Attenuated APC contributes to FGF12 expression and EC progression with an integrated multi-omics analysis

Yunfeng Song  
Tongji University School of Medicine

Cheng Zhong  
Tongji University School of Medicine

Xiang Hu  
Tongji University School of Medicine

Jian Huang  
Tongji University School of Medicine

Yiding Bian  
Tongji University School of Medicine

Qizhi He  
Tongji University School of Medicine

Yiran Li  
liyiran2007@gmail.com  
Tongji University School of Medicine

Research Article

Keywords: endometrial cancer (EC), adenomatous polyposis coli (APC), chromatin remodeling, fibroblast growth factor 12 (FGF12), tumor migration

Posted Date: February 17th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2590191/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

Endometrial cancer (EC) is one of the most common gynecological cancer worldwide. The high-order chromatin structure plays an important role in gene expression regulation. In our previous research, the chromatin remodeling-related gene APC (adenomatous polyposis coli gene) is frequently mutated in endometrial cancer. In this study, we aimed to figure out the role of the APC gene in the chromatin remodeling of endometrial cancer and cancer progression.

Results

The level of APC expression decreased in EC and cell migration assays revealed that APC know-down KLE cells showed increased cell migration ability. An integrated multi-omics analysis, including RNA-sequencing (RNA-seq), assay for transposable accessible chromatin by high-throughput sequencing (ATAC-seq) and Hi-C, between the control cell and APC and knockdown KLE cell was performed and revealed that FGF12 was identified as a differentially expressed gene (DEG) in the switched compartments, cell-specific boundaries, and loops by comparing hierarchical structures and highly expressed in APC knockdown KLE cells. Moreover, high expression of FGF12 indicated a poor prognosis.

Conclusions

APC expression decreased in EC tissues and loss of APC in EC promotes cell migration. Moreover, loss of APC gene expression may reprogram the chromatin architecture to increase FGF12 gene expression, activate tumorigenesis-rated AKT and MAPK (Erk1/2) signaling, and promote endometrial cancer progression. In addition, a high level of FGF12 expression indicated a poor prognosis in EC patients, which provides a novel therapeutic target for EC with low expression level APC.

Background

Endometrial cancer (EC) is one of the most common gynecological cancer worldwide (1), with incidence increasing at a rate of 3.7% per year in China. Although early diagnosis, surgery, and chemotherapy have reduced the mortality rate of endometrial cancer, the mortality rate of endomembrane cancer in China was still high, at a rate of 21.8% in 2015 (2). Endometrial cancer has become a major disease that seriously threatens women's lives and reproductive health. The low-grade, non-ultra mutated tumors usually have a worse prognosis than other subtypes of endometrial cancer. High-order chromatin structure plays important role in gene expression regulation. In our previous research, the mutations in chromatin remodeling-related genes (APC, KMT2A, JAK2, et al) and in DNA-repair-related genes are frequent in this subtype of endometrial cancer (3). While the mechanism by that APC regulates gene expression and promotes cancer progression via chromatin remodeling in EC was still unknown.
Chromatin remodeling is an important part of epigenetic changes, and damages to chromatin remodeling mechanisms lead to the accumulation of epigenetic abnormalities, which ultimately leads to the occurrence and progression of cancer (4). Chromosomes have distinct territories in the cell nucleus (5). Chromatin was divided into two different parts, compartments A and B, according to the analysis of chromatin interactions. Genes expression was active in compartment A, which localized toward the nuclear interior. In contrast, compartment B is localized toward the nuclear periphery, has a compact conformation, and usually shows inactive gene expression (6). Topologically associating domains (TADs) are domains of preferential internal chromatin interactions (7). In addition, genes located at the TADs boundaries might be related to tumorigenesis owing to genomic structure alternations. Moreover, chromatin loops form the basis of TADs and regulate gene expression. The alternations of chromatin compartments switch, TADs boundary and loops were associated with developmental diseases and cancers (8–10). In cancers, abnormal activation of cellular signaling pathways transmits unstable signals to the nucleus, which ultimately alters the cell's chromatin organization and transcriptional output. How chromatin and its organizers respond to abnormal carcinogenic signals in cancer cells remains to be studied (6).

APC (adenomatous polyposis coli), localized on chromosome 5q21, is a tumor suppressor gene associated with chromatin remodeling (3, 11). APC protein has multiple functional regions, and the oligomeric region retains at least 171 amino acids for protein binding and exerts dominant inhibition (12). The armadillo repeat region is the most conserved region, binding to IQGAP1, PP2A, Asef, and KAP3 to stimulate cell migration and adhesion (13). The 15- or 20- residue repeat region and SAMP repeat region of APC negatively regulate the Wnt pathway by the degradation of β-catenin (14). Although APC is generally considered to be one of the destruction complexes involved in the regulation of β-catenin degradation in the cytoplasm, Popow et al. found that there were more than 150 undiscovered APC interaction proteins after mass spectrometry analysis of APC protein co-precipitation, and about half of them were not related to the β-catenin pathway (15). Loss of APC leads to spindle dysfunction in mitosis, inducing genomic and chromosomal instability (16). Deka et al. discovered that APC protein could bind directly to A/T-enriched DNA sequences and revealed that APC proteins can affect genome modification by regulating DNA structure or regulating the binding of proteins on DNA (17). In addition, The basal region and C-terminal region bind to microtubules, directly or indirectly interact with EB1, stabilize microtubules and centromeres, and promote chromosome aggregation (18). Therefore, we aimed to research the role of APC in EC chromatin remodeling.

In this study, we revealed that low expression of APC promotes EC cell migration. To investigate the role of APC in EC, we perform an integrated multi-omics analysis, including RNA-sequencing (RNA-seq), assay for transposable accessible chromatin by high-throughput sequencing (ATAC-seq) and Hi-C, between the control cell and APC and knockdown KLE cell. Our findings revealed that FGF12 was identified as a differentially expressed gene (DEG) by comparing hierarchical structures and highly expressed in APC knockdown KLE cells. Moreover, high expression of FGF12 indicated poor prognosis in EC patients.
Results

**APC expression was attenuated in EC, which inhibited EC cell migration.**

We analyzed the role of APC in endometrial cancer patients with the TCGA database using UALCAN (http://ualcan.path.uab.edu). The expression of APC decreased in EC, contrasted with normal tissue (Fig. 1A). In addition, the clinical EC tissue samples showed decreased APC expression levels in cancer tissue, compared with cancer adjacent tissue using Western blot (n = 4 per group) (Fig. 1B). Next, the expression levels of APC in EC cell lines were measured using western blot and RT-qPCR and KLE cell line showed a higher APC expression level among 7 EC cell lines (Fig. 1C and D). To research the function of the APC gene in EC, we constructed APC knockdown KLE cell line. Western blot and RT-qPCR confirmed that APC was downregulated in KLE (Fig. 1E and F). Next, we researched the effects of APC on cell proliferation and migration of KLE. Cell counting kit-8 assay showed that there was no difference in proliferation between NC and APC knockdown KLE cells (Fig. 1G). While knockdown of APC in EC cell line (KLE) presented increased migration ability compared to EC cells with empty vector (Fig. 1H).

**The DEGs between the control cells and APC knockdown KLE cells.**

RNA-seq was used to compare the transcriptomes of control cells and APC knockdown KLE cells. Compared with the control cells, the heatmap and volcano plot revealed that 3154 upregulated DEGs and 3653 downregulated DEGs were identified in APC knockdown KLE cells, using change fold FC > 1.2 or FC < 10/12 and P-value < 0.01 as criteria for the screening of upregulated or downregulated genes (Fig. 2A and B). The gene ontology (GO) analysis showed that DEGs were related to intracellular organelle and intracellular anatomical structure (Fig. 2C). Additionally, the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis found TNF, mTOR and MAPK signaling pathways were enriched in APC knockdown KLE cells (Fig. 2D). Compared with the control cells, we revealed 377 increased DARs and 1579 decreased DARs in APC knockdown KLE cells. Besides, the DEGs in DARs were identified and presented in the volcano plot (Fig. 2E). The GO analysis revealed that DEGs were associated with protein serine/threonine kinase activity cell and cell communication (Fig. 2F). Additionally, the KEGG enrichment analysis found ECM receptor and p53 signaling pathways were enriched in APC knockdown KLE cells (Fig. 2G).

**The chromosomal structure variations between the control cells and APC knockdown KLE cells.**

To Compared with the control cells, 8 obvious structural variations regions (labeled with black boxes) of inter-chromosomal interactions were identified in APC knockdown KLE cells, indicating chromosomal translocation in endometrial cancer progression, such as chr3 and chr8 (Fig. 3A). For example, 3 obvious interaction regions R6 (between Chr3 R2 and Chr8 R5), R7 (between Chr3 R1 and Chr8 R4)and R8 (between Chr3 R1 and Chr8 R3) were observed in Hi-C maps. The interaction signal (B1) between position A1 (chr8:102270000) and A2 (chr8:121550000) suggested a large inversion region in Chr8 exactly close neighbor to the translocation region (Fig. 3B). In addition, another intra-chromosomal interaction signal (B2, CNV region) in chr8 (111550000-118550000bp, Fig. 3C and D) and an intra-chromosomal interaction...
signal in chr4 were also found (Fig. 3E). In the CNV region, the volcano plot revealed that 356 upregulated DEGs and 667 downregulated DEGs have been identified in APC knockdown KLE cells (Fig. 3F), including some tumorigenesis-associated genes, such as FGF12, VEGFA and MAPK11. The GO analysis revealed that genes in the CNV region were related to cell surface receptor signaling and cell migration (Fig. 3G). TNF, ECM-receptor interaction and VEGF signaling pathways were enriched in APC knockdown KLE cells by the KEGG analysis (Fig. 3H).

The variations of the compartment, TAD boundary, and loop between the control cells and APC knockdown KLE cells

During EC progression, dynamic switches occur in chromatin compartments. Compared to the control cells, switched compartments were found in every chromosome in APC knockdown KLE cells (Fig. 4A). We identified 56 DEGs with loci transformed from compartment A to B (22 downregulated and 34 upregulated) and 79 DEGs with loci switched from compartment B to A (54 downregulated and 25 upregulated; Fig. 4B). The GO and KEGG analysis revealed that they were associated with the cytoskeleton, phenylalanine, tyrosine and tryptophan biosynthesis, and nucleotide excision repair in cancer (Supplementary Fig. 1A and B). Compartments are composed of topologically associating domains (TADs), which are self-interacting regions and remain stable under different physiological conditions and cell types. The split or newly formed TAD boundaries might induce interactions between enhancers and promoters to alter gene expression. TAD boundaries presented higher GC content, and more chromosome-enriched open regions and genes than TADs interiors in KLE cells (Supplementary Fig. 1C and D). Compared to the control cells, we identified 790 TAD merges, 288 TAD splits, and 243 TAD rearrangements in APC knockdown KLE cells.

Next, the volcano plot showed 150 downregulated and 63 upregulated DEGs located in the APC knockdown KLE cell-specific boundaries, including FGF12, RAB3B and CA9 (Fig. 4E). The GO and KEGG analysis presented that these DEGs were associated with cell-cell adhesion plasma-membrane adhesion molecules, Hippo signaling, VEGF signaling and ECM receptor interaction (Supplementary Fig. 1E and F). Enhancers activate promoters via chromatin loops to regulate gene expression. We detected 2577 and 327 loops for the control cells and APC knockdown KLE cells. Loops related to enhancers upregulate gene expression. We identified 522 and 678 promoter-enhancer loops in the control cells and APC knockdown KLE cells, respectively (Fig. 4F). Besides, we revealed more promoter loops and enhancer loops in APC knockdown KLE cells than in the control cells (Fig. 4F). Moreover, we identified 446 control cell-specific loops and 602 APC knockdown KLE cells-specific loops, respectively (Fig. 4G). In control cells-specific loops, 50 APC knockdown KLE cells-upregulated genes and 74 APC knockdown KLE-downregulated genes were found, along with 49 APC knockdown KLE cells-upregulated genes and 34 APC knockdown KLE cells-downregulated genes in APC knockdown KLE cells-specific loops, such as FGF12, DIRC3, and ERBB4 (Fig. 4H). The GO and KEGG analysis indicated that these DEGs were associated with cell surface receptor, VEGF, ECM-receptor interaction and p53 signaling (Supplementary Fig. 1G and H).
APC knockdown in KLE cells upregulated FGF12 expression via rearranging the chromosomal structure, which predicts a worse outcome in EC.

To explore the potential downstream genes of APC, we identified the overlapped DEGs resided in the switched compartments, TADs, and loops in the control cells and APC knockdown KLE cells. 12 overlapped DEGs were identified, including FGF12, SLIT2, CDH6, SLIT3, SUGCT, CPED1, KCNU1, ZNF365, RAD52, GPC5, MX1 and SRPX (Fig. 4I). We found most significant changes in TADs and loops near FGF12 among 12 overlapped DEGs (Fig. 5A and Supplementary Fig. 2). In addition, FGF12 was highly expressed in APC knockdown KLE cells (Fig. 5B, C and D). P-AKT and P-ERK1/2 have been activated in APC knockdown KLE cells (Fig. 5C and D). Moreover, a high expression level of FGF12 predicts a worse prognosis in EC patients ((p = 0.0011, Fig. 5E).

Discussion

Endometrial cancer is one of the major diseases that seriously influence women's lives and reproductive health. Chromatin remodeling plays an important role in gene expression alternation and cancer progression. Multiple recent studies have identified an increased frequency of gene alternations associated with chromatin remodeling in malignancies. In our previous research, chromatin remodeling-related genes, including APC, are frequently altered in this subtype of endometrial cancer and the APC mutation rate was high (18%) in 99 endometrial cancer samples (3). A previous study found the methylation of the APC gene was involved in the occurrence of endometrial cancer (19). Recent studies have demonstrated that APC deficiency induces endometrial hyperplasia and endometrial carcinogenesis by preventing estrogen signaling in the endometrial epithelium (20). In this study, we observed that APC was downregulated in EC cancer tissues and knockdown of APC in KLE cells promotes cell migration. While there is no difference in cell proliferation between control cells and APC knockdown KLE cells. In this comparative integrated multi-omics analysis, RNA-sequence revealed that DEGs between control cells and APC knockdown KLE cells were associated with intracellular organelle and intracellular anatomical structure, TNF, mTOR and MAPK signaling pathways, which were associated with tumorigenesis. In addition, the DEGs in DARs were involved in protein serine/threonine kinase activity cell, cell communication ECM receptor and p53 signaling pathways were enriched in APC knockdown KLE cells. Moreover, we identified obvious structural variations in 8 regions of APC knockdown KLE cells and found significant associations with cell surface receptor signaling, cell migration, TNF, ECM-receptor interaction and VEGF signaling pathways, influencing angiogenesis and tumorigenesis. Additionally, we identified that APC alternated compartment switches, TADs rearrangements and loop changes, and influenced FGF12 gene expression.

FGR12, fibroblast growth factor 12, is a member of the fibroblast growth factor (FGF) family. Members of the FGF family are associated with a wide range of mitotic and cell survival activities and are involved in a variety of tumorigenesis and progression processes including tumor growth, migration and invasion. The FGF family members were divided into three major groups: canonical FGFs (FGF1, FGF4, FGF7, FGF8 and FGF9), endocrine FGFs (FGF19, et al), fibroblast growth factor homologous factors (FHFs, FGF12,
FGF13 and FGF14) and intracellular FGFs, including the FGF11 subfamily (21, 22). FGF12 lacks the N-terminal signal sequence, but it contains clusters of basic residues that have been shown to function as nuclear localization signals. FHFs including FGF12 were reported to be exclusively intracellular proteins whose function is independent of cell surface FGFRs (23). FGF12 is highly expressed in multiple cancers, including gastric cancer, esophageal cancer, colorectal cancer and bladder cancer (24, 25). In colorectal cancer, FGF12 promotes CRCs cell migration, invasion, and stemness by activating the PI3K/AKT signaling pathway (24). In this study, FGF12 was highly expressed in APC knockdown KLE cells with increased cell migration ability and activation of P-AKT and P-ERK1/2. Moreover, a high level of FGF12 indicated a poor prognosis in EC patients.

**Conclusion**

In conclusion, the expression level of APC decreased in EC and promotes EC cell migration. A comparative integrated multi-omics analysis of gene expression, open chromatin region, and chromatin organization generated for the control cell and APC knockdown KLE cell indicated the association between APC-related chromatin remodeling and EC tumorigenesis but also identified a novel potential therapeutic target gene FGF12, altered by APC-related chromatin remodeling. In addition, the high expression level of FGF12 indicated poor prognosis in EC patients, which provides a novel potential strategy for the therapy of EC with APC loss.

**Methods**

**Gene expression and survival overall analysis with The Cancer Atlas (TCGA) database**

The UALCAN (http://ualcan.path.uab.edu) (26) was applied to analyze APC expression and Kaplan-Meier survival curve analysis of FGF12 in the TCGA Uterine Corpus Endometrial Cancer database, which includes 546 EC and 35 normal tissue specimens.

**Western Blotting**

Tissue protein and cell lines protein samples were measured using western blotting according to previous research (3). Antibodies for western blotting include rabbit anti-APC (1:1000; Abcam, 40778), AKT (1:1000; Cell Signaling Technology, 9272), p-AKT(1:1000; Cell Signaling Technology, 8696), ERK1/2 (1:1000; Cell Signaling Technology, 4695), p-ERK1/2 (1:1000; Cell Signaling Technology, 8696), FGF12 (1:1000; Affinity, DF2493) and anti-β-Actin (1:1000; Cell Signaling Technology, 4670) antibodies.

**RNA isolation and quantitative RT–PCR**
Fresh 4 EC and paired 4 tumor-adjacent tissues were acquired from female patients admitted at the Shanghai first maternity and infant hospital (Shanghai, China) from 2020 to 2021. Total RNA was extracted and measured according to previous research (3). Reverse transcription reactions and quantitative RT-PCR of mRNA were performed according to the manufacturer’s protocol (TaKaRa). Expression data of mRNA were normalized to β-actin or GAPDH using the 2^{-ΔΔCT} method. The primer of β-actin, GAPDH, APC and FGF12 was purchased from RiboBio Company (Guangzhou, China).

**Cell Culture**

The human endometrial cancer cell line KLE was purchased from the Chinese Academy of Sciences. and was cultured in Dulbecco's modified Eagle's medium (DMEM) /F12 medium (Hyclone), supplemented with 10% FBS and 1% PenStrep (100 U/mL penicillin and 100 µg/mL streptomycin; Gibco) at 37°C, 5% CO2.

**APC knockdown KLE cell line generation**

The shRNAs lentivirus targeting APC were purchased from Genechem Company (Shanghai, China). KLE cells were infected with lentivirus over 48 h. Polybrene was applied to screen out the stable cell line.

**Cell Proliferation And Migration**

KLE cells were seeded in 96-well plates (3*10^3 cells, per well) for 24 h, 48 h and 96 h. Cell numbers were determined using cell counting kit-8 with a microplate spectrophotometer (Thermo Fisher Scientific) at 450 nm. For migration, KLE (5*10^4 cells) cells were seeded to the upper chambers. After incubation for 16 h, each chamber was stained with AM (calcein-acetoxymethylester, calcein-AM, Thermo Fisher Scientific). The migrated cells were imaged by fluorescence analysis (Nikon, Tokyo, Japan).

**Integrated Multi-omics Analysis**

The integrated multi-omics analysis included RNA-seq, ATAC-seq, and Hi-C data analysis, which were performed by the Frasergen Company (Shanghai, China). The analysis methods refer to the previous research (27). The RNA-seq experiments were committed with three biological replicates. DEGs were identified using the edgeR (v.3.23.1) algorithm, with criteria of false discovery rate (FDR) < 0.01 and |log fold-change (logFC)| > 1.

ATAC-seq analysis was performed according to the research published (28). In brief, cell samples were collected and lysed on a rotary stirrer for 10 min at 4°C. The cell suspension is filtered with a 40um cell strainer and then washed once with cold RSB buffer. Approximately 50,000 nuclei are added to the transposable reaction for labeling. DNA for Tn5 transposability is purified by AMPure DNA beads and PCR amplification. The final qualified library is sequenced on the Illumina Novo-seq platform (San Diego,
CA, United States) in PE150 mode. The differentially accessible regions (DARs) were performed by DiffBind (v2.10.0) with criteria of FDR < 0.01 and |log fold-change (logFC)| > 1.

The Hi-C analysis was committed, referring to the previous research methods (29). In brief, samples are crosslinked with 1% concentration formaldehyde for 10 min at room temperature, followed by quenching with 0.125M final concentration glycine for 5 min. Next, lyse the crosslinked cells. Endogenous nucleases were inactivated with 0.3% SDS and chromatin DNA was cleaved with 100 U MboI (NEB), labeled with biotin-14-dCTP (Invitrogen), followed by ligation with 50 U T4 DNA ligase (NEB). After uncrosslinked, we extract the ligated DNA via the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. Next, purified DNA is cut into fragments 300 to 500 bp in size and further flat-end repair, flat-end phosphorylation, and linker ligation, followed by biotin-streptavidin reaction pull-down DNA fragments and PCR amplification for purification. Finally, the Hi-C library was quantified and sequenced on the Illumina Nova-seq platform (San Diego, CA, USA). The copy number variations (CNV) were verified by using HiNT (https://github.com/parklab/HiNT). The CscoreTool (v 1.1) was applied to identify A and B compartments on 100kb resolution. An insulation score algorithm (arguments: --is100000 --ids 40000) was applied to identify the locations of TAD boundaries of each sample and to determine the location and numbers of TADs (30). The chromatin loops analysis was according to the previous study (27).

**Statistical Analysis**

All data are presented as the means ± SEMs. F-test and Brown-Forsythe test were applied for homogeneity of variance. the Mann-Whitney U test, Student's t-test, Kruskal-Wallis H test, and one-way ANOVA were conducted with GraphPad Prism (v.8.1.2) software and SPSS (v.26.1.2). Statistical significance was considered at P < 0.05 and indicated in the figures by *P < 0.05, **P < 0.01 and ***P < 0.001, ****P < 0.0001.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Clinical Research Ethics Committee of the College of Medicine, Tongji University. Written informed consent was received from participants before inclusion in the study.

**Consent for publication**

The authors confirm that we have obtained written consent from the patients to publish this manuscript.

**Availability of data and materials**

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

**Competing interests**
The authors have declared that no conflict of interest exists.

**Funding**

The study was supported by the National Natural Science Foundation of China (grant numbers 32270952, 32070583, 81472427, 81672574, 81702547, 82002723, and 82172975), the Shanghai Health System Outstanding Talents Program (grant number 2018YQ23), the Shanghai New Frontier Technology Project (grant number SHDC12015110), the Shanghai Rising-Star Program (grant number 22QC1400700), the Shanghai Sailing Program (grant number 22YF1434700), and the Shanghai Municipal Medical and Health Discipline Construction Projects (grant number 2017ZZ02015).

**Author's Contributions**


**Acknowledgments**

The authors would like to acknowledge the helpful suggestions concerning this study received from their colleagues.

**References**


Figures
**Figure 1**

**APC was downregulated in EC, which inhibited EC cell migration.** A, APC expression levels in EC and normal tissue. The data are from the TCGA database and shown in box and whisker plots (****p<0.0001; Kruskal-Wallis H test). B, The protein levels of APC in EC adjacent tissue and EC tissue. C, The protein levels of APC in EC cell lines. D, The APC expression levels in EC cell lines were measured by qRT-PCR, and the values are presented as the means±SEMs. E, F Western blot and qRT-PCR analyses of APC knockdown in stably transfected in EC cell line KLE (**p<0.01; Student’s t-test). G, Representative results of the proliferation after knockdown of APC in EC cell KLE (One-way ANOVA). H, Representative results of the transwell migration assays after knockdown of APC in EC cell KLE (****p<0.0001; One-way ANOVA).
Data analysis of RNA-seq and ATAC-seq. A, The heatmap of DEGs between the control cells and APC knockdown KLE cells. B, Volcano plot of DEGs. Fold change (FC) values > 1.2 or <12/10 and a P-value <0.01 were set as the filter criteria. Green dots represent downregulated proteins; red dots represent upregulated proteins. C, D, GO and KEGG analysis of the identified DEGs. E, the volcano plot of DEGs in DARs the control cells and APC knockdown KLE cells. F, G, GO and KEGG analysis of DEGs in DARs.
Figure 3

The chromosomal structure variations between the control cells and APC knockdown KLE cells. **A,** The heatmap of the whole genome between the control cells and APC knockdown KLE cells, labeled black boxes for inter-chromosomal translocations. **B,** The Hi-C maps between the control cells and APC knockdown KLE cells. **C, D,** The heatmap of Chr8 between the control cells and APC knockdown KLE cells. **E,** The heatmap of Chr4 between the control cells and APC knockdown KLE cells. **F,** The volcano plot of
DEGs in CNV region in APC knockdown KLE cells. G, H, The GO and KEGG analysis of DEGs in the CNV region.

Figure 4

Figure 5

APC knockdown in KLE cells promoted FGF12 expression via altering the chromosomal structure, which predicts a worse outcome in EC. A, The differences of transcriptome, epigenetics, and hierarchical structures among genomic regions of FGF12 between the control cells and APC knockdown KLE cells. B, The level of FGF12 expression in the control cell and APC knockdown KLE cells by RT-qPCR (****p<0.0001, One-way ANOVA). C, D, The protein level of FGF12, P-AKT, T-AKT, P-ERK1/2 and ERK1/2 in
the control cell and APC knockdown KLE cells by western blot (**p<0.01, ***p<0.001, ****p<0.0001, One-way ANOVA). E, The Kaplan-Meier test and Log-rank test were used to analyze the effect of FGF12 expression level on the overall survival (OS) of EC patients (p=0.0011).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplemetalFig1.png
- SupplemetalFig2.png