

BTG1 inhibits malignancy as a novel prognosis signature in endometrial carcinoma

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Abstract

Background Endometrial carcinoma (EC) is one of the three major malignant tumors of the female reproductive system. In recent years, the incidence and mortality rate of EC have increased. B-cell translocation gene 1 (BTG1) is an anti-proliferation gene that regulates the occurrence and development of a variety of tumors, but there is no research regarding this gene in EC.

Methods Based on The Cancer Genome Atlas (TCGA) database, we used a variety of bioinformatics tools and databases to explore the expression and prognosis of BTG1. We verified expression and prognosis of BTG1 in EC using qRT-PCR and analyzed the relevant clinicopathological parameters. We functionally enriched BTG1 and related genes in EC patients through the bioinformatics website and analyzed miRNA targets of BTG1 and interacting protein networks. Cell proliferation, wound healing, transwell invasion, and cell apoptosis assays were used to detect the effects of BTG1 on the malignant biological behavior of endometrial carcinoma cells (ECCs). The effect of BTG1 on the epithelial-to-mesenchymal transition (EMT) process was detected using western blot.

Results We analyzed the expression and prognosis of BTG1 based on TCGA and found that low expression of BTG1 was associated with poor EC prognosis. The qRT-PCR suggested that BTG1 had low expression in EC. BTG1 expression was significantly correlated with overall survival (OS) shortening. Clinicopathological analysis suggested that expression of BTG1 was related to invasion depth and the International Federation of Gynecology and Obstetrics (FIGO) stage. EC pathological tissue type, fertility history, lymphatic metastasis, menopause, estrogen receptor (ER), progesterone receptor (PR), and age of diagnosis were not related. Functional enrichment analysis showed that BTG1 plays an important role in regulating embryonic development, tumorigenesis, apoptosis, and cell cycle. Biological behavior experiments suggest that BTG1 inhibits proliferation, migration, and invasion of ECCs, and promotes apoptosis of ECCs. Western blot indicated that BTG1 inhibited the EMT process of ECCs.

Conclusions BTG1 plays an important role in the occurrence and development of EC. BTG1, as a tumor suppressor gene, shows low expression in EC, which can inhibit malignant biological behavior and EMT process of ECCs. In addition, BTG1 can be used as a potential prognostic biomarker for EC.

Background

Endometrial carcinoma (EC) is the sixth most common cancer among women worldwide, and one of the most common tumors in the female reproductive system ^[1]. In recent years, the incidence of EC has increased, and the mortality rate has also increased ^[2]. The mortality rate of EC is directly related to the poor prognostic factors driving tumor recurrence ^[3].

B-cell translocation gene 1 (BTG1) is a member of the TOB/BTG protein family and is an anti-proliferation gene. Studies have shown that the protein encoded by the TOB/BTG protein family can inhibit cell proliferation, induce cell apoptosis, and induce cell cycle arrest ^[4-5]. It can also prevent tumor

neovascularization and inhibit the expression of vascular endothelial growth factor [6]. The protein product of BTG1 is involved in various cellular processes such as cell division, DNA repair, transcriptional regulation, and messenger RNA stability. BTG1 can also affect the developmental process and adult differentiation and help maintain homeostasis under conditions of cell stress [7]. A large number of studies have shown that BTG1 is associated with various diseases. BTG1 overexpression inhibits the activity of ATF4 and inhibits the expression of the SCD1 gene related to fatty acid synthesis BTG1 transgenic mice are resistant to hepatic steatosis caused by high carbohydrate diet [8]. MIR301A levels in IECS of patients with active inflammatory bowel disease increased. MIR301A reduces the expression of BTG1, reduces the integrity of mouse colonic epithelium, promotes inflammation, and promotes tumorigenesis [9]. Changes in BTG1 expression may play a role in the development of ovarian cancer by regulating the proliferation, migration, invasion, cell cycle, and apoptosis of ovarian cancer [10]. In addition, lidocaine inhibits cervical cancer cell proliferation and induces apoptosis by regulating the lncRNA-MEG3/miR-421/ BTG1 pathway [11].

However, BTG1 has not been studied in EC. In this study, we used multiple bioinformatics databases to objectively analyze the expression of BTG1 in EC, its impact on EC prognosis, and analyze the expression of BTG1 in the function regulation network. In addition, we studied the expression of BTG1 in EC tissues and its relationship with clinical pathological parameters and prognosis. Through in vitro studies we explored the effect of BTG1 on the malignant biological behavior of endometrial cancer cells (ECCs) and the epithelial-to-mesenchymal transition (EMT) process.

Materials And Methods

Oncomine database analysis

This study used the Oncomine database (<http://www.oncomine.org>) [12]. It can be used to analyze gene expression differences, find outliers, and predict co-expression. Through this database, we analyzed the expression of BTG1. The cut-off setting was set to P value <0.05.

GEPIA database analysis

GEPIA (<http://gepia.cancer-pku.cn>) [13] is a website based on TCGA and GTEx projects cancer data mining. We analyzed the differential expression of BTG1 in tumors and normal tissues through GEPIA. Gene name: BTG1.

UALCAN database analysis

UALCAN (<http://ualcan.path.uab.edu>) [14] is a TCGA database online analysis and mining website, built on PERL-CGI, javascript, and css. Based on this database, we analyzed the relative expression of BTG1 in EC tissue and normal endometrial tissue. We analyzed the relative expression in different subgroups of EC, including: individual cancer stages, patient's weight, patient's age, histological subtypes, TP53

mutation status, patient's race, and menopause status. In addition, UALCAN was used to analyze the prognosis of EC.

Survival analysis

Kaplan–Meier (KM) plotter (<http://kmplot.com>)^[15] is an online tool for analyzing the prognosis of tumor patients. According to the expression of BTG1, EC patients were divided into a BTG1 high-expression group and a BTG1 low-expression group. The follow-up time was set at 120 months.

LinkedOmics database analysis

In this study, the LinkFinder module of the LinkedOmics database (<http://www.linkedomics.org/login.php>)^[16] was used to study differentially expressed genes related to BTG1 with nTM = 176 in the TCGA UCEC dataset. The Pearson correlation coefficient was used to statistically analyze the results. LinkFinder displays the results in the form of volcano and heat maps.

Metascape analysis

Metascape (<http://metascape.org>) [17] is a powerful gene function annotation analysis tool. We used Metascape to analyze the enrichment of the top 500 genes of BTG1, and its related differential expression through processes and pathways. Among them, $P \leq 0.01$, the minimum count was 3, and the enrichment factor was >1.5 , to obtain significant statistical differences.

GeneMANIA analysis

GeneMANIA (<http://www.genemania.org>)^[18] can predict gene function and analyze gene lists to look for interaction networks between genes, relationships between genes, and also analyze protein-protein, protein-DNA interactions, pathways, physiological and biochemical reactions, co-expression, and co-localization. Through GeneMANIA, we obtained a network of interactions between genes with significant differences in BTG1.

miRDB analysis

miRDB (<http://mirdb.org>)^[19] is an online database for miRNA target prediction and functional annotation. All targets in miRDB were predicted by the bioinformatics tool MirTarget. We used miRDB to find all miRNAs that bind to BTG1.

Human tissue specimens

All EC tissue samples and normal endometrial tissue samples were from patients undergoing total hysterectomy at Shengjing Hospital affiliated to China Medical University. The diagnosis of EC was evaluated by two experienced clinical pathologists based on FIGO for histological diagnosis and tumor grading. None of the patients received chemotherapy, radiotherapy and hormones and treatment before

surgery. All patients received informed consent, and were approved by the Ethics Committee of Shengjing Hospital Affiliated to China Medical University.

Extraction of RNA, reverse transcription, and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tissues and cells by TRIzol reagent (Takara, Beijing, China). The complementary DNAs (cDNAs) for the lncRNAs and mRNAs of interest were reverse-transcribed from 2 µg of total RNA using PrimeScript RT-polymerase (Takara). qRT-PCR was performed using SYBR-Green Premix (Takara) and specific PCR primers (Sangon Biotech, Shanghai, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The mRNA expression was observed by calculating $2^{-\Delta\Delta CT}$. The primer sequences are listed in the Table S1.

Cell culture and transfection

BTG1's overexpression plasmid and knockdown plasmid were purchased from GeneChem (Shanghai, China), and both were transfected with jetPRIME® in vitro DNA and siRNA Transfection Reagent (PolyPlus-transfection, France) for subsequent experiments. The related sequences can be found in Table S2

Cell proliferation assay

In this study, EdU cell proliferation assay kit (Ribobio, Guangzhou, China) was used to detect the effect of BTG1 on cell proliferation. In a 96-well plate (Guangzhou Jet Bio-Filtration Co., Ltd. Guangzhou, China), 8000 cells were seeded per well. After 24 h of culture, the cells were transfected, and the culture continued for 48 h. The EdU solution was diluted with cell culture medium at a ratio of 1000:1, 100 µLEdU was added to each well, and the plate was incubated at 37 °C. in 5% CO₂ for 2 h. Staining was with Apollo fluorescent dye. Cellular DNA replication activity was detected by fluorescence microscopy.

Wound healing assay

Using logarithmic growth phase cells. 8×10^5 cells/well were seeded a 6-well plate (Guangzhou), and the cells were transfected after 24 h of culture. At 5 h after transfection, the culture medium was discarded, and a 200 µL pipette tip was used to gently stroke the plate. PBS was used to wash away floating cells. A5% FBS culture medium was added, and cells were observed by microscopy and photographed (0 h cell scratches). the position coordinates were marked on the bottom of the plate to facilitate the subsequent positioning for photographs. Cells were photographed at the same location 48 h. The scratch area was calculated to obtain the migration percentage.

Transwell invasion assay

Transwell filter inserts (8 µm pore size; Corning, New York, USA) were pre-coated with Matrigel at 37 °C for 30 min. Complete medium (500 uL) was added to the wells of the 24-well plate, and 200 uL of pure medium cell suspension was added to the chamber. After incubation for 24 h, cells were fix with 4% poly-

oxymethylene for 30 min, and then stained with 0.1% crystal violet for 30 min. Cells were imaged under an inverted fluorescence microscope with an image acquisition system (Nikon, Japan).

Cell apoptosis assay

After the cells were transfected, 10^6 cells were collected from each group. After washing once in PBS, the cells were stained with PE Annexin V Apoptosis Detection (BD Pharmingen™, New Jersey, USA) for 15 min at room temperature, and analyzed by flow cytometry (BD FACSCalibur, New Jersey, USA) to assess the proportion of apoptotic cells.

Western blot

After the cells were harvested, proteins were extracted with the protein extraction kit (Beyotime Biotechnology, Shanghai, China). The samples were separated by 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and then transferred to poly-vinylidene difluoride membranes (Millipore, Massachusetts, USA). The membrane was incubated overnight at 4 °C in diluted primary antibodies against E-cadherin (ProteinTech Group, Chicago, USA), N-cadherin (ProteinTech Group), Vimentin (ProteinTech Group, Chicago, USA). The membrane was then visualized using Quantum One imaging software (Bio-Rad, California, USA). GAPDH was used as an intranuclear control.

Statistical analysis

The data are expressed as mean \pm SEM. All statistical analyses were performed using GraphPad Prism 8.0 Software (La Jolla, CA, USA) and SPSS version 22.0 software (Abbott Laboratories, Chicago, IL, USA) through the two-sided Student's t-test or one-way analysis of variance (ANOVA). When $P < 0.05$, the difference was considered statistically significant.

Results

Using Oncomine database, GEPIA, and UALCAN to analyze the expression of BTG1

We obtained data from the BTG1 study in 453 different types of tumors through the Oncomine database. A total of 43 studies suggested statistical differences in BTG1 mRNA levels between tumors and normal tissues, of which 21 studies showed that BTG1 expression levels in tumors were significantly increased, and another 22 studies showed BTG1 expression levels in tumors significantly reduced. Compared with normal tissues, BTG1 expression was increased in brain and CNS cancer, cervical cancer, head and neck cancer, kidney cancer, and other cancers. BTG1 showed low expression in breast cancer, colorectal cancer, leukemia, lymphoma, ovarian cancer, and sarcoma. In addition, the expression of BTG1 in lung cancer was increased in one study and decreased in two studies (Figure 1A). Through GEPIA, we obtained the relative expression of BTG1 in each tumor and normal tissues (Figure 1B-C).

Next, we conducted a subgroup analysis of UCEC cases through UALCAN, which included 546 UCEC cases. The results showed that, compared with the normal control group, BTG1 mRNA expression was

low in primary endometrial cancer tissues ($P = 1.91589999420927E-09$; Figure 2A). The expression level of BTG1 in stage 4 endometrial carcinoma was significantly lower than that in stage 1, stage 2, stage 3 (stage1 vs stage 4, $P=3.029100E-04$; stage2 vs stage 4, $P =2.382500E-03$; stage 3 vs stage 4, $P=4.759200E-03$; Figure 2B). For patients of different weights, there was no difference between normal weight, extreme weight, and obese; however, BTG1 expression of extreme obese group is higher than extreme weight or obese (extreme weight vs extreme obese, $P=8.015500E -03$; obese vs extreme obese, $P=2.182600E-03$; Figure 2C). For BTG1 expression in different age groups, the expression level of BTG1 in patients between 61 and 80yearsold was lower than the expression level of BTG1 in patients between 41 and 60 years old (age (41-60 yrs)-vs-Age(61-80 yrs), $P=4.821900E-02$; Figure 2D). For the expression level of BTG1 in different histological subtypes, the expression level of BTG1 in endometrioid was higher than in serous and mixed serous and endometrioid subtypes, but there was no difference in expression between serous and mixed serous or endometrioid. (endometrioid vs serous, $P=1.76350000047343E-07$; endometrioid vs mixed serous and endometrioid, $P=1.958680E-03$; Figure E) Furthermore, the expression level of BTG1 in TP53-Mutant EC was significantly lower than that of TP53-NonMutant EC (TP53-Mutant-vs-TP53-NonMutant; $P=1.71269998183732E-09$; Figure F). There was no difference in the expression of BTG1 between patients of different races or menopause status (Figure 2G-2H).

High expression of BTG1 can improve EC prognosis

In the KM plotter online analysis tool, we set the follow-up time to 120 months. A total of 543 EC cases in the database met these criteria. The results showed that patients with high BTG1 EC had significantly higher OS than patients with low BTG1 EC (HR = 0.52, 0.32–0.85, logrank $P = 0.008$) (Figure 2I). At the same time, in UALCAN, patients with high BTG1 expression (136 patients) had a longer survival time ($P = 0.031$) compared with patients with low/moderate BTG1 expression (407 patients) (Figure 2J). However, survival analysis of patients with different menopause status, body weight, and race indicated that there was no difference in survival time (Figure 2K-M). These results show that patients of EC with high BTG1 expression have a better prognosis.

BTG1 mRNA expression in EC and relative clinicopathological analysis

qRT-PCR detection showed that compared with normal human endometrial tissue, BTG1 had low expression in human EC tissue ($P \leq 0.001$, Figure 3A). Clinicopathological analysis showed the expression of BTG1 was related to invasion depth ($P=0.031$) and FIGO stage ($P=0.012$). The relative invasion depth of BTG1 in EC with invasion depth $\geq 1/2$ showed less expression than EC with an invasion depth $< 1/2$ EC. BTG1 was relatively under-expressed in FIGO stage II, III and IV compared to FIGO stage I (Figure 3B-C). BTG1 and EC pathological tissue type ($P=0.450$), fertility history ($P=0.694$), lymphatic metastasis ($P=0.148$), menopause ($P=0.206$), estrogen receptor (ER) ($P=0.930$), progesterone receptor (PR) ($P=0.163$), and age of diagnosis ($P=0.227$) were not related (Figure 3D-J).

BTG1 expression affects the prognosis of EC

A total of 70 patients with EC were followed up until February 15, 2019. The longest and shortest survival times were 60 months and 1 month, respectively. A total of 13 of 70 patients with EC died. Univariate results indicated that the average survival time of the BTG1 high-expression group was 57.2 months, and the average survival time of the BTG1 low-expression group was 48.9 months. Low expression of BTG1 was significantly correlated with OS shortening ($P = 0.041$) (Figure 3K). These results are consistent with the results we obtained based on TCGA.

Functional enrichment of BTG1 in EC patients

Using the functional module in LinkedOmics, the mRNA-related genes of 176 UCEC patients in TCGA were analyzed. As shown in the volcano graph, there were 2805 genes that significantly positively correlated with BTG1 (dark red dots), and 1267 genes that significantly negatively correlated with BTG1 (dark green dots) ($FDR < 0.01$) (Figure 4A). The heat map showed that the top 50 gene sets were positively and negatively related to BTG1 (Figure 4B–C). We queried the functions of the top 50 gene sets positively and negatively related to BTG1. The results showed that BTG1 plays an important role in regulating embryonic development, tumorigenesis, apoptosis, and cell cycle. The statistical scatter plot of each gene showed the expression of BTG1 and SH3BGRL (Pearson-Correlation: 0.6438, $P=9.797e-14$), PCMTD1 (Pearson-Correlation: 0.6322, $P=3.642e-13$), and TET2 (Pearson-Correlation: 0.6207, $P=1.273e-12$) had a strong correlation (Figure 4D-F).

Next, we used Metascape for GO enrichment analysis to analyze the function of BTG1 and the top 200 related differentially expressed genes. The results showed that BTG1 and its related differentially expressed genes are mainly involved in the ING2 complex, protein-containing complex disassembly, translation, GTPase regulator activity, exchange factor activity, and cellular response to epidermal growth factor stimulus (Figure 5A-B, Table S3). The biological processes of BTG1 and its related genes included protein-containing complex disassembly, translation, cellular response to epidermal growth factor stimulus, and regulation of GTPase activity (Figure 5C-D, Table S4). The molecular functions of BTG1 and its related genes included GTPase regulator activity and guanyl-nucleotide exchange factor activity (Figure 5E-F, Table S5). In order to further analyze the relationship between BTG1 and UCEC, we conducted a PPI network analysis and analysis of important genetic components in MCODE. Research suggests that its biological function includes the following aspects: major pathway of rRNA processing in the nucleolus and cytosol, rRNA processing in the nucleus and cytosol, metabolism of RNA, mRNA splicing, major pathway, mRNA splicing, processing of capped intron-containing pre-mRNA, 55S ribosome, mitochondrial, mitochondrial translation elongation, mitochondrial translation termination, resolution of sister chromatid cohesion, separation of sister chromatids, mitotic prometaphase, ING2 complex, HDACs deacetylate histones, and chromatin organization (Figure 6A-B).

BTG1 miRNA target and interacting protein network in EC

In order to further study the functional targets of BTG1 in EC, we used miRDB to obtain 58 miRNAs that can bind to BTG1 (Table 1). Using the protein interaction network constructed by GeneMANIA (Figure 6C),

we obtained interacting proteins, including HOXB9, a transcription factor involved in cell proliferation and differentiation. We further revealed the mutual regulation of BTG1 and HOXB9 (Figure 6D).

BTG1 inhibits cell proliferation, migration, invasion, and promotes apoptosis in ECCs

In order to study the effect of BTG1 on the malignant biological behavior of ECCs, we first transfected Ishikawa cells and HEC-1A cells with a BTG1 overexpression plasmid, a BTG1 knockdown plasmid, or a corresponding negative control. The transfection efficiency was verified by qRT-PCR. A knockdown plasmid with the best knockdown effect (BTG1-RNAi (6008-2)) was selected for subsequent experiments (Figure S1). Through EDU experiments, we found that overexpression of BTG1 could inhibit the proliferation of Ishikawa cells and HEC-1A cells, while knockdown of BTG1 could promote the proliferation of Ishikawa cells and HEC-1A cells (Figure 7A). We obtained through a wound healing assay that overexpression of BTG1 inhibited the invasion of Ishikawa cells and HEC-1A cells. In contrast, knocking down BTG1 promoted the migration of Ishikawa cells and HEC-1A cells (Figure 7B). The transwell cell invasion assay showed that BTG1 overexpression inhibited the invasion of Ishikawa cells and HEC-1A cells. In contrast, knocking down BTG1 expression promoted the invasion of Ishikawa cells and HEC-1A cells (Figure 7C). Finally, we used flow cytometry to examine the effect of BTG1 knockdown and overexpression on apoptosis in Ishikawa cells and HEC-1A cells. The results showed that BTG1 overexpression promoted apoptosis and BTG1 knockdown suppressed apoptosis (Figure 7D).

BTG1 inhibits the EMT process in endometrial cancer cells

In order to explore the mechanism of BTG1 on the EMT process of ECCs, we detected the expression of E-cadherin, vimentin, and N-cadherin by western blot. Overexpression of BTG1 increased the expression of E-cadherin, while the expression of N-cadherin and vimentin decreased. Knocking down BTG1 reduced the expression of E-cadherin and increased the expression of N-cadherin and Vimentin (Figure 8A). Our results indicate that BTG1 can inhibit the EMT process in ECCs.

Discussion

EC is one of the three major malignant tumors of the female reproductive system ^[20]. The risk factors are related to excessive endometrial exposure to estrogen, age, obesity, hypertension, diabetes, and hereditary nonpolyposis colorectal cancer. At present, the main treatment is still total hysterectomy with bilateral salpingo-oophorectomy. Radiation and chemotherapy can also play a therapeutic role ^[21].

Abnormal expression of BTG1 in various tumors regulates their occurrence and development. The low expression of BTG1 in colon cancer is related to the clinicopathological characteristics, postoperative recurrence and survival of patients ^[22]. BTG1 shows low expression in gliomas, and has a tumor suppressing effect. Its anticancer effect is related to the inhibition of the Wnt/ β -catenin pathway ^[23]. BTG1 also shows low expression in hepatocellular carcinoma, and downregulation of BTG1 is significantly associated with disease-specific survival and relapse-free survival ^[24]. In gastric cancer,

BTG1 is expressed at low levels, and down-regulation of BTG1 leads to poor prognosis, especially in proximal non-diffused and diffuse gastric cancer^[25]. BTG1 also shows low expression in breast cancer, and overexpression of BTG1 has been combined with radiation therapy. By regulating the cell cycle and apoptosis-related signaling pathways, the therapeutic effect of breast cancer can be improved^[26].

We objectively analyzed the expression of BTG1 in EC and its impact on EC prognosis through multiple databases. In addition, we analyzed the expression of BTG1 in the function regulation network. Based on the results of 546 UCEC cases in TCGA, the expression level of BTG1 mRNA in primary EC tissue was significantly lower than that of the normal control group. The expression of BTG1 is related to FIGO stage, age, histological subtypes and TP-53. Then we used the KM Plotter online analysis tool and the UALCAN database to predict that patients with high BTG1 expression had a good prognosis. To further verify the expression of BTG1 in EC, we performed qRT-PCR experiments on 70 EC tissues and 30 normal endometrial tissues. We found that compared with normal human endometrial tissue, BTG1 has low expression in human EC tissue ($P \leq 0.001$, Fig. 3A). Clinicopathological analysis suggests that the expression of BTG1 is related to the depth of invasion and FIGO stage. Expression of BTG1 was lower in EC with an infiltration depth $\geq 1/2$ compared with EC with an infiltration depth $< 1/2$. Expression of BTG1 is low in FIGO stage II, III and IV compared with FIGO stage I EC. However, BTG1 expression and EC pathological tissue type, fertility history, lymphatic metastasis, menopause, ER, PR and age of diagnosis are not related. Then, we followed up with these 70 EC patients. The longest and shortest survival times were 60 months and 1 month, respectively, and 13 out of 70 patients with EC died. We concluded that the average survival time of the high BTG1 expression group was 57.2 months, and the average survival time of the low BTG1 expression group was 48.9 months. Low expression of BTG1 was significantly correlated with OS shortening. Moreover, these results were consistent with the results we obtained based on TCGA. Next, we analyzed the gene sets positively and negatively related to BTG1 through Pearson-Correlation, and found BTG1 plays an important role in regulating embryonic development, tumorigenesis, apoptosis, and cell cycle. In addition, we conducted GO analysis of BTG1 and its related genes, and found that BTG1 is related to protein-containing complex disassembly, translation, cellular response to epidermal growth factor stimulus, and regulation of GTPase activity. We also predicted a miRNA and protein interaction network that binds to BTG1. BTG1 and HOXB9 were found to be closely related. HOXB9 is a transcription factor of the HOX family, and plays an important role in embryo development and tumor progression^[27-30]. Studies have also shown that the expression of HOXB9 in EC is increased, and is related to histological grade and lymph node metastasis^[31].

BTG1 regulates the occurrence and development of various tumors. The low expression of BTG1 may be involved in the occurrence and development of pancreatic ductal adenocarcinoma^[32]. miR-27a-3p regulates the proliferation and apoptosis of colon cancer cells by potentially targeting BTG1^[33]. lncRNA DGCR5 can be used as a ceRNA for BTG1 to inhibit the progress of gastric cancer^[34]. However, there are no studies in EC. In order to study the effect of BTG1 on the malignant biological behavior of ECCs, we detected the effect on proliferation, migration, and invasions of BTG1 in Ishikawa cells and HEC-1A cells

through cell proliferation, wound healing, transwell invasion, and cell apoptosis assays. We found that BTG1 can inhibit ECCs proliferation, migration, and invasion, and promote ECCs apoptosis.

EMT is the process of obtaining mesenchymal features from epithelial cells. Most tumors are affected by EMT during tumor progression. It is generally believed that cancers derived from epithelia are determined by the EMT process^[35]. The effect of BTG1 on EMT in EC is unclear. Therefore, we further studied the effect of BTG1 on EMT, and found that overexpression of BTG1 can inhibit the EMT process. In contrast, knocking down BTG1 promoted the EMT process. But the regulatory mechanism of BTG1 affecting EMT still needs further study.

Conclusion

In summary, based on TCGA database, we utilized a variety of bioinformatics tools and databases to explore the expression and prognosis of BTG1. We used functional enrichment of BTG1 and related genes in EC patients through the bioinformatics website, and analysis of BTG1 miRNA targets and interacting protein networks. We believe that BTG1 plays an important role in the occurrence and development of EC. BTG1, as a tumor suppressor gene, shows low expression in EC, which can inhibit the malignant biological behavior and EMT process of ECCs. We believe that BTG1 can be used as a potential prognostic biomarker for EC.

Abbreviations

EC

Endometrial carcinoma

BTG1

B-cell translocation gene 1

TCGA

The Cancer Genome Atlas

ECCs

endometrial carcinoma cells

EMT

epithelial-to-mesenchymal transition

FIGO

The International Federation of Gynecology and Obstetrics

ER

estrogen receptor

PR

progesterone receptor

KM

Kaplan–Meier

ANOVA

one-way analysis of variance
qRT-PCR
quantitative real-time polymerase chain reaction

Declarations

Authors' contributions

YL and XM designed the study. YL drafted the manuscript. JH and JH performed cell culture. YZ contributed to the data interpretation. All authors participated in manuscript editing. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

This study was approved by the Institutional Review Committee (Ethical No.2017PS292K) of the Shengjing Hospital affiliated to China Medical University, and experimentation was conducted based on the approved guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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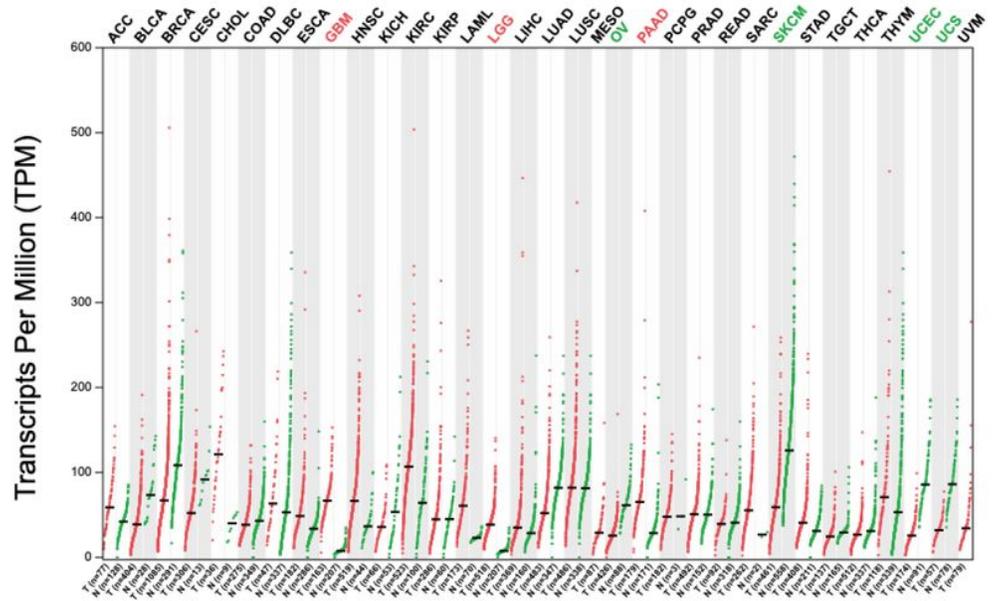
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Figures

A.

Analysis Type by Cancer	Cancer vs. Normal
Bladder Cancer	
Brain and CNS Cancer	9
Breast Cancer	2
Cervical Cancer	1
Colorectal Cancer	7
Esophageal Cancer	
Gastric Cancer	
Head and Neck Cancer	4
Kidney Cancer	5
Leukemia	1
Liver Cancer	
Lung Cancer	1
Lymphoma	8
Melanoma	
Myeloma	
Other Cancer	1
Ovarian Cancer	1
Pancreatic Cancer	
Prostate Cancer	
Sarcoma	1
Significant Unique Analyses	21
Total Unique Analyses	453

B.



C.

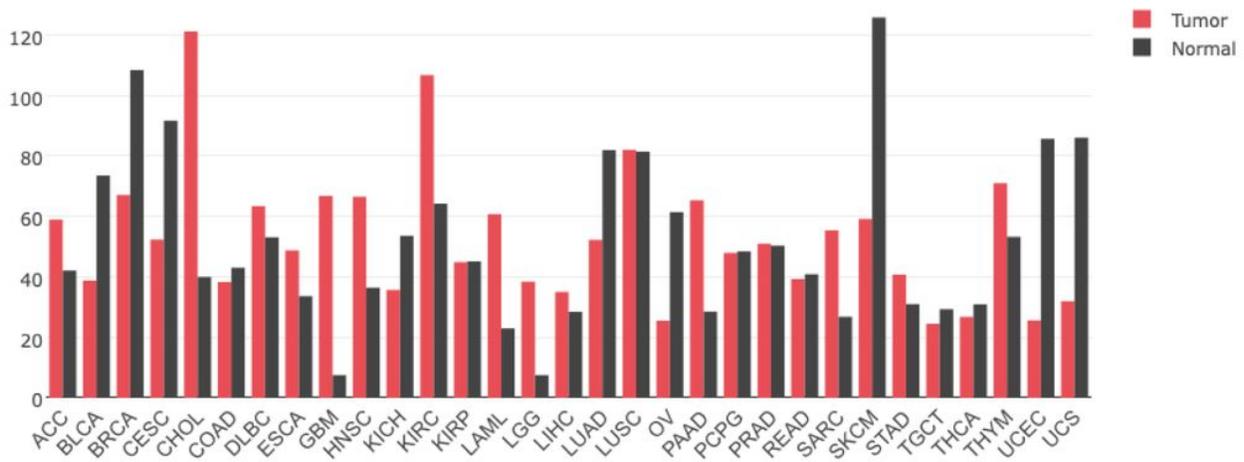


Figure 1

BTG1 expression in cancer and normal tissues. A. Differential expression of BTG1 in cancer and corresponding normal tissues in Oncomine. B. The gene expression profile across all tumor samples and paired normal tissues in GEPIA. (Dot plot) C. The gene expression profile across all tumor samples and paired normal tissues in GEPIA. (Bar plot)

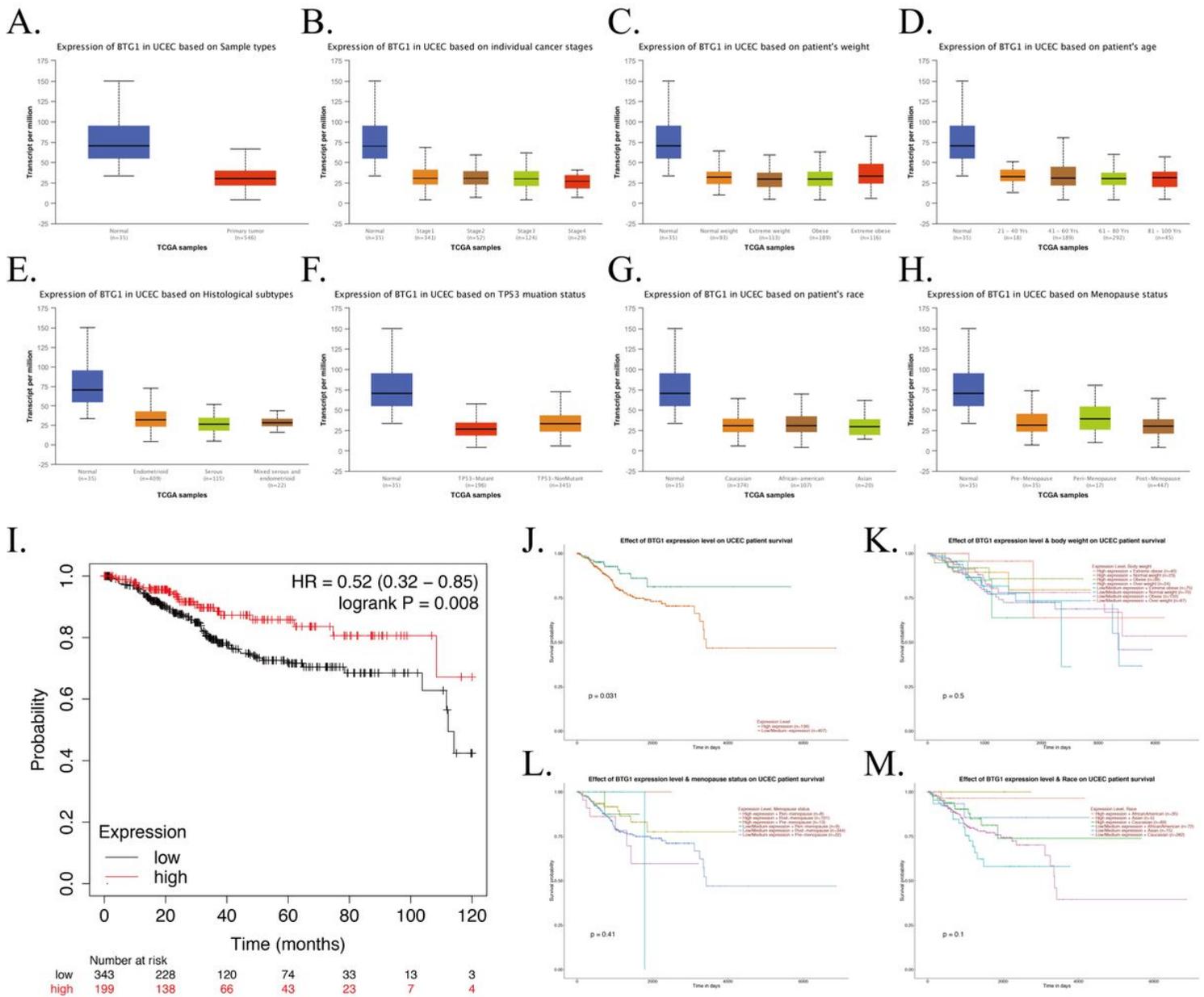


Figure 2

The relationship between BTG1 and various clinical pathological parameters and prognosis in EC. A. Expression of BTG1 in UCEC based on Sample types in UALCAN. B. Expression of BTG1 in UCEC based on individual cancer stages in UALCAN. C. Expression of BTG1 in UCEC based on patient's weight in UALCAN. D. Expression of BTG1 in UCEC based on patient's age in UALCAN. E. Expression of BTG1 in UCEC based on Histological subtypes in UALCAN. F. Expression of BTG1 in UCEC based on TP53 mutation status in UALCAN. G. Expression of BTG1 in UCEC based on patient's race in UALCAN. H. Expression of BTG1 in UCEC based on Menopause status in UALCAN. I. The effect of BTG1 on the prognosis of endometrial cancer in KM plotter online analysis. J. Effect of BTG1 expression level on UCEC patient survival in UALCAN. K. Effect of BTG1 expression level & body weight on UCEC patient survival in UALCAN. L. Effect of BTG1 expression level & menopause status on UCEC patient survival in UALCAN. M. Effect of BTG1 expression level & Race on UCEC patient survival.

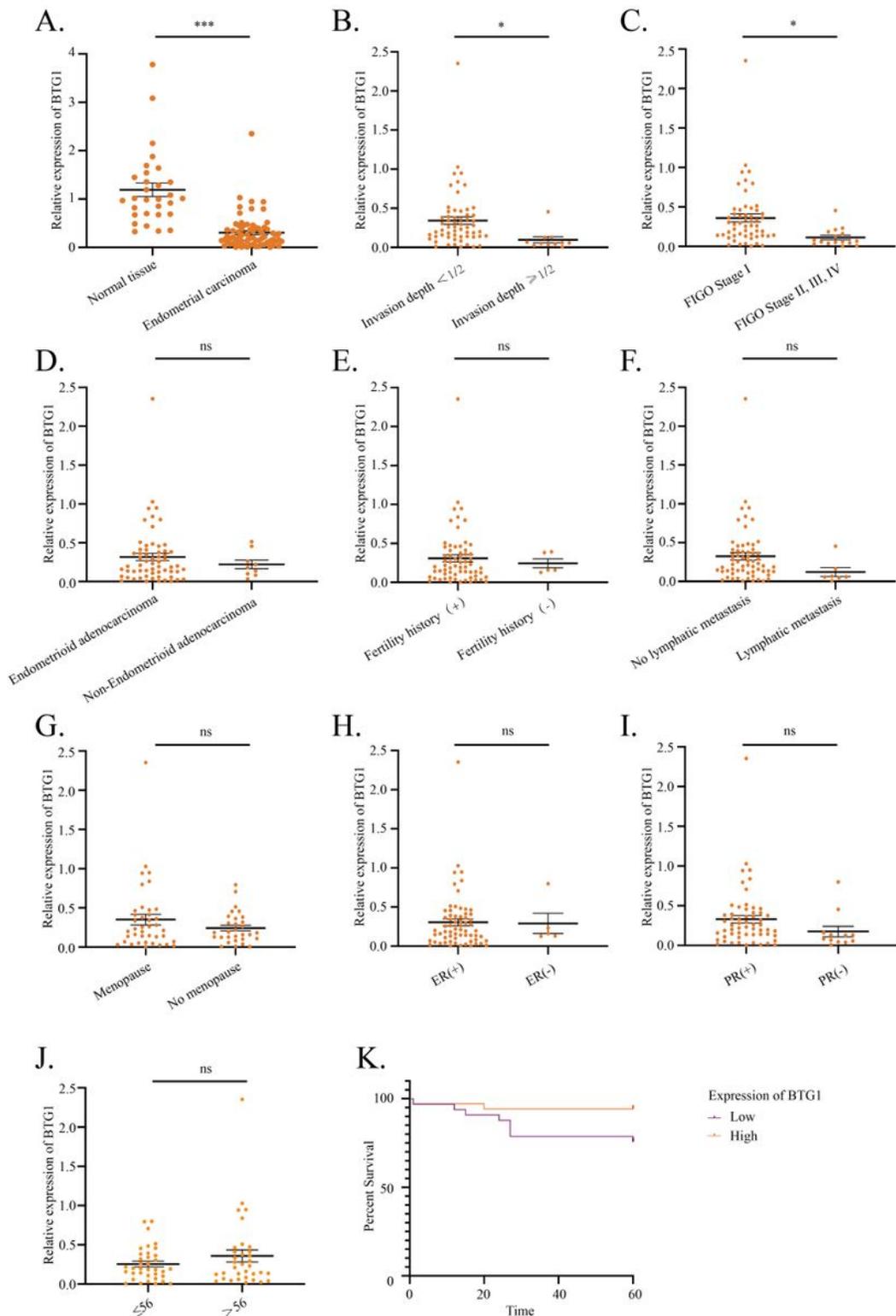


Figure 3

Low expression of BTG1 in EC was significantly correlated with OS shortening. A. qRT-PCR detects the expression of BTG1 in EC samples and normal tissue samples. B. Expression of BTG1 in EC based on invasion depth. C. Expression of BTG1 in EC based on FIGO stage. D. Expression of BTG1 in EC based on pathological tissue type. E. Expression of BTG1 in EC based on fertility history. F. Expression of BTG1 in EC based on lymphatic metastasis. G. Expression of BTG1 in EC based on menopause. H. Expression of

BTG1 in EC based on ER. I. Expression of BTG1 in EC based on PR. J. Expression of BTG1 in EC based on age. K. Effect of BTG1 expression level on EC patient survival. * P < 0.05 ** P < 0.01 *** P < 0.001

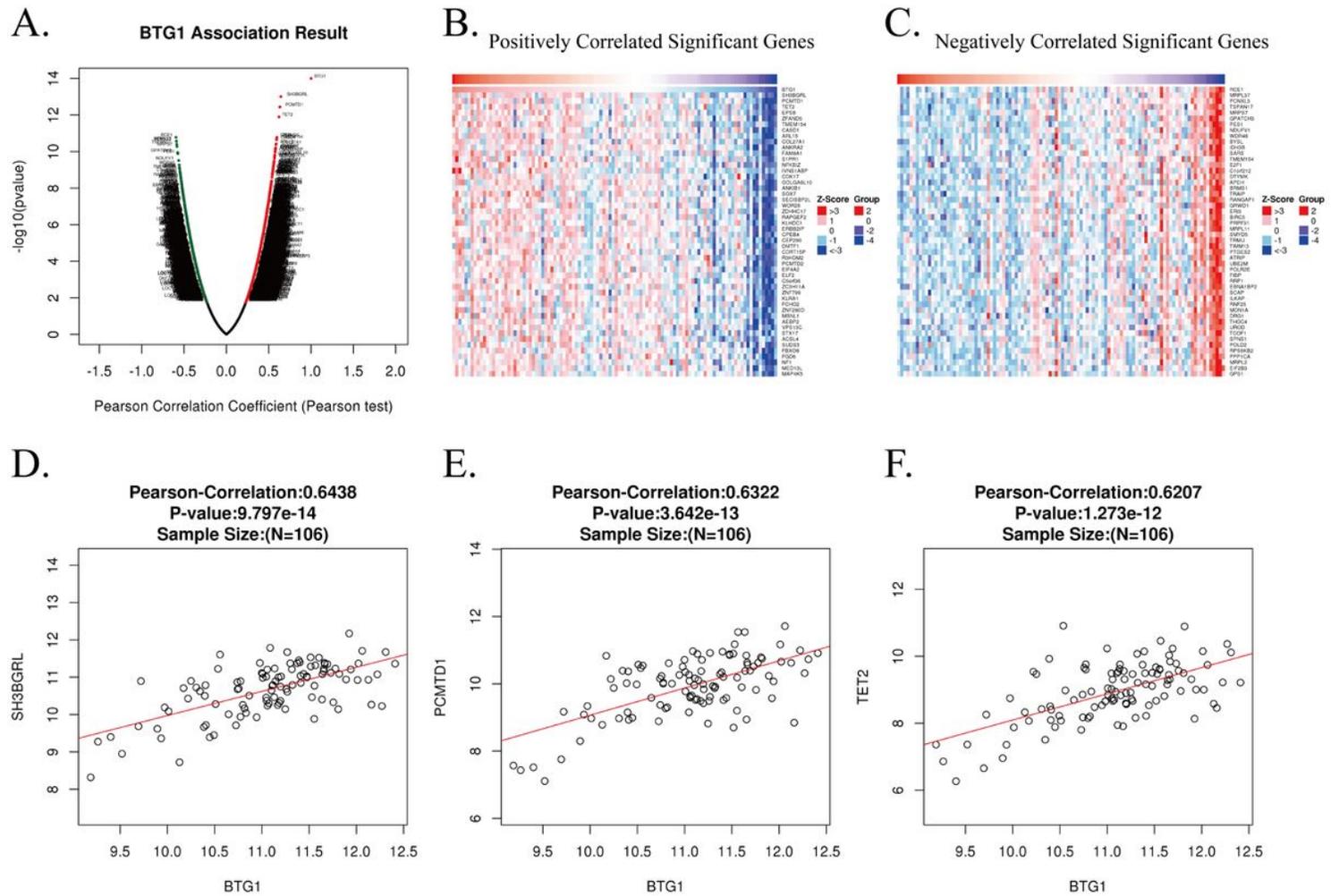


Figure 4

Genes related to BTG1 in LinkedOmics. A. BTG1 association results. (volcano graph) B. Positively correlated significant genes. (heat map) C. Negatively correlated significant genes. (heat map) D. Pearson-Correlation analyzes the correlation between BTG1 and SH3BGRL. E. Pearson-Correlation analyzes the correlation between BTG1 and PCMTD1. F. Pearson-Correlation analysis of the correlation between BTG1 and TET2.

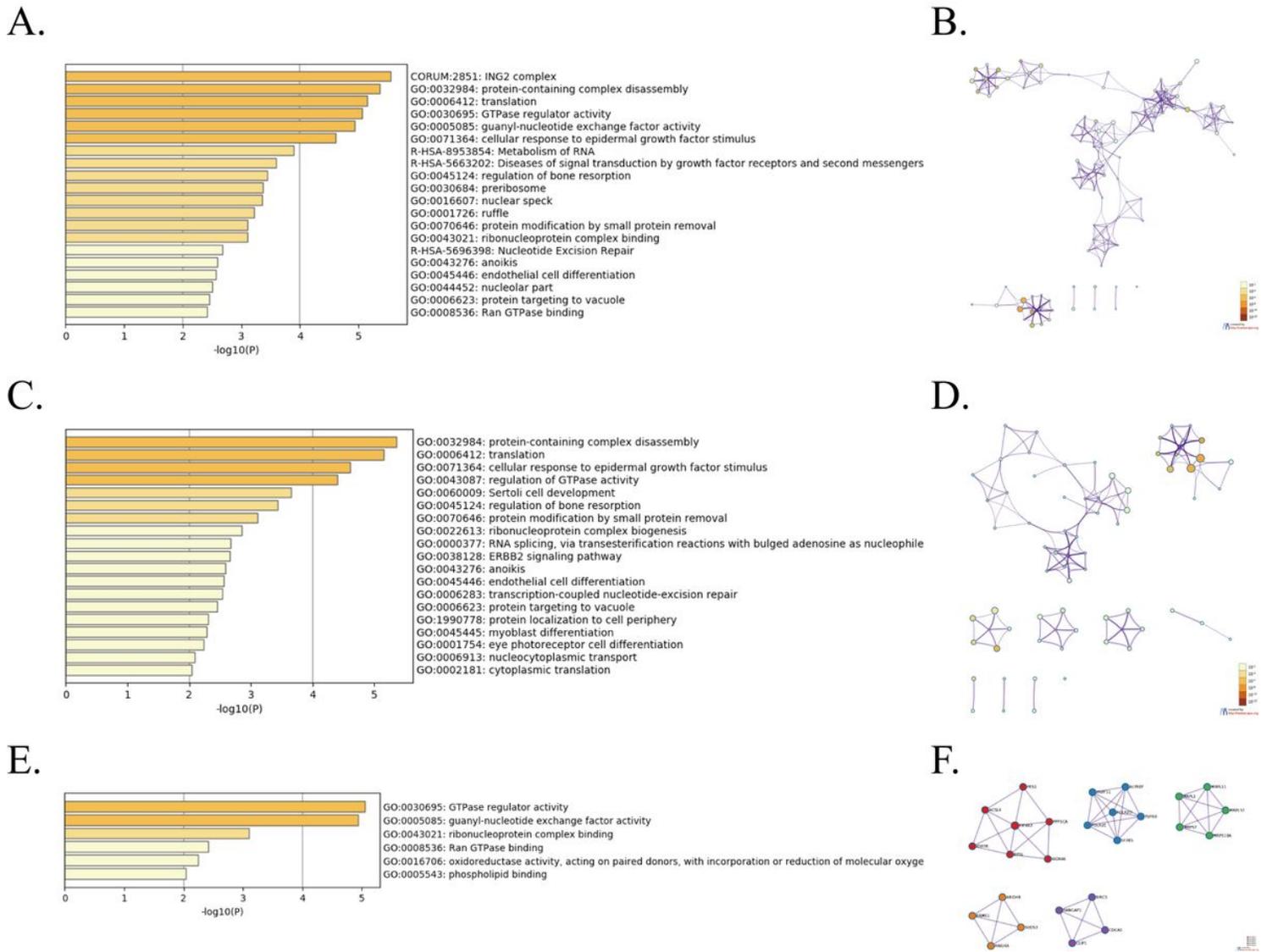


Figure 5

GO annotation of BTG1 related genes in EC. A-B. The cellular components of genes related to BTG1 are enriched through bar graphs and networks. C-D. Related biological processes related to BTG1 genes through bar graphs and networks. E-F. The molecular functions of genes related to BTG1 are enriched through bar graphs and networks.

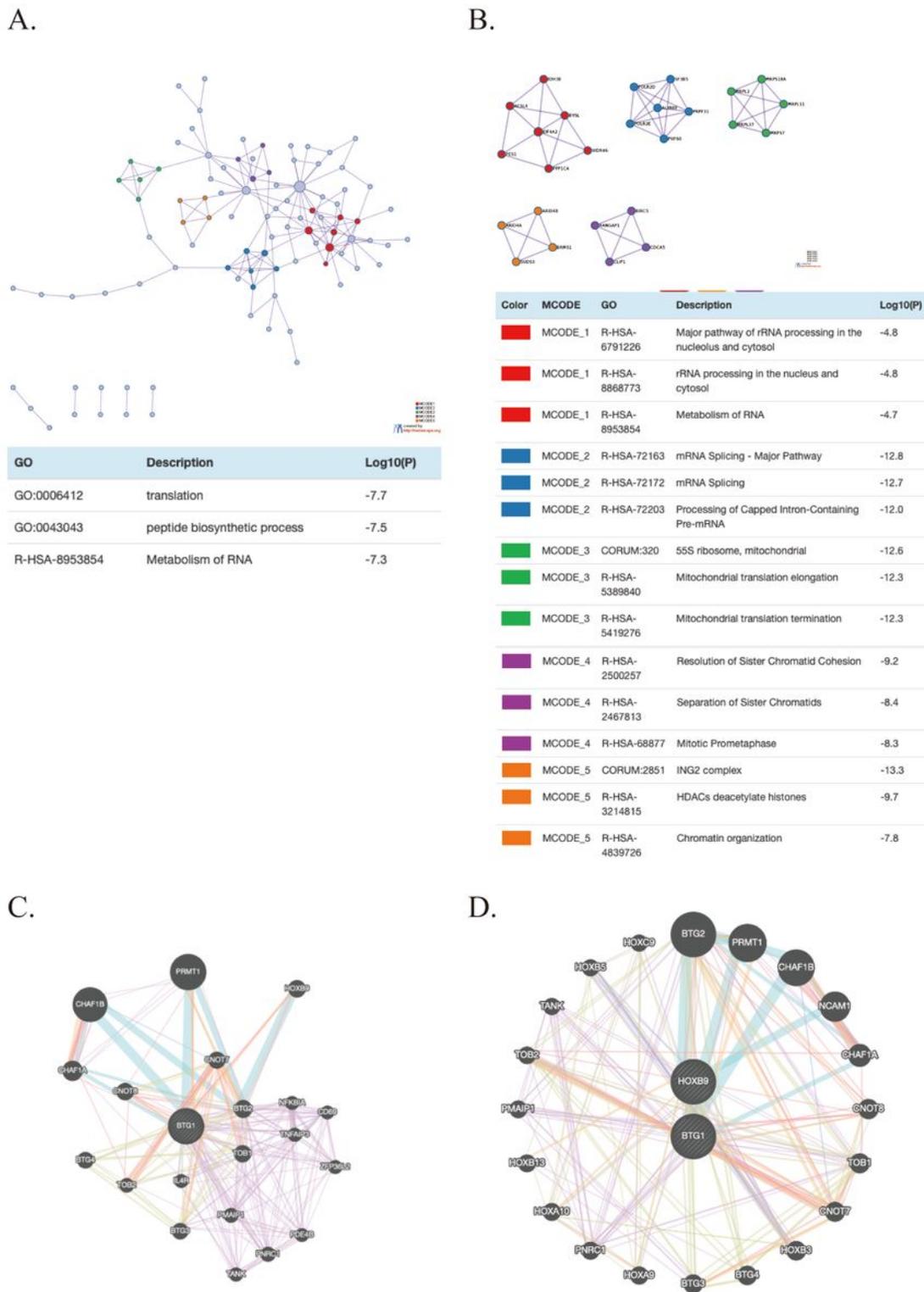


Figure 6

Protein-protein interaction (PPI) network. A. PPI network in Metascape. B. Independent feature richness analysis of five MCODE components in Metascape. C. The protein interaction network constructed by GeneMANIA. D. Mutual adjustment of BTG1 and HOXB9.

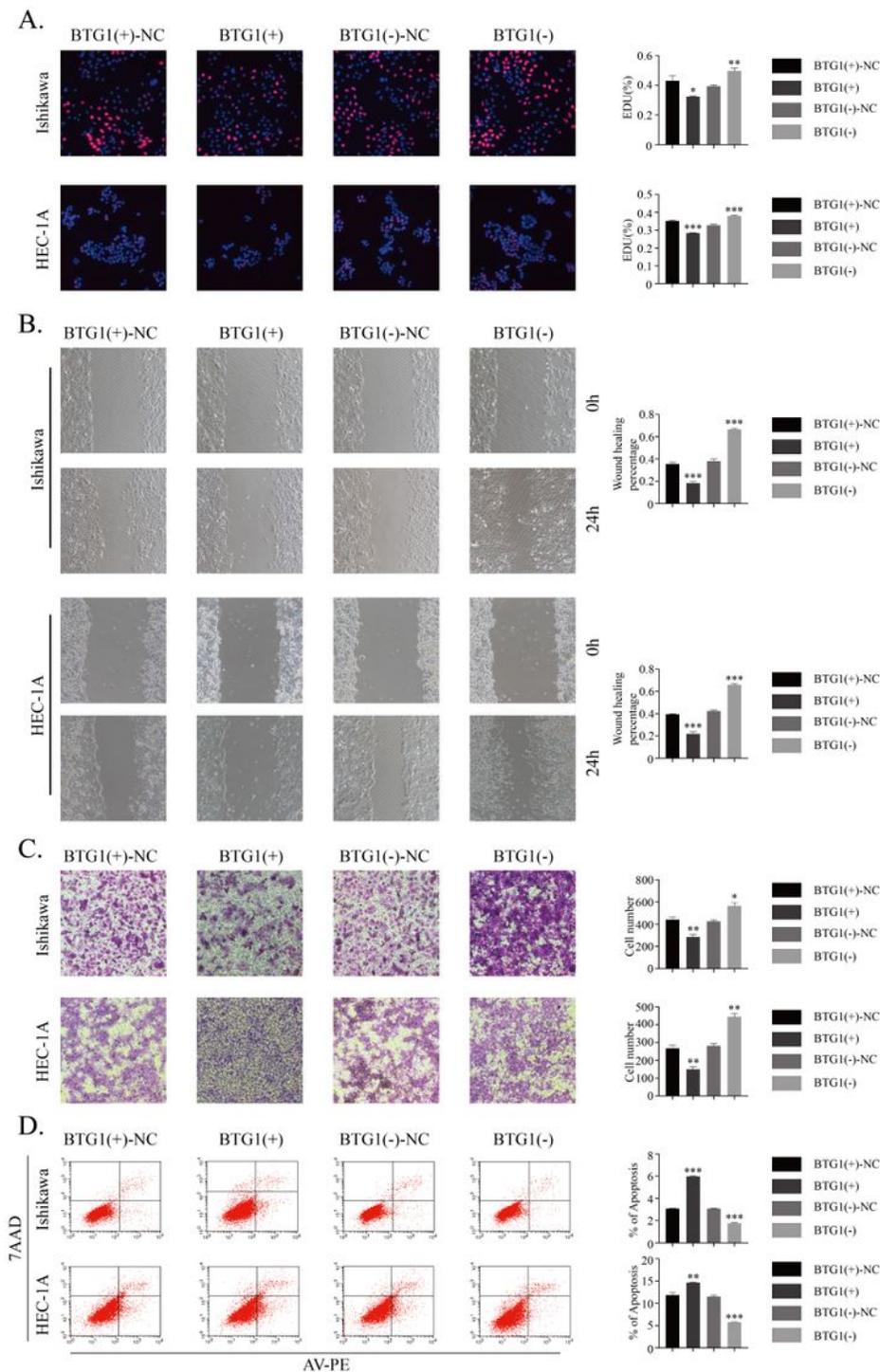


Figure 7

BTG1 inhibits the malignant biological behavior of ECC. A. The EDU test was used to determine the effect of BTG1 on the proliferation of Ishikawa and HEC-1A cell lines. B. Wound healing assay is used to determine the effect of BTG1 on the migration of Ishikawa and HEC-1A cell lines. C. Transwell invasion assay to determine the effect of BTG1 on the invasion ability of Ishikawa and HEC-1A cell lines. D. Cell

apoptosis assay was used to analyze the effect of BTG1 on the apoptosis of Ishikawa and HEC-1A cell lines. The data are expressed as mean \pm SEM (n = 3, each group). *P<0.05, **P<0.01 and ***P<0.001.

A.

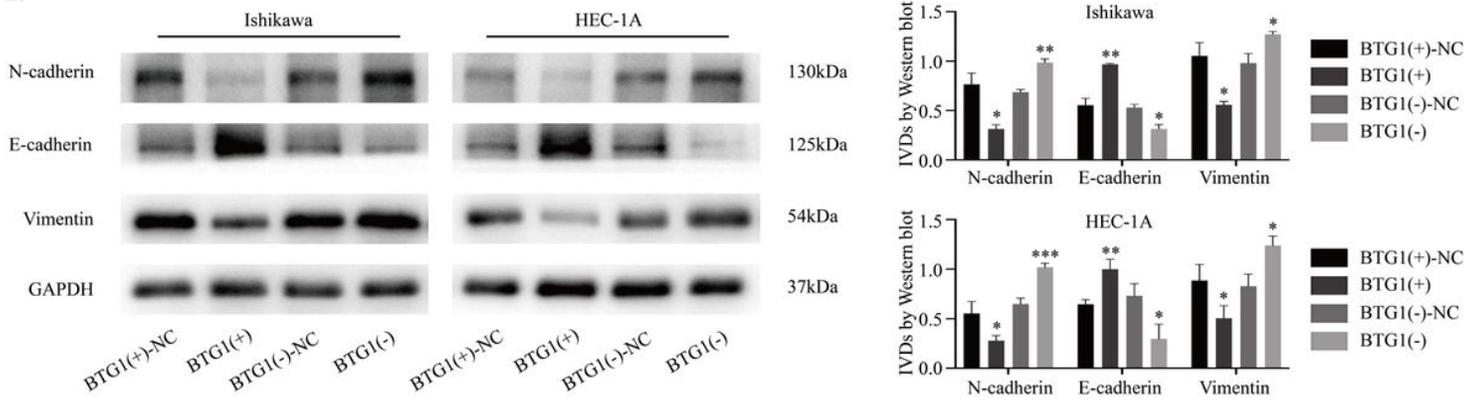


Figure 8

BTG1 suppresses the EMT process of ECC. A. The effect of BTG1 on EMT related indicators N-cadherin, E-cadherin and Vimentin. The data are expressed as mean \pm SEM (n = 3, each group). *P<0.05, **P<0.01 and ***P<0.001.

Supplementary Files

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