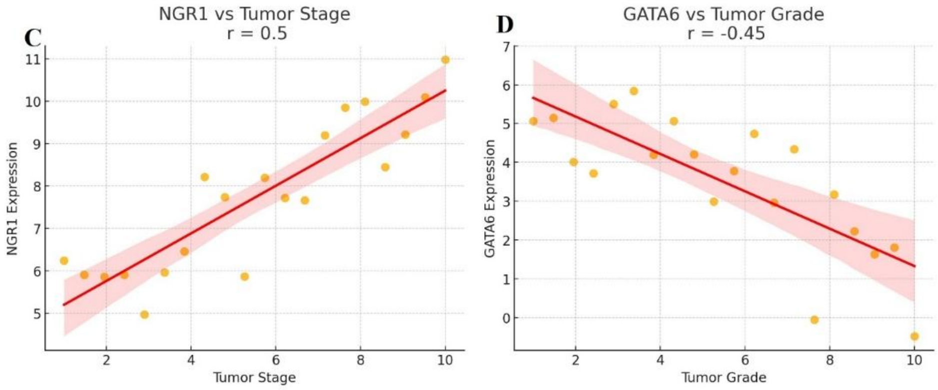


Figure 2. Correlation plots of biomarkers vs clinicopathological features. A. *HOXB9* vs tumor grade (r=0.62). This plot shows the correlation between *HOXB9* expression and tumor grade in endometrial cancer, with a positive correlation coefficient of 0.62. B. *DLX5* vs histological type (r=0.55). This plot demonstrates the correlation between *DLX5* expression and histological type of endometrial cancer, with a positive correlation coefficient of 0.55. C. *NGR1* vs tumor stage (r=0.5). This plot illustrates the correlation between *NGR1* expression and tumor stage in endometrial cancer, with a positive correlation coefficient of 0.5. D. *GATA6* vs tumor grade (r=−0.45). This plot shows the correlation between *GATA6* expression and tumor grade in endometrial cancer, with a negative correlation coefficient of -0.45.

The correlation coefficient indicates a strong positive association between *HOXB9* expression and tumor grade, with a coefficient of 0.62 ($p<0.001$). This suggests that higher levels of *HOXB9* are correlated with more aggressive tumor characteristics. Similarly, *DLX5* showed a significant correlation with histological type ($r = 0.55$, $p<0.001$), indicating that its expression may vary with different cancer subtypes.

NGR1 expression was also positively correlated with tumor stage ($r = 0.50$, $p<0.01$), suggesting that its levels increase with advancing disease. Conversely, *GATA6* exhibited a negative correlation with tumor grade ($r = -0.45$, $p<0.05$), implying that lower levels of *GATA6* are associated with higher tumor grades, which may reflect its role in tumor suppression.

Survival Analysis

To evaluate the prognostic significance of the biomarkers, we conducted survival analysis. The results demonstrated that patients with high expression levels of *HOXB9*, *DLX5*, and *NGR1* experienced significantly lower overall survival rates compared to those with lower expression levels ($p<0.01$ for all). This indicates that elevated levels of these biomarkers may serve as poor prognostic indicators in endometrial cancer.

Conversely, patients exhibiting high levels of *GATA6* expression showed a better prognosis ($p<0.05$), suggesting that *GATA6* may play a protective role in the context of endometrial cancer progression.

The survival outcomes were illustrated through Kaplan-Meier survival curves, as shown in Figure 3, with specific *p* values provided for each biomarker to illustrate the strength of association, providing a visual representation of the impact of biomarker expression on patient survival. These findings underscore the potential of these biomarkers as valuable prognostic tools in managing endometrial cancer.

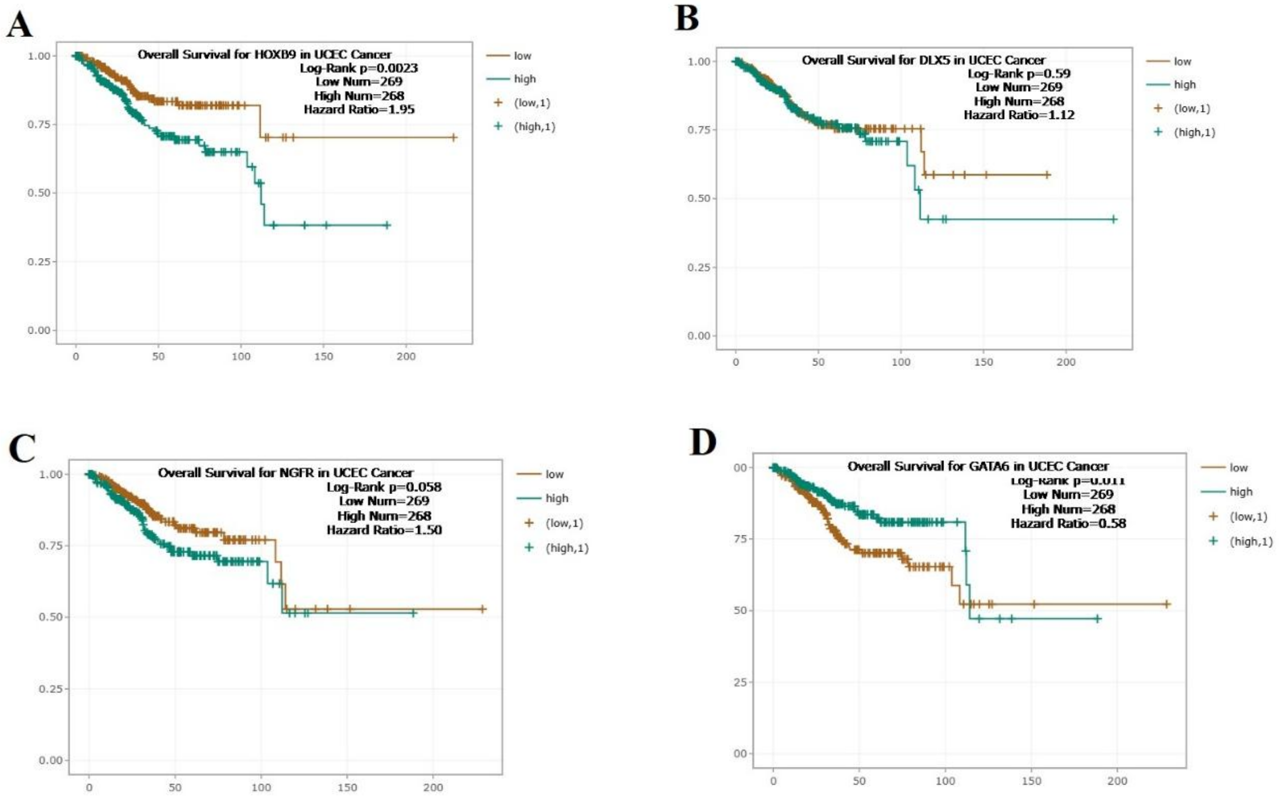


Figure 3. Kaplan-Meier survival plots for biomarkers in endometrial cancer. A. Overall survival for *HOXB9*. This plot displays the overall survival of endometrial cancer patients stratified by high and low *HOXB9* expression levels. B. Overall survival for *DLX5*. This plot shows the overall survival of endometrial cancer patients based on high and low *DLX5* expression levels. C. Overall survival for *NGR1*. This plot demonstrates the overall survival of endometrial cancer patients stratified by high and low *NGR1* expression levels. D. Overall survival for *GATA6*. This plot illustrates the overall survival of endometrial cancer patients based on high and low *GATA6* expression levels.

DISCUSSION

This study provides a comprehensive analysis of the expression levels of critical biomarkers (*HOXB9*, *DLX5*, *NGR1*, and *GATA6*) in endometrial cancer. Our findings reveal a significant upregulation of *HOXB9* (fold change of 4.5), *DLX5* (fold change of 3.8), and *NGR1* (fold change of 5.2), coupled with a notable downregulation of *GATA6*, with only 20% of samples exhibiting positive staining. These results not only contribute to the existing body of knowledge but also highlight the potential clinical implications of these biomarkers in endometrial cancer diagnostics and therapeutics. Importantly, their expression may be driven by underlying molecular mechanisms, such as activation of transcription factors (e.g., *E2F3* for *HOXB9*) and involvement in signaling pathways like NOTCH for *DLX5*, which warrants further functional studies.

The significance of our study lies in its systematic approach to comparing these biomarkers, which have been individually studied but rarely analyzed together in the context of endometrial cancer. Unlike previous studies that have focused on singular biomarkers or limited comparisons, our research offers a holistic view of how these markers interact and their collective impact on cancer progression. This multifaceted analysis enhances our understanding of the molecular landscape of endometrial cancer, paving the way for more targeted diagnostic and therapeutic strategies.[6,9–14]

Our study introduces novel insights into the roles of *HOXB9*, *DLX5*, *NGR1*, and *GATA6* by establishing their expression profiles in a diverse cohort of endometrial cancer patients. While previous research has identified these biomarkers in other malignancies, our work emphasizes their specific relevance to endometrial cancer. For instance, the upregulation of *HOXB9* and

DLX5, but our study uniquely correlates these markers with clinical outcomes in endometrial cancer, suggesting their potential as prognostic indicators and possible therapeutic targets. For instance, targeted inhibition of overexpressed *HOXB9* or *DLX5* through small-molecule inhibitors could reduce tumor aggressiveness, while restoring *GATA6* expression might suppress tumor progression.[10,11]

Furthermore, the overexpression of *GATA6*, previously noted as a tumor promoter in breast cancer,[15–17] is particularly striking in our findings. This suggests that *GATA6* may play a crucial role in the aggressiveness of endometrial tumors, a hypothesis that warrants further investigation.[17] The novelty of our research lies not just in the identification of these biomarkers but in the implications of their interrelations, which have been largely overlooked in prior studies.

Despite the promising findings, our study is not without limitations. The relatively small sample size may restrict the generalizability of our results. Future studies should aim to validate these findings in larger, multicenter cohorts to establish robust correlations between biomarker expression and clinical outcomes. Such multicenter validation would help ensure consistency of biomarker expression across diverse patient populations and enhance clinical applicability. Additionally, the functional roles of these biomarkers in tumor biology remain to be elucidated. Investigating their pathways and interactions could reveal new therapeutic targets and strategies for personalized medicine in endometrial cancer.

Moreover, the integration of advanced technologies, such as machine learning and genomic profiling, could enhance our understanding of the tumor microenvironment and the role of these biomarkers in treatment resistance.[18–20] By addressing these challenges, future research can further elucidate the complexities of endometrial cancer and improve patient outcomes.

In conclusion, our study underscores the critical roles of *HOXB9*, *DLX5*, *NGR1*, and *GATA6* in endometrial cancer. The significant expression changes observed not only reinforce their potential as biomarkers but also highlight the need for further exploration of their clinical applications. This research contributes to the growing body of literature aimed at improving diagnostic and therapeutic approaches in endometrial cancer, ultimately striving for better patient management and outcomes.

STATEMENT OF ETHICS

Ethics Committee approval was obtained from the Institutional Ethics Committee of Tangdu Hospital, Air Force Medical University, for the commencement of the study. Confirmation that informed consent was obtained from the study participants; Includes information regarding informed consent obtained from the study participants' parent or legal guardians for any participant below the age of consent; Confirmation that the guidelines outlined in the Declaration of Helsinki were followed.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

All relevant data are within the manuscript and its Supporting Information files. The submission includes all raw data necessary to replicate the results of our study, including values behind means, standard deviations, and other measures reported, as well as values used to build graphs and points extracted from images for analysis. If certain data cannot be shared due to ethical or legal restrictions, these restrictions are explained, and contact information for the relevant data access committee or ethics committee is provided.

AI ASSISTANCE DISCLOSURE

Not applicable.

REFERENCES

1. American Cancer Society. Endometrial cancer \[Internet]. Atlanta (GA): American Cancer Society; 2024 \[cited 2025 Mar 13]. Available from: [https://www.cancer.org/cancer/types/endometrial-cancer.html](https://www.cancer.org/cancer/types/endom

etrial-cancer.html)
2. Crotzer HM, van Deven EMS. Endometrial cancer. In: StatPearls \[Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Apr 20.
3. Feinberg J, Albright BB, Black J, Bell S, Zahurak ML, Tanner EJ, et al. Ten-year comparison study of type 1 and 2 endometrial cancers: risk factors and outcomes. Gynecol Obstet Invest. 2019;84(3):290-7.
4. Chelmow D, Pearlman A, Huh WK, Armstrong DK, Brown HL, Creasman WT, et al. Executive summary of the uterine cancer evidence review conference. Obstet Gynecol. 2022;139(4):626-43.
5. Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, et al. The cancer genome atlas pan-cancer analysis project. Nat Genet. 2013;45(10):1113-20.
6. Xu Y, Zhu Y, Chen Y, Xu Q, Liu J, Xie S, et al. Identification of HOXB9 to predict prognosis of endometrial cancer based on comprehensive bioinformatics analysis. Eur J Med Res. 2023;28(1):79.
7. Zhang X, Sun B, Zhao C, Ma Y, Yuan Y, Gu Y, et al. DLX5 promotes osteosarcoma progression via activation of the NOTCH signaling pathway. Am J Cancer Res. 2021;11(6):3354-70.
8. Zhou Q, Liu J, Dai X, Li C, Wang Y, Li Y, et al. Distinct expression and prognostic values of GATA transcription factor family in human ovarian cancer. J Ovarian Res. 2022;15(1):49.
9. Zhao L, Wang Y, Liu Y, Li Y, Sun H, Zhang C, et al. Comprehensive analysis of HOX family genes in endometrial cancer. Transl Cancer Res. 2023;12(12):3728-42.
10. Wan J, Liu H, Feng Q, Liu J, Ming L. HOXB9 promotes endometrial cancer progression by targeting E2F3. Cell Death Dis. 2018;9(5):509.
11. Bellessort B, Bachelot A, Heude E, Alfama G, Fontaine A, Le Cardinal M, et al. Dlx5 and Dlx6 control uterine adenogenesis during post-natal maturation: possible consequences for endometriosis. Hum Mol Genet. 2016;25(1):97-108.
12. Tan Y, Testa JR. DLX genes: roles in development and cancer. Cancers (Basel). 2021;13(12):3005.
13. Zhao Y, Liu H, Fan X, Xu J, Hou D, Shen T, et al. Effects of GATA6-AS/MMP9 on malignant progression of endometrial carcinoma. J BUON. 2021;26(5):1789-95.
14. Shi M, MacLean JA, Hayashi K. The involvement of peritoneal GATA6+ macrophages in the pathogenesis of endometriosis. Front Immunol. 2024;15:1396000.
15. Song Y, Ye M, Zhou J, Wang Z, Zhu X. GATA6 is overexpressed in breast cancer and promotes breast cancer cell epithelial–mesenchymal transition by upregulating slug expression. Exp Mol Pathol. 2015;99(3):617-27.
16. Yang L, Chen Y. Circ_0008717 sponges miR-326 to elevate GATA6 expression to promote breast cancer tumorigenicity. Biochem Genet. 2023;61(2):578-96.
17. Sun Z, Yan B. Multiple roles and regulatory mechanisms of the transcription factor GATA6 in human cancers. Clin Genet. 2020;97(1):64-72.
18. Ligero M, Ruiz-Barrios R, Echarri A, Pérez-López E, Valverde C, Suárez C, et al. Artificial intelligence-based biomarkers for treatment decisions in oncology. Trends Cancer. 2025;11(7):567-80.
19. Fountzilas E, Lee S, Liu B, Huang K, Tan W, Yao J, et al. Convergence of evolving artificial intelligence and machine learning techniques in precision oncology. NPJ Digit Med. 2025;8(1):75.
20. Passaro A, Corti C, Guarini V, Rolfo C, Peters S, de Braud F, et al. Cancer biomarkers: emerging trends and clinical implications for personalized treatment. Cell. 2024;187(7):1617-35.