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IQGAP3 can Serve as a Promising Biomarker for the Diagnosis of Endometrial Cancer

Wenyao Liu[1], Qinghong Meng[2], Chengcheng Du[3], Chang Sun[4], Shujuan Hou[2], Yu Li[5,*]

[1]Department of Health Care, the Second Affiliated Hospital of Qiqihar Medical University, 161006 Qiqihar, Heilongjiang, China
[2]Department of Obstetrics and Gynecology, the Second Affiliated Hospital of Qiqihar Medical University, 161006 Qiqihar, Heilongjiang, China
[3]Department of Gynecology, Qiqihar Jianhua Hospital, 161000 Qiqihar, Heilongjiang, China
[4]Department of Health Care, Qiqihar Institute of Engineering, 161003 Qiqihar, Heilongjiang, China
[5]Experimental Teaching Center of Medical Function, Qiqihar Medical University, 161006 Qiqihar, Heilongjiang, China
*Correspondence: liyu2664@qmu.edu.cn (Yu Li)
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Objective: Endometrial cancer (EC), a prevalent malignancy in women's reproductive system, commonly occurs in uterine tissue. Due to its persistently high incidence despite considerable medical advancements, there is a need for the identification of novel diagnostic and treatment biomarkers. This study aims to investigate the potential of IQ motif-containing GTPase-activating protein 3 (IQGAP3) as a biomarker for early diagnosis of endometrial cancer and explore its significance in cervical cancer.
Methods: This study utilized CaSki and Hela cell lines and employed siRNA/shRNA and scrambled control antisense for transfection experiments. However, the expression level of the gene was assessed using qPCR (Quantitative Polymerase Chain Reaction). Additionally, the involvement of IQGAP3 in the cell cycle pathway was elucidated through pathway analysis.
Results: Tumor Immune Estimation Resource (TIMER) 2.0 data analysis showed that *IQGAP3* exhibited significant overexpression in cancer groups (*$p < 0.05$), with elevated levels in EC samples compared to normal tissues (*$p < 0.001$). Furthermore, *IQGAP3* showed significantly close association with survival prognosis ($p < 0.05$). Additionally, we observed that the knockdown of *IQGAP3* inhibited cell proliferation in endometrial cancer cells.**
Conclusion: This study provides valuable insights into the role of *IQGAP3* in cancer diagnosis and treatment. The findings suggest that *IQGAP3* holds potential as a promising biomarker for endometrial cancer diagnosis and as a therapeutic target for its treatment.

Keywords: endometrial cancer; cell cycle proteins; bioinformatics; diagnosis; cell proliferation; cell apoptosis

Introduction

Endometrial cancer (EC), one of the most common malignancies within the female reproductive system, typically originates in the endometrial tissue of the uterus [1,2]. Despite significant advancements in early diagnosis and treatment, the incidence and mortality rates of EC remain high, posing substantial challenges to both patients and healthcare systems [3,4]. Consequently, there is an urgent need for novel biomarkers to enhance the early diagnosis and treatment of EC.

IQ motif-containing GTPase-activating protein 3 (IQGAP3) is a crucial cellular signaling regulator, exhibiting abnormal expression and functionality across various cancer types [5,6]. It serves as a bridge within cells, connecting multiple signaling pathways and participating in essential biological processes such as cell polarity, proliferation, migration, and invasion [7]. This diverse role positions IQGAP3 as a pivotal contributor to the initiation and progression of cancer [8]. Recent studies have shown a significant increase in IQGAP3 expression within endometrial cancer tissues [9,10]. This finding has generated considerable interest because the aberrant expression of IQGAP3 could be closely linked to the pathogenesis of endometrial cancer.

By delving deeper into the role of IQGAP3 in endometrial cancer and comprehensively examining its interactions with various signaling molecules, we aim to gain insights into how IQGAP3 contributes to the growth, differentiation, and metastasis of endometrial cancer cells. This will help uncover its specific role in the pathogenesis of EC, and exploring IQGAP3 as a potential biomarker for endometrial cancer holds crucial clinical significance. Considering the diversity and complexity of endometrial cancer, it is likely that IQGAP3 is not the sole biomarker. Research should investigate the combination of IQGAP3 with other known biomarkers to enhance the accuracy and reliability of diagnosis. Through comprehensive investigation into the role of IQGAP3 in endometrial cancer, we aim to offer new insights and strategies for the early diagnosis, treatment, and prognostic assessment of this prevalent cancer. This work holds the potential to improve the quality of life for patients,

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alleviating the burden on healthcare systems, and providing improved treatment prospects for individuals with endometrial cancer.

Materials and Methods

Data Sources

Data on uterine endometrial carcinoma (EC) were accessed from various datasets within The Cancer Genome Atlas (TCGA) database (https://www.cancer.gov/ccg/research/genome-sequencing/tcga). The Human Protein Atlas database provided the immunohistochemistry (IHC) information. Furthermore, the Tumor Immune Estimation Resource (TIMER) databases were employed to examine the relationship between IQGAP3 expression and the infiltration of immune cells in uterine endometrial carcinoma (UCEC).

TIMER Database Analysis

TCGA database [11] provided mRNA expression levels of *IQGAP3* and clinical data of UCEC patients. Information was retrieved from the National Cancer Institute's TCGA database (https://cancergenome.nih.gov/), and subsequently processed and merged using R software (version 3.6.3, http://www.rproject.org/). This integration yielded a detailed fragments per kilobase of transcript per million reads mapped (FPKM) matrix covering the entire genome, encompassing data from 548 patients diagnosed with uterine endometrial carcinoma and 35 samples of adjacent normal tissue. Based on the median *IQGAP3* mRNA expression level, these 548 EC samples were categorized into two groups: the high-expression group and the low-expression group.

Identification of Differentially Expressed Genes

The genes co-expressed with *IQGAP3* in EC samples sourced in the TCGA database were identified using the R package "stat" with thresholds of |correlation (cor)| >0.3 and $p < 0.05$. Moreover, enrichment analysis was conducted utilizing the "clusterProfiler" package with thresholds of FDR <0.2 and $p < 0.05$. Finally, the outcomes were visualized employing the "ggplot2" package.

Gene Expression Profiling Interactive Analysis (GEPIA) Website Analysis

The differential expression of the *IQGAP3* gene, in both normal endometrial tissue and uterine endometrial carcinoma, was assessed using the GEPIA online analysis [12] website. Furthermore, we analyzed its expression levels in different stages, utilizing TCGA and Genotype-Tissue Expression (GTEx) data. The threshold for differential expression of *IQGAP3* mRNA was set at |log2FC| >1.5 and $p < 0.05$.

Gene Set Enrichment Analysis (GSEA)

GSEA was performed on the *IQGAP3* Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), Panther, Reactome, and Wiki pathways using the GSEA database (http://www.linkedomics.org/admin.php) [13]. The gene enrichment analysis strategy was designed as follows: (1) Initially, the TCGA-UCEC cancer cohort was selected. (2) Subsequently, the dataset for analysis was chosen and evaluated using the Firehose_root mean square error (RSEM)_log2 pipeline, particularly targeting the TCGA-UCEC sample cohort, with a focus on RNAseq data from the HiSeq RNA platform, at the gene analysis level. (3) In the next step, the *IQGAP3* attribute within the search dataset was selected. (4) Subsequently, the target dataset was identified and analyzed employing the Firehose_RSEM log2 pipeline, particularly focusing on the TCGA-UCEC sample cohort, RNAseq data, HiSeq RNA platform, gene analysis level. (5) Finally, the data were analyzed using the Spearman Correlation test. Following these steps, the data were additionally analyzed using the LinkInterpreter tool, particularly to scrutinize the Association Result.

Cell Culture

The CaSki (CL-0048, Procell, Wuhan, China) and Hela (CL-0101, Procell, Wuhan, China) cell lines were acquired and cultured in Dulbecco's Modified Eagle Medium/F12 (PB180329, Procell, Wuhan, China), enriched with 10% fetal bovine serum, 100 µg/mL streptomycin, and 100 U/mL penicillin. The cells were incubated at 37 °C in an environment containing 5% CO_2. Meanwhile, Isoimperatorin was procured from Sigma (CAS-no: 482-45-1, Aldrich, Kansas, MO, USA) and used to treat CaSki and Hela cells at concentrations of 0, 5, 10, 25, 50, and 100 µM. However, the cells were also grown in Dulbecco's Modified Eagle's Medium supplemented with 10% FBS (TMS-016, Aldrich, Kansas, MO, USA), 1% penicillin-streptomycin, and 5 mM glucose, followed by incubation at 37 °C in a humidified 5% CO_2 atmosphere. The cells underwent subculturing when they reached 70–80% confluence. The cells were authenticated through STR profiling and no cross-contamination was identified following mycoplasma testing.

Quantitative Polymerase Chain Reaction (qPCR)

Total RNA was extracted from frozen cells using TRIzol reagent (15596026, Thermo Fisher Scientific, Waltham, MA, USA) and subsequently converted into cDNA utilizing the Transcriptor First Strand cDNA Synthesis Kit (04897030001, Thermo Fisher Scientific, Waltham, MA, USA). Moreover, quantitative real-time PCR was conducted using LightCycler 480 SYBR Green I Master Mix (04887352001, Roche, Basel, Switzerland). For reverse transcription, RNA was quantified, and subsequently, 4 µL of total RNA was mixed with 4 µL of 5× TransScript All-

in-One Super-Mix for qPCR, 1 µL of gDNA Remover, and 20 µL of Rnase-free ddH_2O in an ice bath. The mixture underwent incubation at 42 °C for 15 minutes, followed by heating at 85 °C for 5 seconds to deactivate TransScript RT/RI and gDNA Remover, and finally stored at –20 °C. The resultant cDNA was diluted 10-fold for qPCR analysis. The reaction mixture of qPCR included SybrGreen qPCR Master Mix, primer F (10 µM), primer R (10 µM), and cDNA template. The reaction mixture was seeded in a 96-well plate and amplification was proceeded in an ABI Step-One Plus real-time PCR instrument. The primers used in qPCR were as follows:

IQGAP3-F-5′-AGGGTGATCAGGAACAAGCC-3′, *IQGAP3*-R-5′-ACAGGGTACACTGGAGGCAG-3′; *β-actin*-F-5′-TGACGTGGACATCCGCAAAG-3′, *β-actin*-R-5′-CTGGAAGGTGGACAGCGAGG-3′.

Western Blot

For total protein extraction, cells were lysed using RIPA buffer (89900, Thermo Fisher Scientific, Waltham, MA, USA) followed by centrifugation (12,000 g) of the cell lysates at 4 °C for 30 minutes. Subsequently, the resultant supernatant, containing protein extracts, was collected and underwent protein quantification using the Pierce™ BCA Protein Assay Kit (23225, Thermo Fisher Scientific, Waltham, MA, USA). The proteins were resolved on SDS-PAGE (4561096, Bio-Rad, Herakles, CA, USA) and subsequently transferred onto a polyvinylidene fluoride membrane. The membrane was blocked with 5% non-fat dry milk in TBS containing 0.1% Tween-20 to prevent non-specific binding. After this, the membrane was incubated overnight with primary antibodies, including IQGAP3 (1:200, sc-376021, Santa Cruz Biotechnology, Santa Cruz, CA, USA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:4000, sc-17835, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and β-actin (1:5000, sc-8432, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C on a shaker. The next day, after washing with PBS, the membrane was incubated with secondary antibodies (Mouse anti-human, NBP2-22471; QGAP3 antibody, NBP3-16228, Novus Biologicals, Aurora, CO, USA) for 50 minutes. Finally, the immunoblot was developed in an exposure unit for about 3 minutes, ensuring the bands faced upwards. Protein bands were assessed and quantified using the Odyssey infrared imaging system (LI-COR, LI-COR Biosciences, Jersey, NJ, USA) and grayscale values of the protein bands were determined through Image-Pro Plus. However, the GAPDH and β-actin were utilized as the internal controls.

Lipo2000 Transfection

The CaSki and Hela cells were transfected with 20 µM siRNA/shRNA or scrambled control antisense using Lipofectamine 2000 (11668500, Thermo Fisher Scientific, Waltham, MA, USA) [14]. When the cell density of transfected cells in the logarithmic growth phase reached 30% to 50%, they were divided into the negative control group (siScramble group), *IQGAP3* interference group 1 (si-*IQGAP3*-1 group), and *IQGAP3* interference group 2 (si-*IQGAP3*-2 group). For the transfection of *IQGAP3* siRNA, 5 µL of Lipofectamine 2000 reagent and an equal volume of siRNA were each diluted in 250 µL of OPTI-MEM, following the manufacturer's guidelines. The targets of si-*IQGAP3*-1, si-*IQGAP3*-2, and si-*IQGAP3*-3 were ATGTTGGCTTTGTCATCAA, TCAGAGTTCTTGTCTTGCC, and AGATTGGTCTGCTCGTCAA, respectively. These solutions were left at room temperature for 5 minutes before being combined followed by incubation for an additional 20 minutes. The mixture was then added to a 6-well plate, ensuring even distribution by gentle shaking. The cells were incubated at 37 °C in a 5% CO_2 atmosphere for 6 hours, followed by replacing culture medium containing 10% serum, and subsequently underwent incubation for 48 hours. Post-transfection, cells were evaluated for transfection efficiency and processed for subsequent analysis. Each experiment was conducted in triplicate, using five different transfection reagents along with a control group without any reagent.

MTT Assay

Endometrial cancer cells transfected with siScramble, si-*IQGAP3*-1, and si-*IQGAP3*-2 were seeded in a 96-well cell culture plate at a density of approximately 5×10^3 cells per well, with each group assigning three replicate wells [15]. The cells were incubated at 37 °C in the presence of CO_2 for 24, 48, and 72 hours. Subsequently, 20 µL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) solution (5 mg/mL, orb867674, Biorbyt, Cambridge, UK) was added to each well, followed by incubation at 37 °C for 4 hours. After removing the supernatant, 100 µL of dimethyl sulfoxide (DMSO) was introduced to each well and mixed through gentle shaking for 10 minutes. Finally, the optical density (OD) of each well was determined at 490 nm using a microplate reader. Moreover, the average OD value was assessed by observing five different fields on the microplate membrane. This procedure was replicated three times.

Statistical Analysis

Statistical analyses were performed using R software (version 3.6.3, http://www.rproject.org/) and GraphPad Prism software (version 8.0, GraphPad, San Diego, CA, USA). The data were presented as the mean ± SD. An independent samples *t*-test was used to compare two sample means. Survival analysis utilized the log-rank test, considering $p < 0.05$ as statistically significant (two-tailed). The receiver operating characteristic (ROC) curve was developed employing the 'pROC' package (http://www.rproject.org/). Furthermore, the differences in *IQGAP3* gene expression between EC and normal tissues were assessed utilizing the Mann-Whitney U test and paired *t*-test. Moreover, Kaplan-Meier and Cox analyses were employed for

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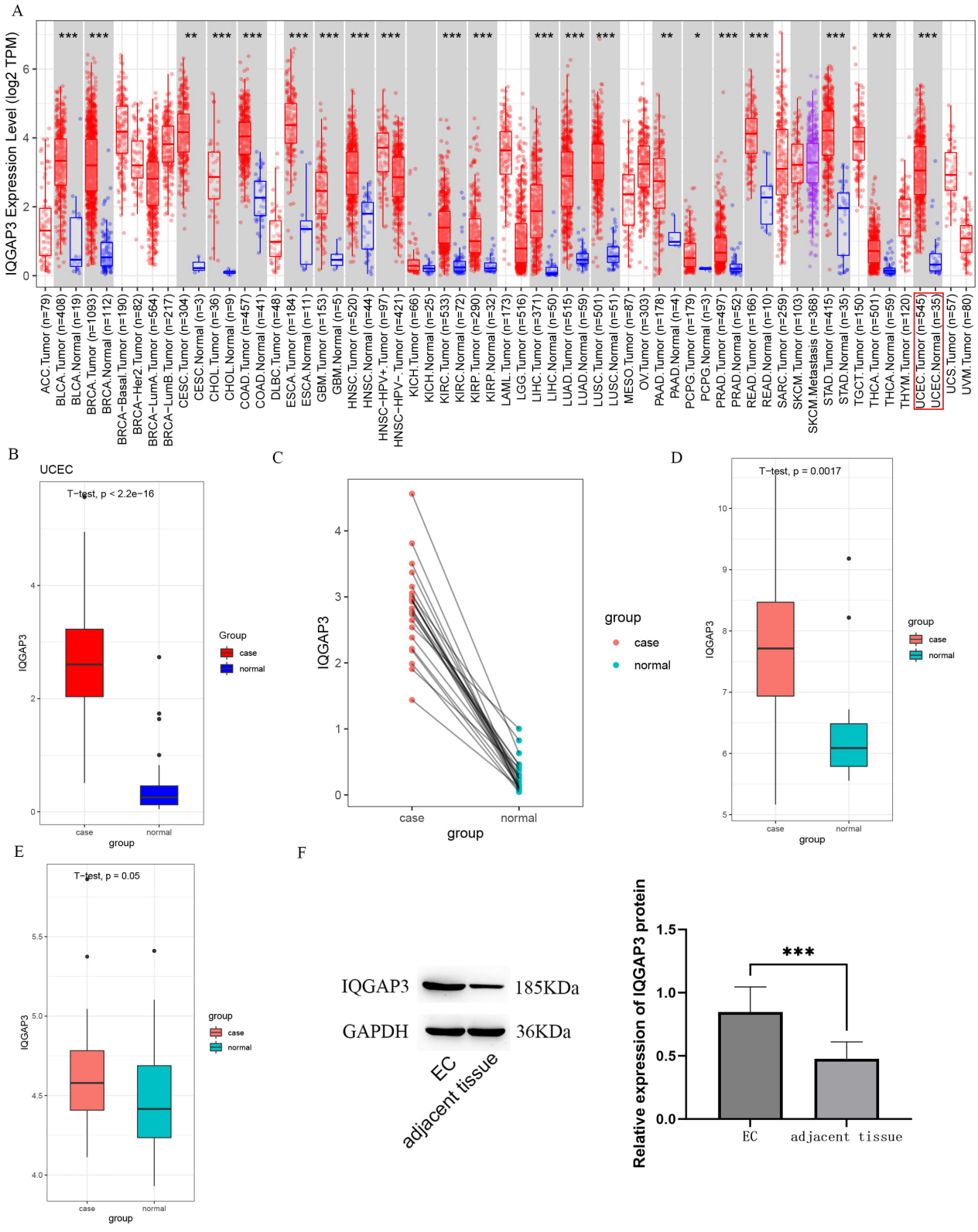

Fig. 1. IQ motif-containing GTPase-activating protein 3 (IQGAP3) was highly expressed in endometrial cancer (EC) samples. (A) Tumor Immune Estimation Resource (TIMER) analysis revealed varying *IQGAP3* expression levels across different tumor types in the The Cancer Genome Atlas (TCGA) database; (B–E) TCGA and TIMER2.0 data (http://timer.cistrome.org/) indicated higher *IQGAP3* expression in EC samples; (F) UALCAN database comparisons showed elevated IQGAP3 protein levels in EC samples compared to normal renal tissues. Note: Significance levels are denoted as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. n = 10. TPM, Transcripts Per Kilobase Million; UCEC, uterine endometrial carcinoma; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

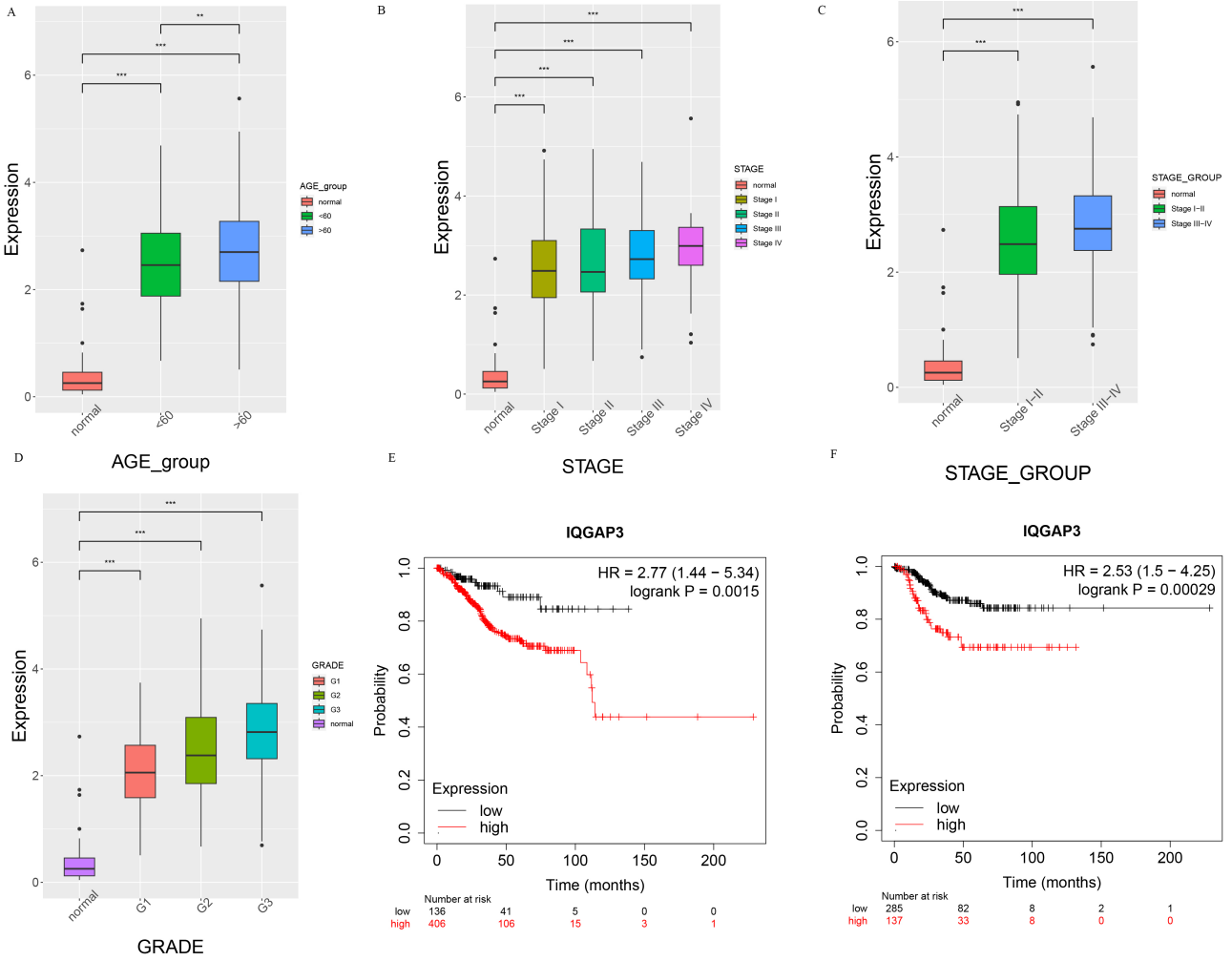

Fig. 2. The correlation of *IQGAP3* with clinical characteristics in the TCGA-uterine endometrial carcinoma (UCEC) dataset. (A) *IQGAP3* mRNA expression and patient age; (B) *IQGAP3* mRNA expression and tumor stage; (C) *IQGAP3* mRNA expression and menopausal status; (D) *IQGAP3* mRNA expression and histological subtypes; (E,F) The association of High *IQGAP3* expression with overall survival (OS) and recurrence-free survival (RFS) in endometrial cancer. Note: Significance levels are indicated as $**p < 0.01$, $***p < 0.001$.

survival studies. Furthermore, multiple group comparisons were determined through one-way ANOVA coupled with Dunnett's test. Statistical significance was set at $p < 0.05$.

Results

Determining the Significance of IQGAP3 in Endometrial Cancer

The TIMER 2.0 database (http://timer.cistrome.org/) revealed upregulation of IQGAP3 in numerous cancers, including endometrial cancer ($*p < 0.05$, Fig. 1A). Using the TCGA-UCEC dataset (https://portal.gdc.cancer.gov/), involving the whole-genome FPKM matrix of 548 endometrial cancer patients and 35 adjacent normal tissues, we found that *IQGAP3* was significantly overexpressed in endometrial cancer ($*p < 0.05$, Fig. 1B). Furthermore, we compared cancer-affected tissues with adjacent tissue samples and observed significantly elevated levels of *IQGAP3* in cancer tissues ($*p < 0.05$, Fig. 1C). Additionally, we downloaded the expression matrices of the whole genome for endometrial cancer patients and adjacent tissues from GSE17025 and GSE106191 datasets and found that *IQGAP3* exhibited significant overexpression in cancer groups ($*p < 0.05$, Fig. 1D,E). Furthermore, Western blot (WB) analysis revealed elevated levels of IQGAP3 in EC samples compared to the normal tissues (Fig. 1F, $***p < 0.001$).

Relationship between IQGAP3 Gene and Clinical Characteristics in TCGA-UCEC Dataset

Using the TCGA-UCEC dataset (https://portal.gdc.cancer.gov/), including the whole-genome FPKM matrix and clinical data of 548 endometrial cancer tissues and 35 adjacent normal tissues, we utilized the "ggplot2" package

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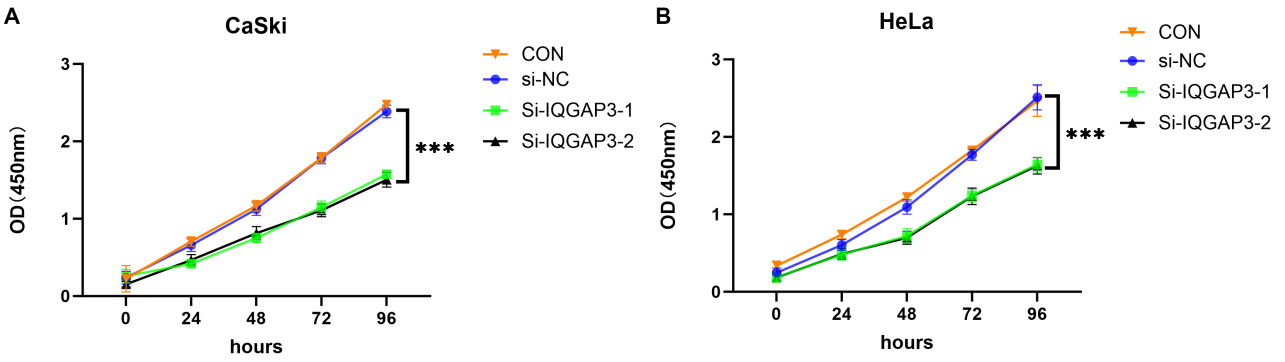

Fig. 3. The impact of *IQGAP3* on endometrial Cancer Cell Proliferation. (A) Knockdown of IQGAP3 inhibits the proliferation of CaSki cells; (B) Knockdown of IQGAP3 inhibits the proliferation of Hela cells. Notes: ***$p < 0.001$. n = 3. CON, control; OD, optical density; si-NC, negative control; si-*IQGAP3*, *IQGAP3* interference.

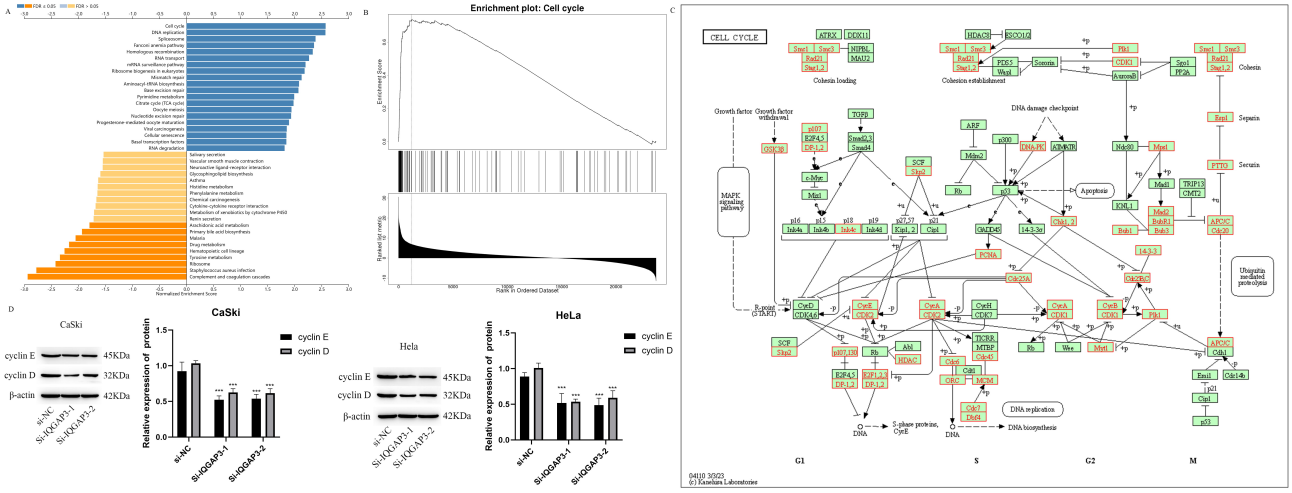

Fig. 4. Gene Ontology (GO) and Western blot Analysis Results of *IQGAP3* in TCGA UECE. (A) mRNA GO analysis of *IQGAP3* expression levels associated with endometrial cancer; (B) Cell cycle (GO: 0098742); (C) Cell cycle pathway diagram; (D) Impact of IQGAP3 knockdown on the expression of Cyclin D and Cyclin E in CaSki cells and HeLa cells. Notes: ***$p < 0.001$.

in R software to generate box plots illustrating the expression of *IQGAP3* in cancer tissues compared to adjacent tissues across different clinical characteristics such as age (less than 60 years, greater than 60 years, and normal group), stage, combined stage, and grade (Fig. 2A–D). Additionally, Kaplan-Meier analysis demonstrated that high *IQGAP3* mRNA expression in EC patients was associated with overall survival (OS) and recurrence-free survival (RFS) in endometrial cancer (Fig. 2E,F) ($p < 0.001$). Furthermore, in endometrioid adenocarcinoma patients, high *IQGAP3* mRNA expression was significantly associated with shortened OS and RFS ($p < 0.001$).

Knockdown of IQGAP3 Promotes Cell Proliferation in CaSki and Hela Cells

The impact of IQGAP3 on endometrial cancer cell proliferation was assessed using an MTT assay. As depicted in Fig. 3A, following the knockdown of IQGAP3, the cell proliferation rate was significantly reduced in both the si-*IQGAP3*-1 and si-*IQGAP3*-2 groups than the negative control (si-NC) and control (CON) groups in CaSki cells. These findings suggest that the knockdown of IQGAP3 can inhibit the proliferation of the CaSki cells. Furthermore, in Hela cells, cell proliferation rate in both the si-*IQGAP3*-1 and si-*IQGAP3*-2 groups were significantly lower than negative control (si-NC) group and CON groups, indicating that knockdown of IQGAP3 can inhibit the proliferation of Hela cells (Fig. 3B).

Molecular Mechanisms of the Key Target Gene IQGAP3

Based on the median expression levels of IQGAP3, the 548 patients in the TCGA-UCEC dataset were divided into an upregulated group and a downregulated group (Fig. 4A). Bayesian analysis was conducted using the "limma" package in R software. Subsequently, an online Gene Set Enrichment Analysis (GSEA) using WebGestalt (WEB-based GEne SeT Analysis Toolkit, https://www.we

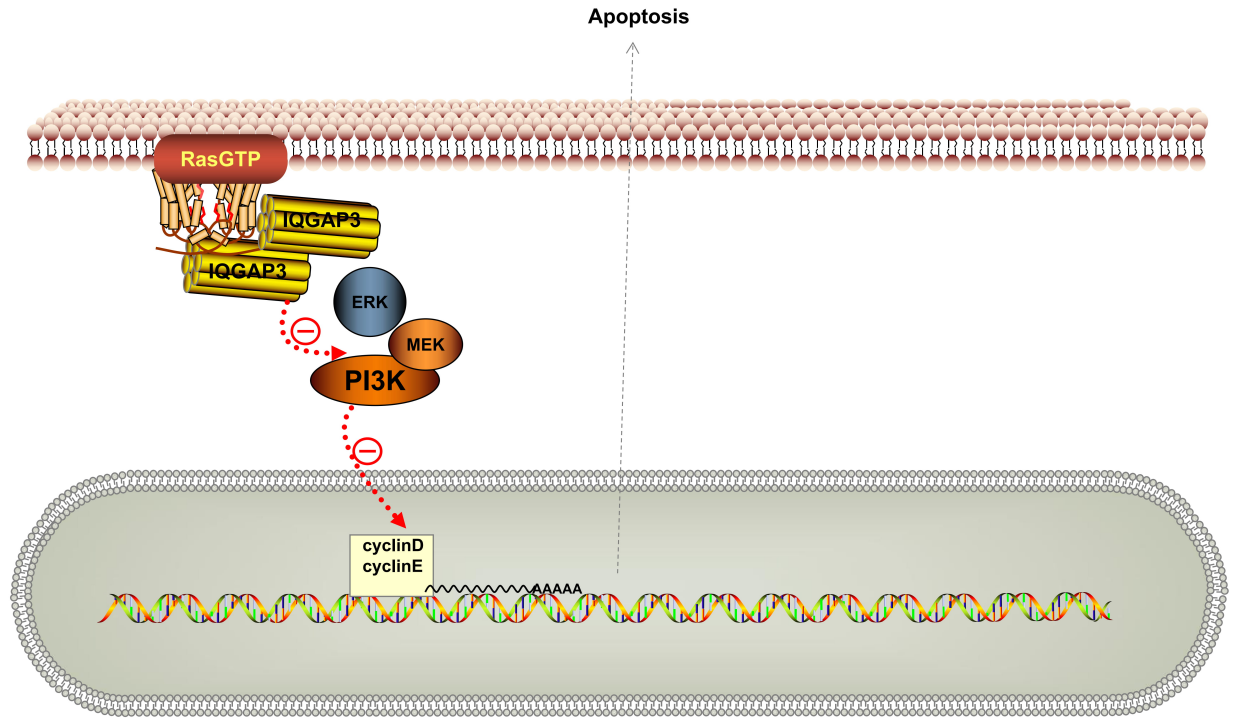

Fig. 5. Knocking down IQGAP3 can arrest the cell cycle and promote apoptosis by reducing the expression of cyclin D and cyclinE. ERK, Extracellular signal-regulated kinase 1/2.

bgestalt.org/) revealed associations with the cell cycle pathway (Fig. 4B,C). Compared to the siScramble group, the knockdown of IQGAP3 significantly decreased the expression levels of cell cycle-related protein markers, including Cyclin D and Cyclin E, closely associated with EC development (Fig. 4D). These findings suggest that *IQGAP3* gene silencing leads to the cell cycle arrest, thereby inducing tumor cell apoptosis.

Discussion

EC is one of the most common malignancies in the female reproductive system [16,17]. Early diagnosis of EC is crucial for effective treatment and improved patient outcomes [18,19]. This study focuses on the role of IQGAP3 as an early diagnostic biomarker as well as its clinical significance in endometrial cancer. We observed a significant increase in IQGAP3 expression in cervical cancer, which correlates with survival outcomes. Additionally, IQGAP3 overexpression is linked to enhanced cell proliferation and reduced apoptosis. Pathway analyses suggest a substantial correlation between IQGAP3 and the cell cycle pathway. Consequently, the study concludes that IQGAP3 exhibits potential as an early screening marker for endometrial cancer and may serve as a viable target for therapeutic interventions.

The findings of this study are consistent with previous study indicating the significance of IQGAP3 in cancer diagnosis and treatment [20]. IQGAP3 is a scaffold protein that plays a crucial role in cell signaling, adhesion, and motility. Overexpression of IQGAP3 has been reported across various types of cancer, including cervical cancer, ovarian cancer, and breast cancer. The association of IQGAP3 with the cell cycle pathway suggests its potential role in regulating cell proliferation and cell cycle progression [21]. Previous studies [22,23] have shown that IQGAP3 is overexpressed in various types of cancer, including cervical cancer, ovarian cancer, and breast cancer. The overexpression of IQGAP3 has been associated with increased cell proliferation, decreased cell apoptosis, and poor prognosis in cancer patients. These findings are consistent with the outcomes of the current studies [24,25], which shows a significant overexpression of IQGAP3 in cervical cancer and its close association with survival prognosis. Similarly, the association of IQGAP3 with the cell cycle pathway has been documented in previous studies. Moreover, its interaction with various cell cycle proteins, including cyclin D1, cyclin E, and CDK4 has been found. These interactions suggest its potential role in regulating cell cycle progression and proliferation (Fig. 5) [26]. Fig. 5 was created using Adobe Illustrator software (version CC 2022, Adobe Systems Incorporated, San Jose, CA, USA). The current study's pathway

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analysis reveals a strong association between IQGAP3 and the cell cycle pathway, further supporting the role of IQGAP3 in regulating cell proliferation [27]. Furthermore, previous studies have demonstrated IQGAP3 as a potential biomarker for cancer diagnosis and a therapeutic target for cancer treatment [28,29], including ovarian cancer diagnosis. Moreover, IQGAP3 has also been suggested as a potential therapeutic strategy for cancer treatment. The current study's findings suggest that IQGAP3 holds potential as a promising biomarker for endometrial cancer diagnosis and as a therapeutic target for its treatment [30]. In summary, existing research has highlighted the significance of IQGAP3 in cancer diagnostics and therapy. Our study aligns with prior findings, substantiating the viability of IQGAP3 as both a diagnostic biomarker and a therapeutic target in cancer. Future research is necessary to corroborate these results and to further investigate the application of IQGAP3 in cancer diagnosis and treatment.

The application of IQGAP3 as a biomarker for endometrial and cervical cancers investigated in this study faces certain limitations regarding its applicability across different types of cancers and populations due to biological diversity. The research is at an early stage and needs further clinical trials to assess IQGAP3's clinical applicability, a process that can be time-consuming and costly. Additionally, the study provides limited insight into the detailed mechanisms through which IQGAP3 influences cancer progression and patient outcomes. The antibodies used in this study have all been validated by the manufacturer using gene knockout cell lines, ensuring their specificity. Consequently, this study did not utilize a blank control group. Nevertheless, the deficiency represents a potential limitation for this study. Furthermore, there's uncertainty regarding the feasibility of specifically targeting IQGAP3 in therapeutic interventions without affecting other critical proteins or pathways.

Conclusion

In summary, this study offers crucial insights into the role of IQGAP3 in cancer diagnosis and therapy. The findings indicate that IQGAP3 could be an effective biomarker for diagnosing endometrial cancer and as a therapeutic target for its treatment. However, further research is essential to validate these findings and to further explore IQGAP3's potential in cancer diagnosis and treatment.

Availability of Data and Materials

All experimental data included in this study can be obtained by contacting the first author if needed.

Author Contributions

WL and YL designed the research study. QM, CD, SH provided help and advice on the experiments. YL, CS analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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