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Original Paper

The Role of Basic Leucine Zipper and W2 Domain Containing 2 (BZW2) in Endometrial Cancer: Implications for Tumor Progression and Patient Outcomes

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Abstract

Background: This study investigates the role of basic leucine zipper and W2 domain-containing 2 (BZW2) in endometrial cancer (EC) and its implications for tumor progression and patient outcomes. We assessed the expression of BZW2 in endometrial cancer (UCEC) and its correlation with tumor progression, clinical pathological features, and patient prognosis.

Methods: We utilized RNA sequencing data from the TCGA and UALCAN databases to analyze BZW2 expression patterns in EC and its association with tumor grade, stage, patient age, and prognosis. Additionally, we explored the molecular relationships between BZW2, various signaling pathways, and immune cell markers. The role of BZW2 in endometrial cancer cell proliferation and migration was validated through cellular experiments.

Results: Our analysis revealed elevated BZW2 expression in EC, which correlated significantly with tumor grade, stage, and patient age. Prognostic analyses indicated that high BZW2 expression was associated with reduced overall survival and progression-free survival. Molecularly, BZW2 was linked to several signaling pathways and immune cell markers, while cellular experiments confirmed its role in

promoting endometrial cancer cell proliferation and migration.

Conclusion: The multifunctional role of BZW2 in endometrial cancer highlights its potential as both a therapeutic target and a prognostic biomarker. Our findings provide compelling evidence supporting BZW2 as a promising target for the treatment and prognosis evaluation of UCEC, thereby guiding future research and clinical applications.

Keywords

BZW2, Endometrial Cancer (EC), Tumor Progression, Prognostic Biomarker, Cell Proliferation

1. Introduction

Endometrial cancer (EC) is the leading gynecological malignancy in developed countries, posing a significant threat to women's health (Urick & Bell, 2019). Its incidence has been steadily rising, compounded by a concerning trend toward younger age at diagnosis. The American Cancer Society estimates that tens of thousands of new cases are diagnosed annually, establishing EC as the fourth most common cancer among women (Crosbie, Kitson, Mcalpine, Mukhopadhyay, Powell, & Singh, 2022). Although many cases are diagnosed at an early stage due to symptomatic presentation—resulting in relatively favorable five-year survival rates—late-stage diagnoses remain prevalent, largely due to the lack of effective screening biomarkers (El-Ghazzi, Durando, Giro, & Herrmann, 2023). Consequently, patients, particularly those with high-grade or serous histological subtypes, often face poorer prognoses characterized by more aggressive disease courses and higher mortality rates (Njoku, Barr, & Crosbie, 2022).

The molecular landscape of EC is intricate, involving multiple signaling pathways that contribute to tumorigenesis and disease progression (Bostan, Mihaila, Roman, Radu, Neagu, Bostan, et al., 2024). Identifying key molecular players and elucidating their roles in EC is essential for enhancing our understanding of the disease and developing targeted therapies. One such player is the basic leucine zipper transcription factor, BZW2, which belongs to the bZIP superfamily. BZW2 possesses a distinct molecular architecture that includes an alkaline region and a leucine zipper, enabling specific DNA binding and modulation of downstream gene expression (Jin, Liao, Zhang, Yang, & Zhao, 2019). Previous studies have indicated that BZW2 is overexpressed in various cancer types and correlates with tumor aggressiveness and poor patient outcomes (Hu, Huang, Tao, Li, Kuang, Liu, et al., 2023). Its role in oncology is becoming increasingly prominent, with accumulating evidence suggesting that BZW2 overexpression is closely associated with malignant behaviors such as proliferation, migration, invasion, and resistance to apoptosis. In osteosarcoma, BZW2 facilitates the transition from the G2 to M phase of the tumor cell cycle by activating the Akt/mTOR signaling pathway (Cheng, Li, Zhu, Yuan, Yang, & Fan, 2017). Similarly, in hepatocellular carcinoma, elevated BZW2 expression correlates with increased malignant phenotypes, which can be mitigated by its suppression (Jin, Liao, Zhang, Yang, & Zhao, 2019). In muscle-invasive bladder cancer, silencing BZW2 not only inhibits cell growth but also induces G1 phase arrest and apoptosis (Gao, Yu, Zhang, Yu, Sun, & Li, 2019). Furthermore, in colorectal cancer,

BZW2 overexpression is implicated in cancer cell proliferation through the activation of the ERK/MAPK signaling pathway (Agarwal, Afaq, Bajpai, Behring, Kim, Varambally, et al., 2023; Huang, Chen, Fan, Ai, & Sheng, 2020). These findings underscore the multifaceted role of BZW2 in the progression of various malignancies.

In this study, we aimed to investigate the expression patterns of BZW2 in endometrial cancer (EC) and its correlation with clinical and pathological features, as well as patient prognosis. We hypothesized that BZW2 may serve as a potential therapeutic target and prognostic biomarker in EC. To test this hypothesis, we conducted a comprehensive analysis utilizing data from The Cancer Genome Atlas (TCGA) and UALCAN databases and performed cellular experiments to assess the functional impact of BZW2 on endometrial cancer cells. Our findings provide valuable insights into the role of BZW2 in EC and its potential clinical applications.

2. Materials and Methods

2.1 Pan-Cancer Expression Analysis

We downloaded and organized RNA sequencing (RNA-seq) data from 33 tumor projects in The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov) and extracted transcript per million (TPM) formatted data. Data visualization was performed using the ggplot2 package in R software. Groups with fewer than three samples or a standard deviation (SD) of zero were excluded from statistical analysis but were included for visualization purposes.

2.2 Expression of BZW2 in Endometrial Cancer

Utilizing endometrial cancer (EC) data from TCGA, we analyzed the expression differences of BZW2 between tumor tissues and adjacent non-tumor tissues. Data visualization was conducted using the ggplot2 package in R software. Additionally, UALCAN (http://ualcan.path.uab.edu/) was employed to analyze BZW2 mRNA expression levels in uterine corpus endometrial carcinoma (UCEC).

2.3 Association of BZW2 Expression with Pathological Parameters

Patients were stratified based on the median BZW2 expression level in UCEC, as identified from TCGA. We compared the distributions of clinical variables between high and low expression cohorts to discern any significant disparities. Furthermore, we investigated the correlation between BZW2 expression levels and clinical pathological features to evaluate BZW2's potential as an independent prognostic factor.

2.4 Kaplan-Meier Survival Analysis

Survival analysis was conducted using the survival package in R, which included fitting survival regression models and testing the proportional hazards assumption. Results were visualized using the survminer and ggplot2 packages. We analyzed the relationship between BZW2 gene expression and overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) in EC patients. The diagnostic value of BZW2 in EC was assessed through receiver operating characteristic (ROC) curve analysis.

2.5 Functional and Pathway Analysis of BZW2 in Endometrial Cancer (EC)

We conducted Gene Ontology (GO) analysis, encompassing biological processes, molecular functions, and cellular components, as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for the top 71 genes most closely associated with BZW2 (corrected p-values < 0.05). Additionally, Gene Set Enrichment Analysis (GSEA) was performed using the MSigDB Collections database (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp) to identify the biological processes influenced by BZW2 and its role in the development of EC.

2.6 Correlation between BZW2 Expression and Tumor Immune Cell Infiltration

Using the single-sample Gene Set Enrichment Analysis (ssGSEA) algorithm from the GSVA R package (version 1.46.0), we assessed the immune infiltration status in EC based on the markers provided in a relevant Immunity article (Bindea, Mlecnik, Tosolini, Kirilovsky, Waldner, Obenauf, et al., 2013). We then analyzed the correlation between BZW2 expression and tumor immune cell infiltration.

2.7 Cultivation and Maintenance of Endometrial Cancer Cell Lines

Human endometrial cancer cell lines, Ishikawa and ECC-1, were obtained from the China Center for Type Culture Collection (Wuhan, China). Both cell lines were cultured in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin, maintained at 37℃ in a 5% CO2 incubator.

2.8 Transfection of Endometrial Cancer Cells

Transfection was performed using a laboratory-prepared transfection reagent. Briefly, cells were plated in 6-well plates at a density of 70%–90% one day prior to transfection. DNA (2 μg) was diluted in 100 μL of serum-free medium and mixed with an equal volume of MAX reagent, also diluted in serum-free DMEM. This mixture was incubated at room temperature for 20 minutes before being added to the cells. After 48 hours of incubation at 37℃ in a 5% CO2 environment, subsequent experiments were conducted.

2.9 Construction of BZW2 Knockout Cell Line

We employed the CRISPR/Cas9 system to knockout the target gene in cells. A lentiviral vector containing gRNA specific to the gene of interest was constructed using Zhang Feng's online tool. The gRNAs were cloned into the digested lentiCRISPR v2 vector. Positive clones were selected, and the vector was subsequently transfected into the target cells. Following the removal of puromycin selection pressure, single-cell clones were isolated and expanded for further analysis.

2.10 Construction of BZW2 Overexpressing Cell Line

Total RNA was extracted from endometrial cancer cells and reverse-transcribed into complementary DNA (cDNA), which served as the template for PCR amplification. The PCR products were purified through agarose gel electrophoresis and ligated with the digested vector. Positive clones were selected, and the plasmid was extracted for subsequent analysis.

2.11 Cell Proliferation Using CCK-8

Endometrial cancer cells and BZW2 knockdown cells were trypsinized, counted, and plated in 96-well plates at a density of 1,000 cells per well, with six replicates for each group. A CCK-8 mixed solution

was added to the cells, which were then incubated for 1 hour. Absorbance was measured at 450 nm using a microplate reader at daily intervals over 7 consecutive days.

2.12 Cell Migration Using Wound Healing

Cell migration was assessed using the scratch wound assay. Cells were seeded in 6-well plates and then scratched with a 10 μL pipette tip. They were cultured in serum-free medium, and the migration distance was measured and photographed at specified intervals. ImageJ software was utilized for quantification and analysis of the migration data.

2.13 Clonogenic Potential by Colony Formation

Endometrial cancer cells and BZW2 knockdown cells were trypsinized and counted. A total of 600 cells were plated in 6-well plates, with three replicates for each group. After 14 days of culture, the plates were stained with 0.25% crystal violet, and the number of colonies was counted.

2.14 Protein Expression Analysis by Western Blot

Endometrial cancer cells were collected, and total protein was extracted using a standard protein extraction protocol. Protein concentration was determined using a BCA assay or similar method. Equal amounts of protein were loaded onto SDS-PAGE gels for electrophoresis, followed by transfer to nitrocellulose or PVDF membranes. Membranes were blocked and incubated with primary antibodies overnight at 4 ℃, followed by incubation with secondary antibodies for 1 hour at room temperature. Protein bands were visualized using chemiluminescent detection or other appropriate methods.

3. Results

3.1 BZW2 Expression Patterns across Tumor Types and Endometrial Cancer

Our pan-cancer analysis of RNA sequencing data from the TCGA database revealed significantly elevated BZW2 mRNA expression in 16 different tumor types, including endometrial cancer (Figure 1A). In endometrial cancer, we observed a marked increase in BZW2 protein expression compared to adjacent normal tissues (Figure 1B). Consistent with these findings, analysis from the UALCAN database indicated that the median mRNA expression level of BZW2 was substantially higher in UCEC tissues than in normal controls ($P < 0.001$) (Figure 1C). Collectively, these observations suggest that BZW2 may play a crucial role in the pathogenesis of various cancers, with a particularly significant impact in endometrial cancer.

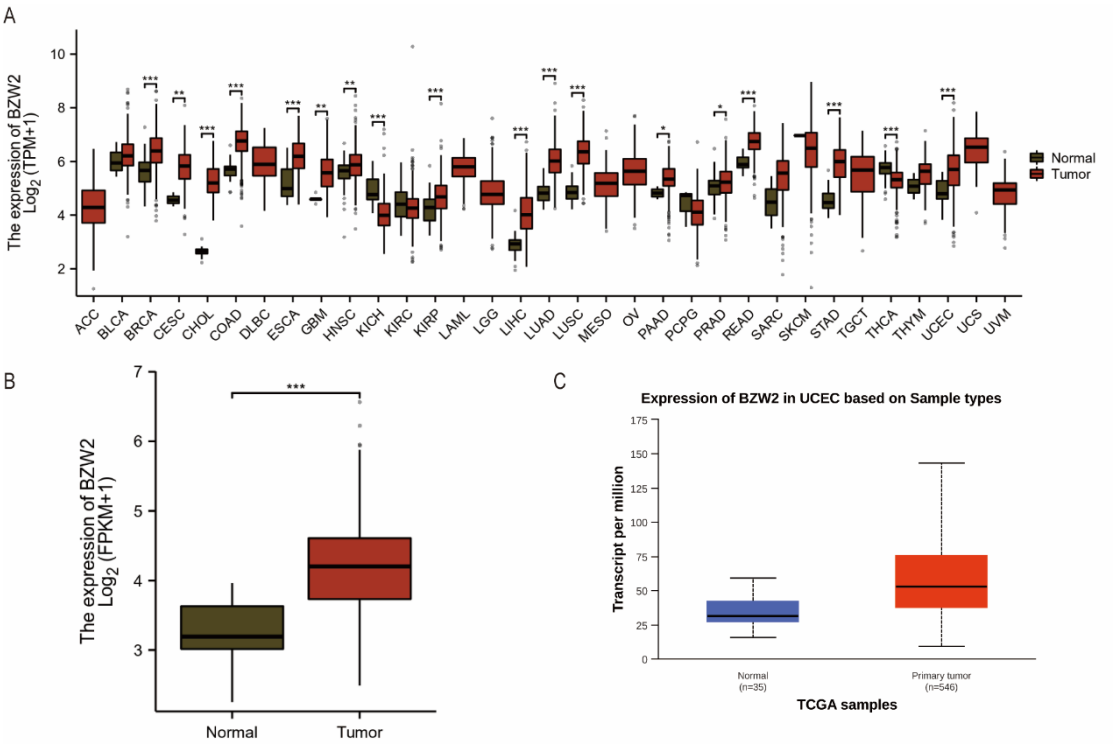

Figure 1. Elevated BZW2 Expression in Various Tumor Types and Endometrial Cancer (A) Pan-cancer Analysis Depicting BZW2 Expression Levels across 33 Tumor Types from TCGA. (B) Comparative Analysis of BZW2 Protein Expression in Endometrial Cancer (EC) Tumor Tissues and Adjacent Non-neoplastic Tissues (TCGA Database). (C) Assessment of BZW2 mRNA Expression Disparity between EC Tumor Tissues and Adjacent Non-neoplastic Tissues Utilizing UALCAN Database Analysis.

3.2 Clinical Feature Correlations with BZW2 Expression in Endometrial Cancer

BZW2 expression was significantly higher in endometrial cancer patients compared to non-tumor individuals (Figure 2A). Notably, patients aged 60 years and above exhibited significantly higher levels of BZW2 compared to those under 60 (Figure 2B). Additionally, BZW2 levels increased with tumor grade in uterine corpus endometrial carcinoma, indicating a potential role in tumor progression (Figure 2C).

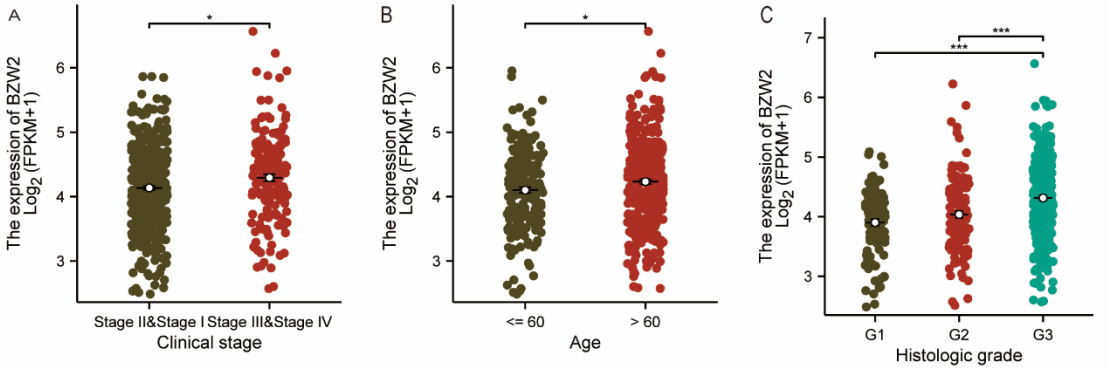

Figure 2. Analysis of the Correlation between BZW2 Expression Levels and Clinical Pathological Characteristics (A) BZW2 Expression Levels by Clinical Stage. (B) BZW2 Expression Levels by Patient Age. (C) BZW2 Expression Levels by Tumor Grade.

3.3 Construction of Nomogram and Diagnostic and Prognostic Value of BZW2

We constructed a nomogram to predict the 1- to 5-year survival probabilities of patients with varying BZW2 expression levels, which demonstrated good calibration (Figure 3A-B). Kaplan-Meier survival analysis indicated that patients with high BZW2 expression had significantly lower overall survival (OS) (log-rank P = 0.041, HR = 1.54), disease-specific survival (DSS) (log-rank P = 0.057, HR = 1.63), and progression-free interval (PFI) (log-rank P = 0.028, HR = 1.48) compared to those with low BZW2 expression (Figure 3C-E). The area under the ROC curve for BZW2 in predicting endometrial cancer (EC) was 0.881, indicating high predictive accuracy for the diagnosis of EC, with a cut-off value of 3.842-0.920 (Figure 3F).

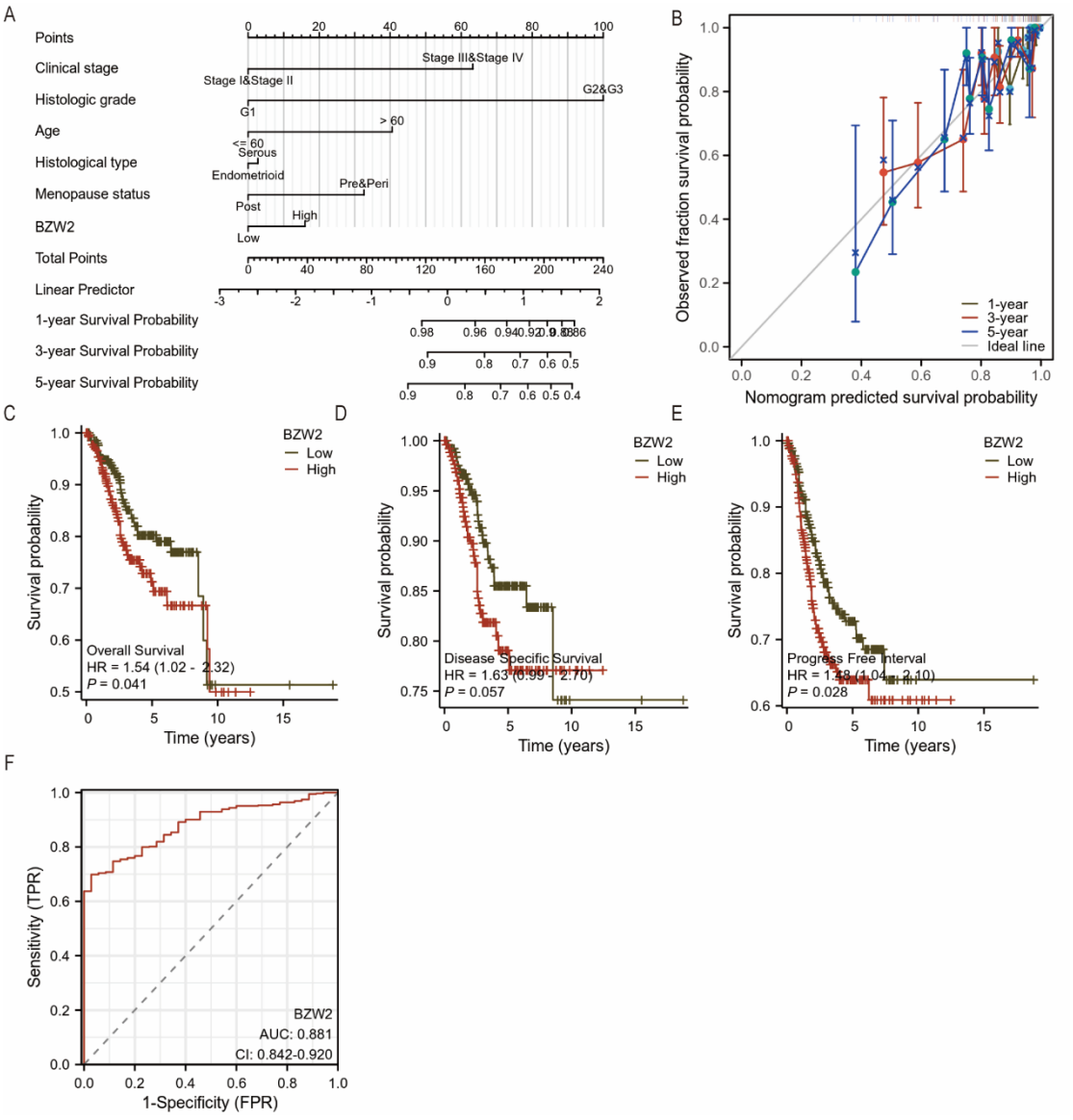

Figure 3. Prognostic Value of BZW2 Uterine Corpus Endometrial Carcinoma (UCEC) Patients (A and B) Prognostic Nomogram and Calibration Curve Assessing Survival Rates over 1-5 Years for Patients with Varying BZW2 Expression Levels and Clinical Features. (C-E) Relationship between BZW2 Expression and Overall Survival (OS), Disease-Specific Survival (DSS), and Progression-Free Interval (PFI) in UCEC Patients Based on TCGA Data. (F) Diagnostic ROC Curve of BZW2 in EC.

3.4 Functional Enrichment Analysis of BZW2-related Genes

Based on the median expression level of the BZW2 gene in endometrial cancer (EC) from the TCGA database, the EC cohort was stratified into two groups: the BZW2 high expression group and the BZW2 low expression group. A total of 71 differentially expressed genes were identified, adhering to the screening criteria of $|logFC| > 1.5$ and $p < 0.05$. Subsequent enrichment analyses were performed on

these genes to elucidate their roles in the development of EC, particularly concerning BZW2.

Gene Ontology (GO) enrichment analysis revealed that BZW2-associated genes are involved in crucial biochemical processes and molecular interactions within EC. These include interactions with G-protein coupled receptors, binding to histone deacetylases, modulation by hormones, fatty acid binding, and activities related to neuropeptide hormones. These functions are essential for cellular signaling, gene expression regulation, and the control of metabolic pathways. KEGG pathway analysis identified two significant pathways: Neuroactive ligand-receptor interaction and Long-term depression (LTD). The former may influence tumor cell behaviors such as proliferation, migration, and invasion, while the latter is potentially linked to the adaptability and plasticity of tumor cells, affecting their responses to environmental stimuli. Both pathways are implicated in the progression of endometrial cancer, as illustrated in Figure 4A.

Additionally, advanced Gene Set Enrichment Analysis (GSEA) indicated that elevated BZW2 expression in uterine corpus endometrial carcinoma (UCEC) correlates with the activation of pathways such as MYC_TARGETS_V2, PROTEIN_SECRETION, UNFOLDED_PROTEIN_RESPONSE, and INTERFERON_ALPHA_RESPONSE. Conversely, high BZW2 expression suppresses pathways related to MYOGENESIS and ALLOGRAFT_REJECTION, as shown in Figures 4B-G. The activation of these pathways due to increased BZW2 expression is believed to play a pivotal role in regulating cell growth, survival, metabolism, and immune responses, thereby potentially driving the onset and progression of EC (Figure 4B-G).

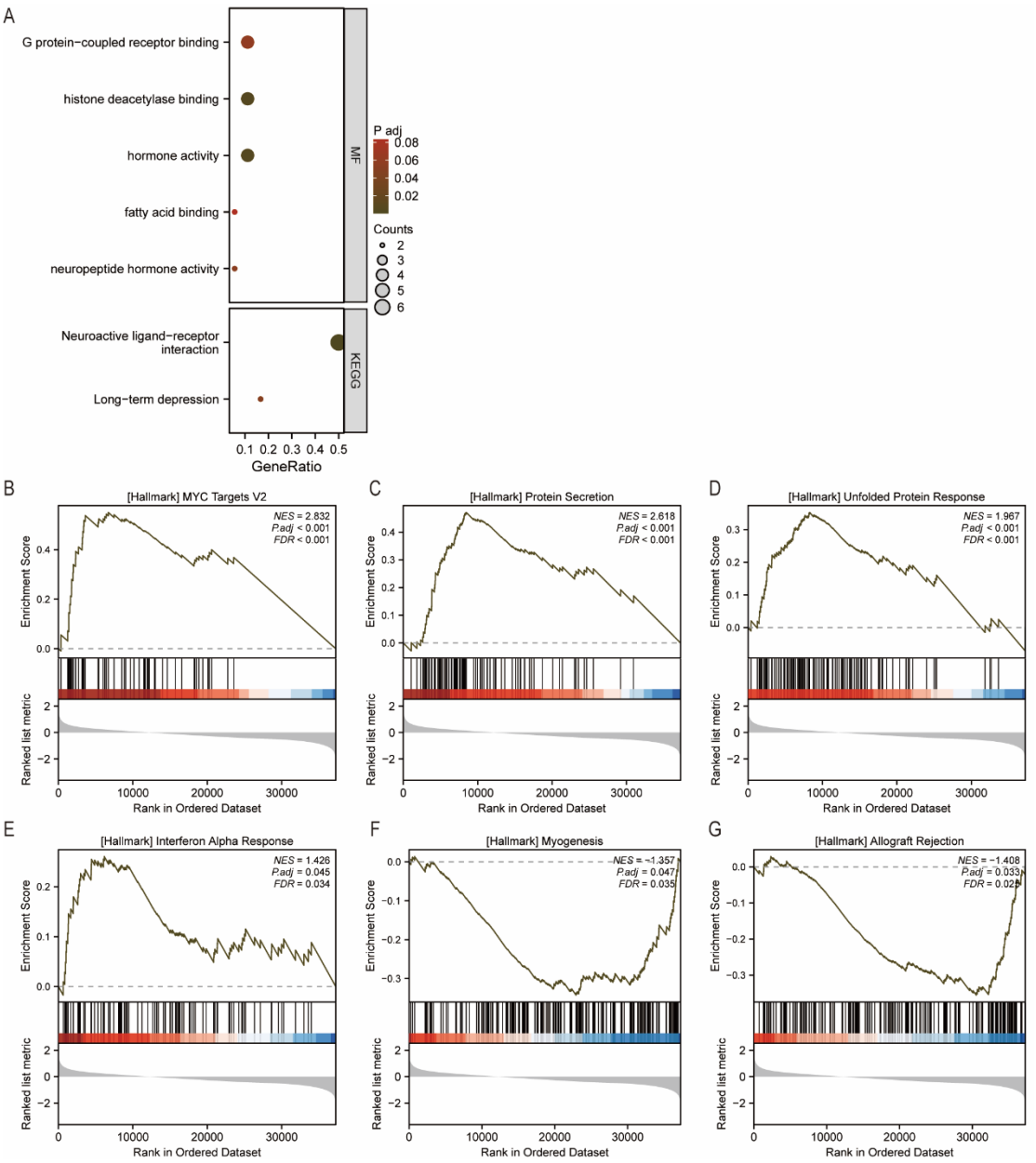

Figure 4. GO Enrichment Analysis, KEGG Enrichment Analysis, and GSEA Enrichment Analysis of BZW2-related Genes (A) Gene Ontology (GO) Enrichment Analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Analysis Results. (B-G) Gene Set Enrichment Analysis (GSEA) Enrichment Analysis Findings.

3.5 Correlation between BZW2 Expression and Tumor Immune Cell Infiltration in Endometrial Cancer (EC)

Our analysis revealed that BZW2 expression exhibited a negative correlation with several immune cell markers, including B cells, CD8 T cells, cytotoxic cells, dendritic cells, eosinophils, and natural killer (NK) cells. Conversely, BZW2 expression was positively correlated with macrophages, T helper cells, and Tγδ cells (Figure 5A-B). These correlations imply that BZW2 may play a significant role in

modulating the tumor immune microenvironment in EC.

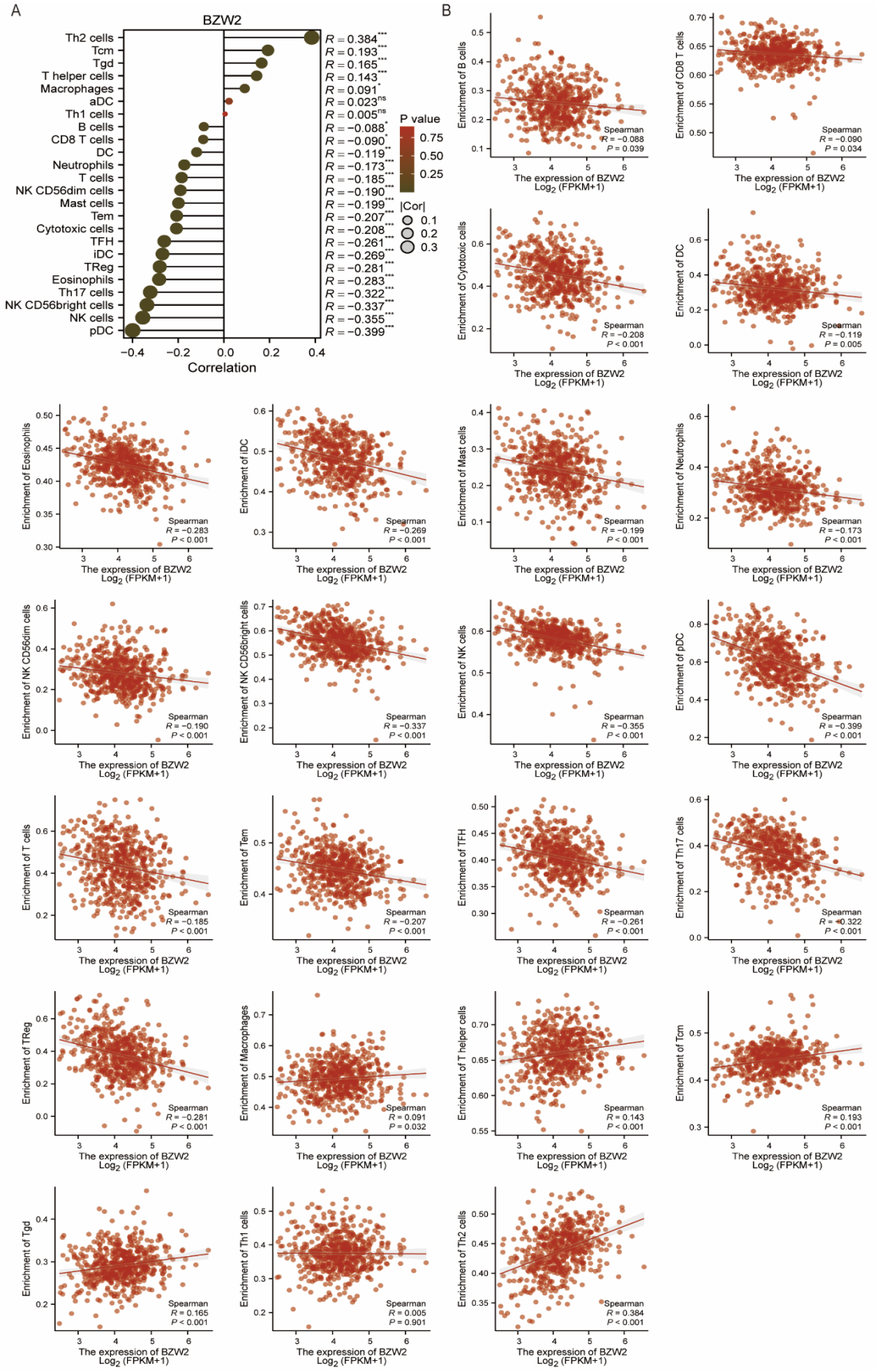

Figure 5. Relationship between BZW2 Gene Expression and Tumor Immune Cell Infiltration in EC (A-B) Correlation between BZW2 Expression and Immune Cell Markers in Endometrial Cancer (EC) Tumors.

3.6 Development of BZW2 Knockdown Cell Lines

We successfully established BZW2 knockdown cell lines using the CRISPR/Cas9 system in both Ishikawa and ECC-1 cell lines. Western blot analysis confirmed the efficient knockdown of BZW2 in these cell lines (Figure 6A-B).

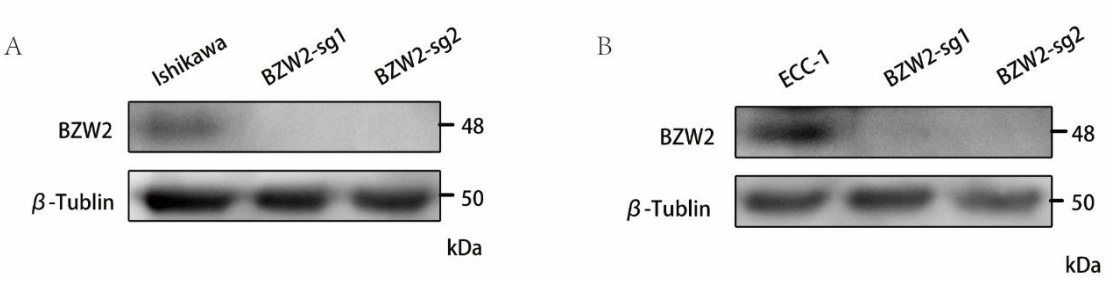

Figure 6. Western Blot Analysis Demonstrating BZW2 Expression Levels Following Knockdown in Ishikawa and ECC-1 Cells β-Tubulin was Utilized as a Loading Control.

3.7 Impact of BZW2 Knockdown on Endometrial Cancer Cell Proliferation

CCK-8 assays indicated that BZW2 knockdown significantly inhibited cell proliferation in a time-dependent manner. Similarly, colony formation assays revealed that BZW2 knockdown markedly reduced the clonogenic ability of Ishikawa and ECC-1 cells (Figure 7A-C). These findings underscore the critical role of BZW2 in promoting the proliferation of endometrial cancer cells.

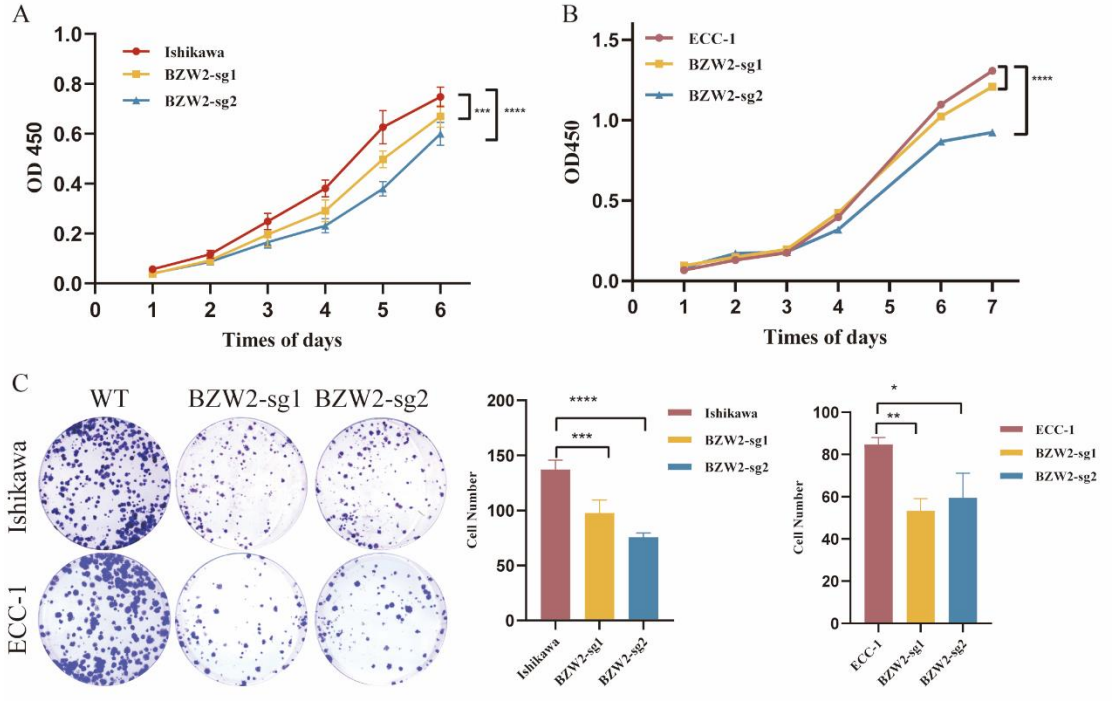

Figure 7. Effects of BZW2 Knockdown on the Viability of Ishikawa and ECC-1 Cells (A- B) Wild-type and BZW2-knockdown Ishikawa and ECC-1 Cells were Seeded into 96-well Plates with Six Replicates per Group. Cell Viability was Assessed at Designated Time Points from Day 1 to Day 7 Using the CCK-8 Assay, and the Data were Statistically Analyzed. ***p < 0.001. (C) The Impact of BZW2 Knockdown on the Proliferation of Ishikawa and ECC-1 Cells was Evaluated by Plating 600 Wild-type and BZW2-knockdown Cells in 6-well Cell Culture Plates, with Three Replicates Per Group. After Two Weeks, Colonies were Stained with Crystal Violet, and the Number of Monoclonal Colonies was Quantified. The Left Panel Displays Photographic Images, while the Right Panel Presents Statistical Results from Three Independent Experiments. *p < 0.05, **p < 0.01, ***p < 0.0001.

3.8 Effect of BZW2 Knockdown on Cell Migration

Wound healing assays (Figure8A) revealed that BZW2 knockdown significantly impaired the wound closure rate in endometrial cancer cells in a time-dependent manner. In parallel, Transwell migration assays (Figure8B, C) demonstrated that BZW2 knockdown markedly reduced the migratory ability of Ishikawa and ECC-1 cells. Collectively, these findings underscore the critical role of BZW2 in promoting the migration of endometrial cancer cells.

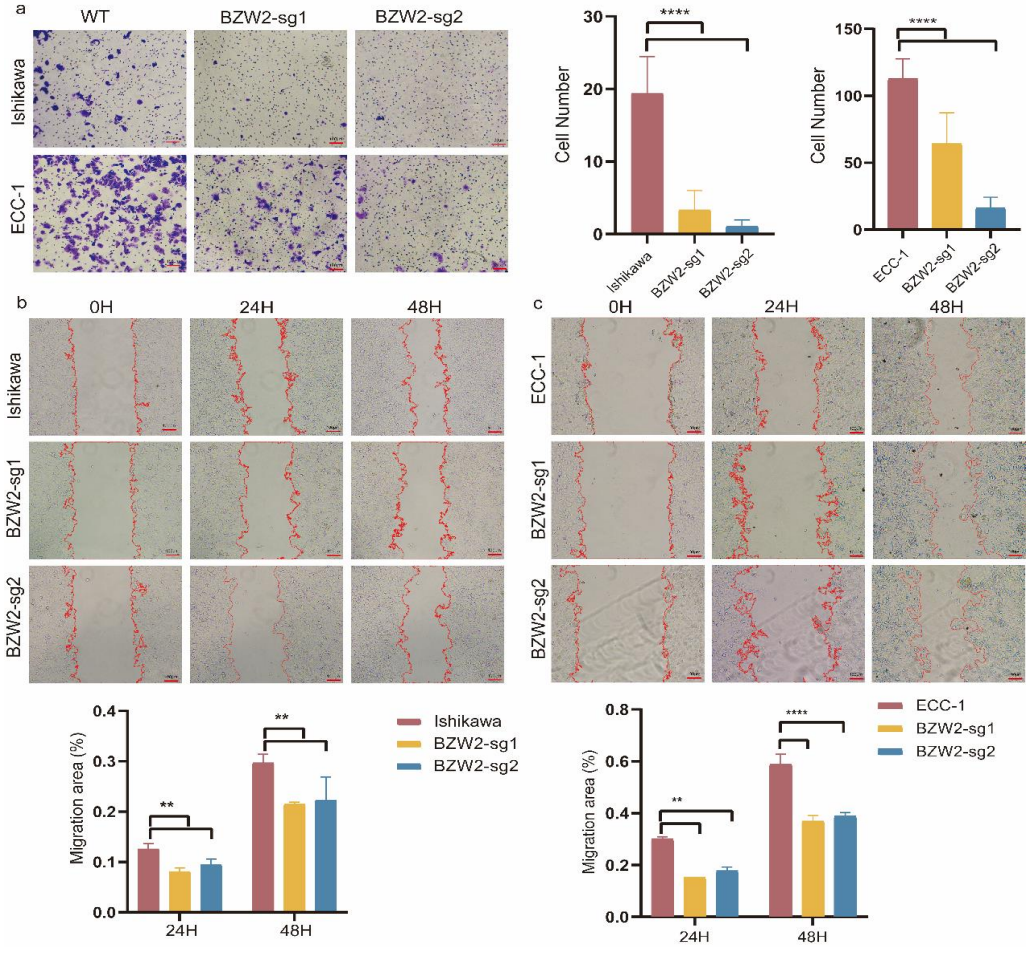

Figure 8. Impact of BZW2 Knockdown on Cell Migration Rates (A) Equal Numbers of Wild-type Ishikawa and ECC-1 Cells, as well as Their BZW2-knockdown Counterparts, were Seeded in Transwell Chambers. After One Day, the Upper Layer of Cells was Gently Removed Using a Cotton Swab, while the Lower Layer Cells were Fixed and Stained for Quantification. (B, C) A Defined Number of Wild-type Ishikawa and ECC-1 Cells, along with their BZW2-knockdown Counterparts, were Plated in 6-well Plates. On the Second Day, following Cell Adherence, a Scratch was Introduced, and the Cells were Cultured in Serum-free Medium to Evaluate Their Migratory Capacity. *p < 0.05, **p < 0.01, ***p < 0.0001.

3.9 Promotion of Cell Growth and Migration by BZW2 Overexpression

The successful construction of a BZW2-overexpressing Ishikawa cell line, as confirmed by western blot analysis for the efficiency of overexpression, is a crucial step in understanding the role of BZW2 in endometrial cancer cells. Overexpression of BZW2 in Ishikawa cells significantly enhanced their clonogenic ability, as demonstrated by colony formation assays (Figure 9A). This was further supported by CCK-8 cell growth assays (Figure 9B), which showed a marked increase in cell proliferation. Transwell assays (Figure 9C) revealed that the overexpression of BZW2 also significantly boosted the migration capacity of these cells. Lastly, wound healing assays (Figure 9D) indicated that BZW2 overexpression led to a faster rate of wound closure, suggesting an increase in the invasive potential of endometrial cancer cells. These findings collectively underscore the role of BZW2 as a promoter of both growth and invasiveness in endometrial cancer cells.

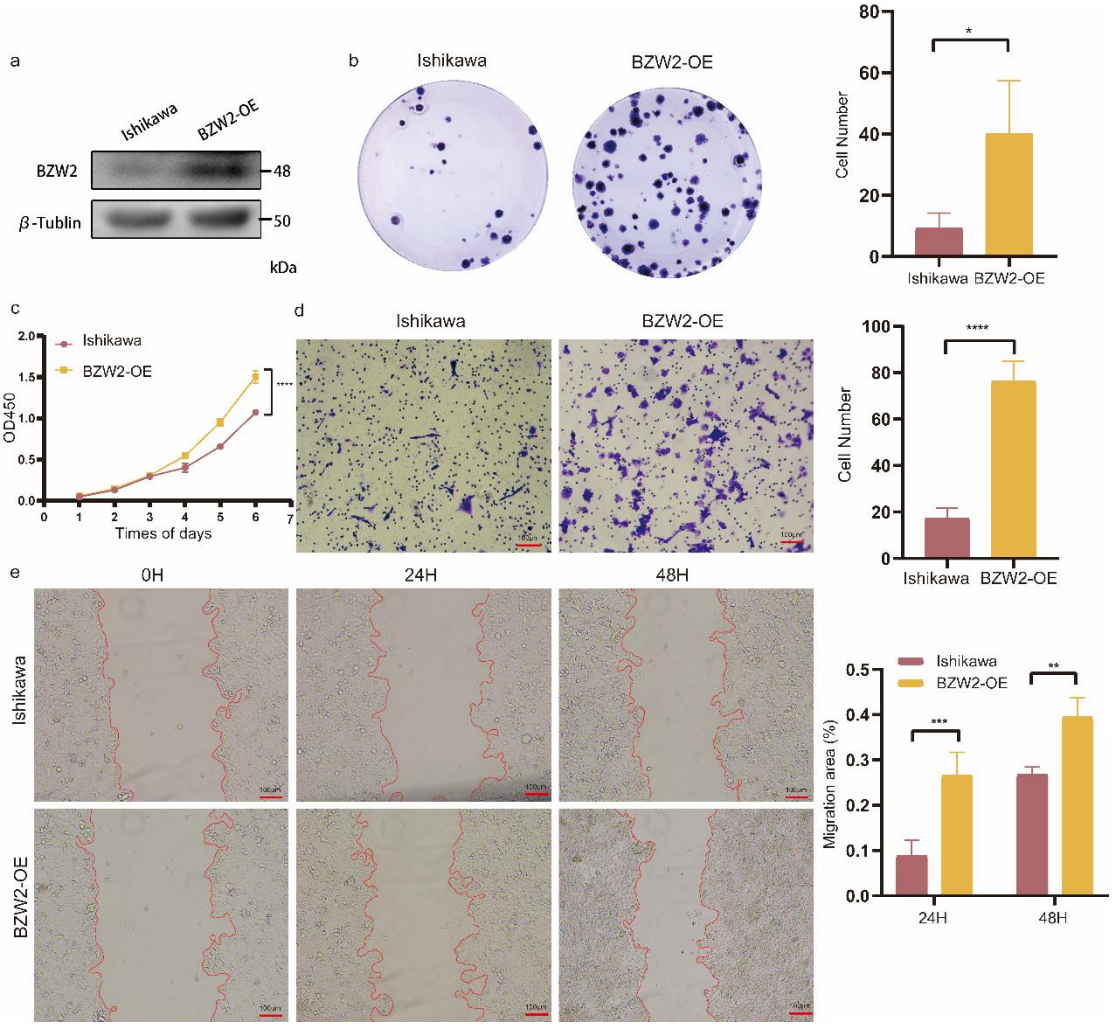

Figure 9. Overexpression of BZW2 Enhances Cell Proliferation and Migration (A) The Expression of BZW2 was Measured by Western Blots after BZW2-overexpression in Ishikawa Cells. β-Tublin was Used as a Loading Control. (B) Cloning Formation Assay. (C) CCK-8 Cell Proliferation Assay. (D) CCK-8 Cell Proliferation Assay. (E) Wound Healing Assay.

4. Discussion

The present study provides compelling evidence that BZW2 serves as a promising therapeutic target and prognostic biomarker in endometrial cancer (EC). Our analysis of BZW2 expression across various cancer types, with a particular focus on EC, reveals significant overexpression that correlates with advanced tumor grade and stage (Jin, Liao, Zhang, Yang, & Zhao, 2019). This correlation suggests BZW2's involvement in the aggressiveness of the disease, which is further substantiated by its association with poorer patient outcomes (Agarwal, Afaq, Bajpai, Behring, Kim, Varambally, et al., 2023).

Survival analysis indicates that elevated BZW2 expression is linked to reduced overall survival, disease-specific survival, and progression-free interval in EC patients (Cheng, Li, Zhu, Yuan, Yang, &

Fan, 2017). These results are consistent with the hypothesis that BZW2 may play a critical role in tumor growth and metastasis, highlighting its potential as a prognostic indicator in clinical settings.

Our research has expanded on previous findings by highlighting a significant role for BZW2 in endometrial carcinoma (EC), demonstrating its overexpression and association with adverse clinical outcomes (Sato, Masuda, Hu, Tobo, Gillaspie, Niida, et al., 2019). The elevated expression of BZW2 in EC aligns with trends observed in hepatocellular carcinoma (HCC) and lung adenocarcinoma (LUAD), where BZW2 has been recognized as a promoter of tumorigenesis and a predictor of poor prognosis (Kim, Shin, Lee, Bae, Sung, Nam, et al., 2012; Liu, Lv, Qin, Gen, Zheng, Liu, et al. 2012). This consistency across various cancer types suggests that BZW2 may serve as a common denominator in malignant transformation and disease progression.

Molecular analyses indicate that BZW2 is linked to pathways and processes critical for cell signaling, gene expression regulation, and metabolic pathways. Its involvement in neuroactive ligand-receptor interactions and long-term depression pathways implies a complex role in modulating tumor cell behavior and adaptability, which could be pivotal in the progression of EC (Wu, Tao, Wang, Lobie, & Wu, 2017). Notably, the correlation of BZW2 expression with immune cell markers within the tumor microenvironment points to a potential role in immunomodulation. Specifically, the negative correlation with cytotoxic and dendritic cells suggests that tumors with high BZW2 expression may promote an immunosuppressive microenvironment, which could have significant implications for responses to immunotherapies (Liu, Yin, Ouyang, Zeng, Xiao, & Li, 2020).

Functional assays conducted in our study demonstrate that BZW2 knockdown impairs the proliferative and migratory capacities of EC cells, thereby supporting its role as an oncogenic driver (Huang, Tan, Huang, Chen, Lin, & Fu, 2018). These findings are consistent with previous research showing that BZW2 knockdown inhibits cell growth and invasion in HCC (Mendez-Blanco, Fondevila, Garcia-Palomo, Gonzalez-Gallego, & Mauriz, 2018). Furthermore, our data regarding the promotional effects of BZW2 overexpression on cell growth and migration align with its established role in LUAD and colorectal cancer (Fu, Sun, Huang, Quan, Hu, Tang, et al., 2018; Seifer, Su, & Taylor, 2017).

The potential of BZW2 as a therapeutic target in endometrial cancer (EC) is further highlighted by our findings that knockdown of GSK3β can reverse the effects of BZW2 knockdown. This observation suggests that BZW2 may exert its oncogenic effects through the modulation of GSK3β, a well-known regulator of the Wnt/β-catenin signaling pathway (Cheng, Li, Zhu, Yuan, Yang, & Fan, 2017). This pathway is frequently deregulated in various cancers and plays a critical role in essential cellular processes such as proliferation, migration, and immune evasion (Mendez-Blanco, Fondevila, Garcia-Palomo, Gonzalez-Gallego, & Mauriz, 2018; Seifer, Su, & Taylor, 2017). Therefore, our results provide a compelling rationale for future research aimed at exploring the BZW2/GSK3β axis as a potential therapeutic target in EC.

While this study offers valuable insights into the role of BZW2 in EC, it is important to acknowledge certain limitations. The retrospective nature of the clinical data analysis and the reliance on in vitro

experiments may limit the generalizability of our findings. Future studies employing larger cohorts and in vivo models are essential to validate these results and to delve deeper into the mechanisms underlying BZW2 action. Nevertheless, this research lays a solid foundation for future investigations and underscores the promise of BZW2 as both a therapeutic target and a prognostic biomarker in endometrial cancer.

In conclusion, by integrating the findings from our study with existing literature, BZW2 emerges as a critical promoter of EC progression and a promising therapeutic target. The consistent association of BZW2 with cancer aggressiveness and poor prognosis across various cancer types underscores its potential as a universal cancer biomarker. Further research is imperative to fully elucidate the role of BZW2 in EC and to explore its therapeutic potential.

5. Conclusion

In conclusion, the present study delineates the multifaceted role of basic leucine zipper and W2 domain-containing 2 (BZW2) in endometrial cancer (EC), underscoring its potential as both a therapeutic target and a prognostic biomarker. Through the analysis of BZW2 expression in EC and its correlation with tumor grade, stage, patient age, and prognosis, we have uncovered a significant association between elevated BZW2 levels and the severity of the disease, as well as reduced overall and progression-free survival rates. Molecular analyses have linked BZW2 to various signaling pathways and immune cell markers, while cellular experiments have confirmed its role in promoting EC cell proliferation and migration. Collectively, our findings provide compelling evidence that BZW2 is a promising target for the treatment and prognosis evaluation of uterine corpus endometrial carcinoma (UCEC), guiding future research and clinical applications.

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