Label-Free Proteomic Analysis Uncovers SMC1A Expression is Down-regulated in AUB-E Patients

Yingxian Jia  
Women's Hospital, School of Medicine, Zhejiang University

Jie Luo  
Zhejiang University School of Medicine Women's Hospital

Yibing Lan  
Zhejiang University School of Medicine Women's Hospital

Chunming Li  
Zhejiang University School of Medicine Women's Hospital

Linjuan Ma  
Zhejiang University School of Medicine Women's Hospital

Xiaoming Zhu  
Zhejiang University School of Medicine Women's Hospital

Fei Ruan  
Zhejiang University School of Medicine Women's Hospital

Jianhong Zhou  
Zhejiang University

Keywords: Abnormal uterine bleeding, Human endometrium, Proteomic analysis, Primary endometrial disorder

DOI: https://doi.org/10.21203/rs.3.rs-97402/v1

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

**Background:** While heavy menstrual bleeding (HMB) is a prevalent symptom among women with abnormal uterine bleeding caused by the primary endometrial disorder (AUB-E) seeking gynecologic care, the primary endometrial disorder remains poorly understood.

**Methods:** 5 human endometrial samples from women with AUB-E to healthy women and the age-matched healthy women were selected. Proteins from the samples were analyzed by a linear ion trap (LTQ)-Orbitrap Elite mass spectrometer based label-free proteomic approach.

**Results:** A total of 2138 protein groups were quantified under highly stringent criteria with a false discovery rate of < 1% for protein groups, and 317 proteins were significantly changed between the two groups. Combined the results from enriched analysis and protein–protein interaction network analysis showed that the down-regulation of structural maintenance of chromosomes protein 1 A (SMC1A) in AUB-E patients. validated by western blot and immunohistochemistry.

**Conclusion:** The results indicated novel evidence that SMC1A change of the endometrium in women with AUB-E may be associated with the key biological mechanisms (such as cell proliferation) underlying human endometrial remodeling.

**Background**

Abnormal uterine bleeding (AUB) is one of the most common gynecological diseases in women, accounting for 33% of patients attending the gynecology clinic and two-third cases of hysterectomy[1]. To meeting the requirement of standardized definitions and terminologies, using the acronyms of underlying causes and the mechanisms involved in the genesis of AUB, the "PALM-COEIN" classification system as a suitable system for widespread international use since 2011[2], which have been accepted by the International Federation of Gynecology and Obstetrics (FIGO)[3, 4]. In this system, the COEI group is involved in entities not defined by imaging or histopathology ("non-structural"). AUB-E that occurs in the context of a structurally normal uterus with regular menstrual cycles without evidence of coagulopathy is likely to have an underlying endometrial cause, primary endometrial disorder[4]. This condition is often characterized by heavy menstrual bleeding (HMB), usually at fairly regular intervals and with a pattern of daily menstrual loss similar to normal menses, with ~ 90% of the flow occurring during the first 3 days[5]. Although AUB-E may be implicated in many women, a lack of clinically available specific tests or biomarkers means that practical testing for such disorders is not yet feasible[6], which are still have neglected, inadequate diagnosis in AUB-E patients. It occurs in 9–14% of women of all ages and is responsible for about 25% of gynecological surgeries which results from a universally applicable approach deficiency in medical therapy[7, 8]. However, a well-structured history and examination often help, there is no commercially available testing. Hence, a clear role for understanding the etiology of AUB-E and developing biomarkers.
Accompanied by rapid developments and improvements in the field of mass spectrometry (MS), proteomics has employed for discovering biomarkers and answering multidisciplinary scientific questions in different aspects of medicine, such as the elucidation of pathways affected in disease or prediction for the high-risk individuals of developing the disease[9]. Expression analysis directly at the protein level is necessary to unravel the critical changes that occur as part of disease pathogenesis[10]. Therefore, proteomics provides extremely useful tools for investigating the etiology of patients with AUB-E. So far, however, proteomic analysis has been used in other gynecological diseases, there has not been applied to compare the differential protein expression profile for investigating the etiology of AUB-E[11].

In this study, the human endometrial tissues in the proliferative phases between AUB-E patients (group E) and endometrial healthy women from the control group who without AUB (group C) was carried out by label-free proteomic analysis and sought to identify biomarkers of relevance to the endometrial disorder which were validated by western blot and immunohistochemistry. This study will be likely to provide a reference for further studies that will define proteins important for identifying women with AUB-E. The workflow of this study was shown in Fig. 1.

**Methods**

**Patients and control subjects**

This study was carried out at the Department of Gynecology in Women's Hospital, School of Medicine, Zhejiang University. The selection criteria included an examination to rule out any other known cause of AUB, thus strictly adhering to the definition of AUB-E. All non-pregnant females completed a face-to-face interview to obtain their detailed histories, including demographic characteristics, menstrual and medical history combining pregnancy test, routine hematological and coagulation studies, and transvaginal ultrasound for the exclusion of other possible causes of AUB. The selection criteria were permanent residence in Zhejiang Province, aged between 20 - 40 years with self-reported HMB assessed by the Pictorial Blood Assessment Chart (PBAC)[12]. Any female presenting with systemic diseases like thyroid dysfunction, diabetes mellitus, hypertension, chronic liver, kidney disease and organic genital tract lesion, uterine and ovarian tumors, pregnancy-related causes, intrauterine contraceptive devices, endometriosis, and pelvic inflammatory disease were excluded. Endometrial tissues were obtained by curettage in the proliferative phases of the menstrual cycle (days 7 - 10; n = 5) and examined thoroughly to note the histopathological details and classified into group E.

Controls were selected from women of similar age group with normal menstrual history and no endometrial abnormalities undergoing curettage following laparoscopic sterilization (n = 3), and assessment of tubal patency (n = 2). The proliferative endometrial biopsies were carried out a histopathology diagnosis to exclude leiomyoma, endometriosis, polyp, endometrial inflammatory, endometritis, and malignancy. All women had not received steroid hormone therapy in the last 6 months.

**Sample preparation**
All endometrial tissues (~ 20 mg) from group E (age range 26 - 37, median age 28) and group C (age range 27 - 34, median age 28) were transported immediately to the laboratory in phosphate-buffered saline (PBS) on ice. Samples were extensively washed with PBS to remove any blood and were distributed into two parts. One part was sent for histopathology diagnosis, and another was stored at -80 °C for further proteomic or western blot analysis. The total duration from endometrial biopsy removal to sample freezing was controlled to be in 15 min.

**Protein digestion using the FASP method**

Snap-frozen endometrial tissues were added to SDS lysis buffer (2% SDS, 0.1 M DTT, 0.1 M Tris-HCl, pH 7.6), and homogenized. Following centrifugation (16 000g × 5 min at 4 °C), the supernatant was transferred to a fresh tube and the pellet re-extracted as above. The mixture was incubated in boiling water for another 5 min and sonicated for 20 min and centrifuged at 16 000g × 30 min at 20 °C. The supernatants were collected and determined protein concentration by a NanoDrop® ND-1000 Spectrophotometer.

200 μg of the sample was digested by the filter-aided sample preparation (FASP) procedure as described[13]. Each sample was concentrated at 14 000g × 40 min at 20 °C in 30k Microcon filtration devices (Millipore, USA). Then, 200 μl of urea buffer (8 M urea, 0.1 M Tris-HCl, pH 8.5) was added to the sample and centrifuged again for another 14 min. This step was repeated one more time. The concentrate was mixed with 100 μl of 50 mM iodoacetamide (IAA) in urea buffer and incubated for an additional 40 min at room temperature in darkness. Following centrifugation at 14 000g × 15 min, the sample was diluted with 200 μl of urea buffer and centrifuged two more times. Then, 200 μl of 50 mM NH4HCO3 was added and centrifuged again. This step was repeated twice. Finally, 50 μl of 50 mM NH4HCO3 and trypsin (1:50) was added to the sample, which was then incubated at 37 °C overnight. Eluted peptides were collected by centrifugation followed by two washes with 40 μl of 50 mM NH4HCO3 and vacuum dried. Desalting was then carried out using C18 Zip Tips (Millipore, USA) following the manufacturer’s instructions and the samples were vacuum dried. Finally, peptide digests were resuspended in 10% acetonitrile (ACN) in 0.1% formic acid (TFA) and detected the protein concentration.

**LC-MS/MS analysis**

High-performance liquid chromatography (HPLC) was used for sample separation using the EASY-nLC 1000 (Thermo Fisher Scientific, USA) with a binary buffer system consisting of 0.1% TFA in water (buffer A) and ACN in 0.1% TFA (buffer B). Samples were loaded into the precolumns (20 mm ×75 μm, 3 μm-C18, Thermo scientific EASY column, USA) using an auto-sampler and separated by analytical columns (150 mm × 50 μm, 2 μm-C18, Thermo scientific EASY column, USA) at a flow rate of 10 μl/min. The liquid phase gradients were as follows: 3 - 8% buffer B for 0 - 10 min, 8-20% buffer B for 10 - 120 min, 20 - 30% buffer B for 120 - 137 min, 30 - 90% buffer B for 137 - 143 min, and 90% buffer B for 143 - 150 min. The separated peptides were then analyzed by a linear ion trap (LTQ)-Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, USA) fitted with an electrospray ionization (ESI) source. In the positive ion mode, a full scan range from m/z 300 to 2000 with a resolution of 60000 (200 m/z). The top 20
precursors of the highest abundance in the full scan were selected and fragmented by collision-induced
dissociation (CID) function and analyzed in MS/MS, where a resolution was 15000, a normalized
collision energy was set as 35%. The following dynamic exclusion settings were also used: repeat counts
1; repeat duration 30 s; exclusion duration 60 s. Data were post-processed using the Qual Browser part of
Thermo Scientific Xcalibur 2.2 software.

Data analysis

Unprocessed raw files were searched against the UniProtKB Homo sapiens database comprised of
188386 sequences (www.uniprot.org) by the search engines: PEAKS® Studio 8.0 (Thermo Fisher
Scientific). The search parameters are set as follows: mass tolerance for precursor ion was 10 ppm and
mass tolerance for production was 0.02 Da. Carbamidomethyl (C) was specified as fixed modifications,
Oxidation (M) was specified as dynamic modification, and acetylation was specified as N-terminal
modification in PEAKS® Studio 8.0. A maximum of 2 missed cleavage sites was allowed. To improve the
quality of analysis results, the software further filtered there retrieval results: Combining the identified
PSMs (with the credibility of more than 99%) and protein (contained at least 1 unique peptide) were
retained and performed with false discovery rate (FDR) no more than 1.0%. The protein quantitation
results were statistically analyzed by the t-test. The proteins whose quantitation significantly different
between AUB-E and control groups ($p \leq 0.05$ and fold change (FC) $\geq 1.5$) were defined as differentially
expressed proteins (DEPs). The principal components analysis (PCA) of all samples was also checked.

The functional analysis of DEPs

DEPs were used for volcanic map analysis, cluster heat map analysis, and enrichment analysis of Gene
Ontology (GO, http://www.geneontology.org/), and Kyoto Encyclopedia of Genes and Genomes (KEGG,
http://www.genome.jp/kegg)[14]. The protein-protein interactions (PPI) of probable pathways from
enrichment analysis was predicted using Cytoscape software (Version.3.8.0, https://cytoscape.org/)[15].

Western blotting

Western blot was used to validate the differential expression of structural maintenance of chromosomes
protein 1 A (SMC1A) between group E and C. In brief, each tissue was homogenized for 10 min in RIPA
buffer and protease inhibitor cocktail. Then, the crude extract was sonicated for 1 min and centrifuged at
10000 g × 10 min at 4 °C. The supernatant was detected the protein concentration and denatured at 95 °C
for 10 min. After that, 10 μl of sample ran on a 12% SDS-PAGE, transferred onto polyvinylidene fluoride
(PVDF) membranes, blocked with 5% bovine serum albumin (BSA) for 1 h at room temperature, and
incubated in anti-SMC1A (ab243875, Abcam, Cambridge, UK) antibodies 1:1000 diluted overnight at 4 °C.
After washing with tris buffered saline with Tween 20 (TBST), membranes were incubated with anti-rabbit
IgG horseradish peroxidase-labeled antibody produced in goat (ab6721, Abcam, Cambridge, UK) for 1
hour; then, the fluorescence images were acquired by Super ECL Detection Reagent. Each gel was loaded
with a ladder from 5 to 250 kDa for indicating the molecular weight of the corresponding gel band.
GAPDH protein was used as a loading control to normalize the western blot data, and the normalized
abundance of SMC1A is calculated by dividing the band intensity by the corresponding GAPDH band intensity.

**Immunohistochemistry**

The tissues obtained were subsequently preserved in formalin and paraffin-embedded blocks were made. Routine histological examination using hematoxylin and eosin staining was done to identify any abnormalities. The sections were also immunohistochemically stained for SMC1A (ab243875, Abcam).

Frozen sections of 5 μm thickness of endometrial tissue were prepared and then fixed with 4% paraformaldehyde for 20 min at 4 °C to identify the localization of SMC1A in the endometrium. All subsequent incubations were performed in a humidified chamber. Slides were first blocked with 10% goat serum (Invitrogen, USA) for 30 min at room temperature; then, they were incubated overnight at 4 °C with a rabbit anti-SMC1A antibody (1:200 dilution). After washing, the samples were incubated with FITC labeled goat anti-rabbit IgG (Invitrogen; 1:200 dilution) for 1 h at 37 °C. Then, the samples were incubated with DAPI (5 μg/mL, Dojindo, Japan) for 5 min to counterstain the nucleus. Finally, the stained sections were scanned by the Grundium Ocus® scanner (Grundium, Finland).

**Statistical analysis**

Data were performed using the *t*-tests for means of two groups and the chi-square test for the composition ratios. Continuous and categorical variables were described as mean ± standard deviation (SD) and the number and percentage of subjects, respectively. The Mann–Whitney U test was used if continuous variables were non-normally distributed, and shown as median Median (P_{25}–P_{75}). *p* values ≤ 0.05 were considered statistically significant by the SPSS software (Chicago, IL, version 23.0) and graphed with GraphPad PRISM software (v6.0; GraphPad Software Inc., San Diego, CA).

**Results**

**Baseline data analysis**

Baseline data of all participants were collected and analyzed. There was no significant difference in demography and menstrual characteristic among the two groups (*p* > 0.05) in Table 1. Although there were no statistically significant differences of hematoglobin (Hb), hematocrit (HCT), and baseline FSH levels and duration of flow and menstrual cycle length (all *p* values > 0.05), the PBAC scores showed a significant difference between the two groups (*p* = 0.000) (see Table 2).

**Protein identification**

The experiment detected 2353 protein groups and quantified 1921 different proteins (Supplementary Table S1 and Table S2). The further screening revealed 291 DEPs in AUB-E patients compared with healthy controls, including 140 up-regulated proteins (FC ≥ 1.50, *p* < 0.05) and 151 down-regulated proteins (FC ≤ 0.67, *p* < 0.05) (Supplementary Table S3), and these are presented in heat map format in
Supplementary Figure S1. Moreover, PCA analysis and protein-level volcano plots were indicated in Figure 2 and 3.

**GO and KEGG pathway enrichment analysis of DEPs**

GO enrichment analysis of DEPs revealed in Supplementary Figure S2, the major biological processes (BP) of ‘cellular process’ (41.81%) with a higher proportion of down-regulated DEPs (52, 22.41%), and ‘metabolic process’ (37.93%). Also, the proteins were mostly located in ‘cell part’ (73, 31.47%) in cellular component (CC), and possessed ‘catalytic activity’ (74, 31.90%) in molecular functions (MF). The function of proteins heavily relies on their interactions. Besides, enrichment KEGG analysis revealed some significant pathways: ‘Apoptosis’ (5 proteins), ‘Cell adhesion molecules’ (2 proteins), and ‘Cell cycle’ (4 proteins), these results suggested that the occurrence of AUB-E was the result of multiple pathway dysfunction (as Supplementary Table S4). By PPI analysis, 11 DEPs (P63208, A0A384MR33, P25205, Q13740, B4DGI3, P05198, A0A384P5S9, D0PNI1, Q03252, A0A024RBT2, and P05556) interacted with each other and enriched into a larger protein interaction network (Figure 4), including 4 down-regulated proteins and 7 up-regulated proteins. Among up-regulated proteins, B4DGI3 (TNF receptor-associated factor), A0A384P5S9 (epididymis secretory sperm binding protein) both retrieved no corresponding gene name from the UniProt database (https://www.uniprot.org/) and A0A024RBT2 (hCG_1782202, E/C ratio: 1.79) which proteins do not have related commercial antigen. Meanwhile, P05198 (EIF2S1, E/C ratio: 2.23), D0PNI1 (YWHAZ, E/C ratio: 1.99), Q03252 (LMNB2, E/C ratio: 1.97), P05556 (ITGB1, E/C ratio: 1.77) were up-regulated. Moreover, a down-regulated protein, Q13740 (ALCAM, \( p = 6.71 \)) did not be almost expressed in group E without an E/C ratio. Two down-regulated nucleus proteins: P63208 (SKP1, E/C ratio: 0.34) and P25205 (MCM3, E/C ratio: 0.15) were involved in the cell cycle pathway and have been linked with A0A384MR33 (SMC1A, E/C ratio: 0.32) which was the only DEPs was validated by later study.

**Validation of the biomarker by western blot and immunohistochemistry**

For validating purposes protein, SMC1A protein was analyzed by western blotting and immunohistochemical staining in human endometrial tissues (as Figure 5 and 6). Comparing the SMC1A expression levels between the two groups, SMC1A is low expressed in group E following the same expression pattern observed in the proteomic analysis. Also, immunohistochemical staining of SMC1A was mainly expressed in glandular epithelial cells of the endometrium and partly in the stroma area (see figure 6), and SMC1A was similarly subject to significant decreases in the AUB-E group.

**Discussion**

While AUB is a prevalent symptom among women seeking gynecologic care, endometrial function in the context of menstruation and its disorders is still not fully understood[16]. For the present, the diagnosis of AUB-E depends on careful history taking and exclusion of other contributors[4, 17]. AUB-E is thought to be caused by a local disturbance in endometrial function-deficiencies or excesses of proteins or other entities that have an adverse effect on hemostasis, normal angiogenesis, vascular integrity, or endometrial repair[2, 4]. Nevertheless, the contributions to AUB-E is not completely understood, especially
after the “PALM-COEIN” classification system was applied. In this study, we have focused on proteomic analysis, as a powerful tool to identify proteins possibly involved in pathogenesis, with the hypothesis that functionally important protein changes, will identify biomarkers of relevance to endometrial disorder (AUB-E).

In clinical practice, the diagnosis, evaluation, and treatment of HMB are based upon “patient experience”, the woman's assessment of her blood loss and its impact on her life[7, 18]. Therefore, the PBAC combined with self-reported to quality-of-life issues for women with excessive blood loss were suggested to assess the volume of menstrual blood loss. Although Hb and HCT levels were not significant differences between 2 groups, there were 2 patients presented anemia and all AUB-E subjects complained about a situation of HMB with a significant difference of PBAC scores in Table 2.

Previous studies indicated that if patients performed a symptom of HMB, a primary disorder of mechanisms regulating local endometrial “hemostasis” itself may exist. Other primary endometrial disorders may not manifest in HMB but cause intermenstrual (IMB) or prolonged bleeding. Prolonged bleeding may be a manifestation of deficiencies in the molecular mechanisms of endometrial repair. Such disorders may be secondary to endometrial inflammation or infection, to abnormalities in the local inflammatory response, which was remaining unknown about the role of infection and other local inflammatory disorders against the normal presence of inflammatory cells in the endometrium[17]. It should be noted that AUB-related researches published in the past generally focused on the previous term “dysfunctional uterine bleeding” that could be confused with other causes of AUB[2]. Therefore, the results of these studies were limited. In this study, all participants in group E were excluded from other contributors of AUB, and self-reported predictable and cyclical ovulatory cycles with a situation of HMB. PCA analysis was used to reflect the total difference between groups and the variability among each group. Notably, in our study, the two groups were well discriminated into two blocks. Meanwhile, the protein-level volcano plots of log₂ FC and the p value between AUB-E and control group were shown in Fig. 3 that was very similar to the normal distribution. These results suggested that this experimental procedure was performed without significant bias toward different samples.

SMC1A (also called SMC1L1), a member of the SMC superfamily and core structural component of the cohesin complex, is essential for sister chromatid cohesion, DNA recombination and repair, cell cycle regulation, genomic stability maintenance, and tumorigenesis[19, 20]. However, there was no relative study about SMC1A expression in the human endometrium or any gynecological diseases. Previous studies have reported that SMC1A expression was significantly stronger in colorectal carcinomas than in normal mucosa tissues, which suggested SMC1A played a key role in colorectal cancer development. Adversely, the downregulation of SMC1A generally inhibits cell proliferation, cell cycle progression, and cell migration[21]. Further evidence indicated that the roles of SMC1 in cell proliferation and apoptosis are mediated through the NF-κB signaling pathway[22]. Nevertheless, these studies have raised more questions that need to be answered. In this study, our results revealed that the SMC1A expression of endometrium in women with AUB-E is significantly lower than healthy women by western blotting and immunohistochemical studies. Down-expression of SMC1A protein in the human endometrium may
indicate a genesis of AUB-E. However, details regarding the mechanisms of action require further investigation.

The endometrium is morphologically divided into functional and basal layers. During endometrial repair and proliferation, mitosis occurs in the functional layer of the endometrium, a highly active layer consisting of glands supported by stroma[23]. Immunohistochemical staining in this study demonstrated a change of SMC1A expression mainly located in the glandular epithelium in proliferative-phase endometrium, which indicated disturbances to endometrial remodeling after menstruation may underpin AUB-E. Here, enriched GO and KEGG pathway analysis revealed that SMC1A was down-expressed in the AUB-E group which was involved in the cell cycle pathway. Although absence from related research of SMC1A in human endometrial epithelial cells and, SMC1A knockdown appeared to coincide with cell cycle arrest and/or increased apoptosis in human breast, lung, prostate, and colorectal cancer cells[24,25], which indicated SMC1A protein may enhance the tolerance to DNA damage further supported its effects on cell proliferation and inhibit apoptosis regulated the cell cycle progression[21,22,26]. In this study, the dynamics of SMC1A during the proliferative phase may be closely associated with cell proliferation of endometrial glandular epithelial cells regulated local endometrial “hemostasis”, which caused HMB. The present data also demonstrated a novel mechanism for the cause of AUB-E, as down-expression SMC1A potentially inhibited the gland’s proliferation and regulated the cell cycle progression.

The main limitation of this study was a small number of samples. It is thus essential that any proposed biomarkers are subject to analysis on sufficiently large sample cohorts from multiple sites. Additional studies are needed with molecular functional studies to demonstrate the physiologic roles of SMC1A and provide mechanistic insight. Other limitations included the endometrial changes of architecture and cellular composition during the cycle phases and women may have multiple potential causes of their AUB symptoms that may limit the applicability of this study.

**Conclusion**

Given the prevalence of AUB, there is a clear need for understanding the genesis of AUB for offering the best results of individualized and personalized diagnosis and care. In this study, a label-free proteomic strategy for the first time was employed to compare the proteomics of human proliferative-phase endometrium between AUB-E and control groups to meet the urgent biomedical and research need. A total of 2353 unique proteins were identified and quantified, which includes 317 DEPs statistically significant between the two groups. Enriched KEGG pathway analysis revealed the cell cycle involved by SMC1A down-regulated in the endometrial tissues. Further western blotting and immunohistochemical analysis validated down-regulation of SMC1A located in endometrial glandular epithelial cells in AUB-E patients may demonstrate that SMC1A potentially inhibited the glandular proliferation during endometrial remodeling contributed a situation of HMB. Although there were some limitations, this study provided up-to-date information on the cause of AUB and the potential mechanism of SMC1A in AUB-E patients that is imperative for the advancement of women’s health-related quality of life.
Abbreviations

AUB: Abnormal uterine bleeding; FIGO: International Federation of Gynecology and Obstetrics; MS: mass spectrometry; PBAC: Pictorial blood assessment chart; PBS: phosphate-buffered saline; FASP: filter-aided sample preparation; IAA: iodoacetamide; CAN: acetonitrile; TFA: formic acid; HPLC: High-performance liquid chromatography; LTQ: linear ion trap; FDR: false discovery rate; FC: fold change; DEPs: differentially expressed proteins; PCA: principal components analysis; GO: Gene Ontology; KEGG: Kyoto 179 Encyclopedia of Genes and Genomes; PPI: protein-protein interactions; SMC1A: structural maintenance of chromosomes protein 1A; PVDF: polyvinylidene fluoride; BSA: bovine serum albumin; TBST: tris buffered saline with Tween 20; SD: standard deviation; Hb: hemoglobin; HCT: hematocrit; BP: biological processes; CC: cellular component; MF: molecular functions

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of Women's Hospital, School of Medicine, Zhejiang University located in Hangzhou, Zhejiang Province, China with the Ethical Clearance (No. 20180200). Informed consent was obtained from all subjects.

Consent for publication

All data have consent for publication.

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Science and Technology Planning Project of Zhejiang Province, China (2019C03026).

Authors’ contributions

YXJ participated in the design of the study, performed experiments, and drafted the manuscript. YBL, CML, and LJM participated in the acquisition of data and the collection of clinical samples. JL and FR analyzed and performed the statistical analysis. XMZ participated in the design of the study. JHZ conceived in the study and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.
Acknowledgments

The authors thank all the subjects who participated in this study. Also, we thank all the technologists for their cooperation and contribution to this study.

References

1. Ilavarasi CR, Jyothi GS, Alva NK. Study of the Efficacy of Pipelle Biopsy Technique to Diagnose Endometrial Diseases in Abnormal Uterine Bleeding. J Midlife Health. 2019. 10(2): 75-80.

2. Munro MG, Critchley HO, Fraser IS. The FIGO systems for nomenclature and classification of causes of abnormal uterine bleeding in the reproductive years: who needs them. Am J Obstet Gynecol. 2012. 207(4): 259-65.

3. Fraser IS, Critchley HO, Broder M, Munro MG. The FIGO recommendations on terminologies and definitions for normal and abnormal uterine bleeding. Semin Reprod Med. 2011. 29(5): 383-90.

4. Munro MG, Critchley HO, Fraser IS, FIGO Menstrual Disorders Working Group. The FIGO classification of causes of abnormal uterine bleeding in the reproductive years. Fertil Steril. 2011. 95(7): 2204-8, 2208.e1-3. doi: 10.1016/j.fertnstert.2011.03.079. Epub 2011 Apr 15. PMID: 21496802.

5. Fraser IS, Hickey M, Song JY. A comparison of mechanisms underlying disturbances of bleeding caused by spontaneous dysfunctional uterine bleeding or hormonal contraception. Hum Reprod. 1996. 11 Suppl 2: 165-78.

6. Whitaker L, Critchley HO. Abnormal uterine bleeding. Best Pract Res Clin Obstet Gynaecol. 2016. 34: 54-65.

7. Bryant-Smith AC, Lethaby A, Farquhar C, Hickey M. Antifibrinolytics for heavy menstrual bleeding. Cochrane Database Syst Rev. 2018 Apr 15;4(4):CD000249. doi: 10.1002/14651858.CD000249.pub2. PMID: 29656433

8. Soleymani E, Ziari K, Rahmani O, et al. Histopathological findings of endometrial specimens in abnormal uterine bleeding. Arch Gynecol Obstet. 2014. 289(4): 845-9.

9. Altmäe S, Esteban FJ, Stavreus-Evers A, et al. Guidelines for the design, analysis and interpretation of 'omics' data: focus on human endometrium. Hum Reprod Update. 2014. 20(1): 12-28.

10. Srivastava S, Srivastava RG. Proteomics in the forefront of cancer biomarker discovery. J Proteome Res. 2005. 4(4): 1098-103.

11. Meehan KL, Rainczuk A, Salamonsen LA, Stephens AN. Proteomics and the search for biomarkers of female reproductive diseases. Reproduction. 2010. 140(4): 505-19.

12. Magnay JL, O’Brien S, Gerlinger C, Seitz C. Pictorial methods to assess heavy menstrual bleeding in research and clinical practice: a systematic literature review. BMC Womens Health. 2020. 20(1): 24.

13. Wiśniewski JR, Nagaraj N, Zougman A, Gnad F, Mann M. Brain phosphoproteome obtained by a FASP-based method reveals plasma membrane protein topology. J Proteome Res. 2010. 9(6): 3280-9.
14. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009. 37(1): 1-13.

15. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003. 13(11): 2498-504.

16. Singh S, Best C, Dunn S, Leyland N, Wolfman WL. No. 292-Abnormal Uterine Bleeding in Pre-Menopausal Women. J Obstet Gynaecol Can. 2018. 40(5): e391-e415.

17. Munro MG, Critchley HO, Fraser IS. The flexible FIGO classification concept for underlying causes of abnormal uterine bleeding. Semin Reprod Med. 2011. 29(5): 391-9.

18. Warner PE, Critchley HO, Lumsden MA, Campbell-Brown M, Douglas A, Murray GD. Menorrhagia II: is the 80-mL blood loss criterion useful in management of complaint of menorrhagia. Am J Obstet Gynecol. 2004. 190(5): 1224-9.

19. Zhang Y, Yi F, Wang L, et al. Phosphorylation of SMC1A promotes hepatocellular carcinoma cell proliferation and migration. Int J Biol Sci. 2018. 14(9): 1081-1089.

20. Musio A. The multiple facets of the SMC1A gene. Gene. 2020. 743: 144612.

21. Pan XW, Gan SS, Ye JQ, et al. SMC1A promotes growth and migration of prostate cancer in vitro and in vivo. Int J Oncol. 2016. 49(5): 1963-1972.

22. Li J, He J, Wang Y, Shu Y, Zhou J. SMC1 promotes proliferation and inhibits apoptosis through the NF-κB signaling pathway in colorectal cancer. Oncol Rep. 2019.

23. Gargett CE, Ye L. Endometrial reconstruction from stem cells. Fertil Steril. 2012. 98(1): 11-20.

24. Li J, Feng W, Chen L, He J. Downregulation of SMC1A inhibits growth and increases apoptosis and chemosensitivity of colorectal cancer cells. J Int Med Res. 2016. 44(1): 67-74.

25. Yadav S, Kowolik CM, Lin M, et al. SMC1A is associated with radioresistance in prostate cancer and acts by regulating epithelial-mesenchymal transition and cancer stem-like properties. Mol Carcinog. 2019. 58(1): 113-125.

26. Sarogni P, Palumbo O, Servadio A, et al. Overexpression of the cohesin-core subunit SMC1A contributes to colorectal cancer development. J Exp Clin Cancer Res. 2019. 38(1): 108.

Tables

Table 1. The demographic characteristic of all subjects
### Table 2. The clinical details of all subjects

| Demographic characteristic | AUB-E       | Control     | \( p \) value |
|----------------------------|-------------|-------------|---------------|
|                            | \( n = 5 \) | \( n = 5 \) |               |
| **Age, years**             | 28 (26, 37) | 28 (27, 34) | 1.00\(^a\)    |
| Median (P\(_{25}\)-P\(_{75}\)) |             |             |               |
| **Education, n (%)**       |             |             | 0.421\(^b\)   |
| Above college              | 1 (20)      | 1 (20)      |               |
| Middle school              | 2 (40)      | 4 (80)      |               |
| others                     | 2 (40)      | 0           |               |
| **Employed status, n (%)** | 5 (100)     | 5 (100)     | 1.00\(^b\)    |
| **Income, n (%)**          |             |             | 0.310\(^b\)   |
| \( \leq 5,000 \) RMB       | 4 (80)      | 2 (40)      |               |
| \( > 5,000 \) RMB          | 1 (20)      | 3 (60)      |               |
| **No smoking, n (%)**      | 5 (100)     | 5 (100)     | 1.00\(^b\)    |

\( n \), the number of subjects.

\(^a\) \( p \) value for the Mann–Whitney U-test. \(^b\) \( p \) value for the chi-square test.
| Clinical details                  | AUB-E       | Control     | \( p \) value |
|----------------------------------|-------------|-------------|---------------|
|                                  | n = 5       | n = 5       |               |
| **Menarche, years**              | 14 (1.14)   | 13 (1.00)   | 0.073\(^a\)  |
| Mean (SD)                        |             |             |               |
| **Parity**                       | 1 (1, 1.5)  | 1 (1, 1.5)  | 1.000\(^b\)  |
| Median (\(P_{25}\),\(P_{75}\)) |             |             |               |
| **Height, cm**                   | 159.80 (1.789) | 161.20 (2.049) | 0.283\(^a\) |
| Mean (SD)                        |             |             |               |
| **Weight, kg**                   | 54.80 (5.007) | 55.80 (7.497) | 0.810\(^a\) |
| Mean (SD)                        |             |             |               |
| **Body-mass index, kg/m\(^2\)** | 21.49 (2.28) | 21.46 (2.75) | 0.989\(^a\) |
| Mean (SD)                        |             |             |               |
| **Menstruation duration, days**  | 5.2 (1.80)  | 5.8 (0.84)  | 0.516\(^a\)  |
| Mean (SD)                        |             |             |               |
| **Menstrual cycle length, days** | 30 (29, 31) | 30 (29, 30) | 0.841\(^b\)  |
| Median (\(P_{25}\),\(P_{75}\)) |             |             |               |
| **PBAC score**                   | 151.2 (26.20) | 61.8 (8.927) | 0.000\(^{a,*}\) |
| Mean (SD)                        |             |             |               |
| **Hematoglobin, g/L**            | 130 (88.5, 134) | 136 (129, 138) | 0.151\(^b\) |
| Median (\(P_{25}\),\(P_{75}\)) |             |             |               |
| **HCT**                          | 0.36 (0.07) | 0.4 (0.024) | 0.227\(^a\)  |
| Mean (SD)                        |             |             |               |
| **Baseline FSH, U/L**            | 5.9 (5.1, 8.6) | 5.45 (4.88, 7.60) | 0.690\(^b\) |
| Median (\(P_{25}\),\(P_{75}\)) |             |             |               |

\( n \), the number of subjects; PBAC, the Pictorial Blood Assessment Chart; HCT, hematocrit; \(*: p \leq 0.05\), which denoted a significant difference between AUB-E and Controls.

\(^{a}\) \( p \) value for the \( t \)-tests. \(^{b}\) \( p \) value for the Mann–Whitney U-test.

**Supplementary Tables And Additional Files**

Supplementary Table S1: Quantified protein groups in all human endometrial samples.
Supplementary Table S2: Different proteins in the human endometrium between AUB-E and control.

Supplementary Table S3: DEPs in human endometrium with AUB-E vs. control.

Supplementary Table S4: enrichment KEGG pathway analysis of the DEPs.

Supplementary Figure S1: Heat map of the DEPs between AUB-E and control.

Supplementary Figure S2: Merged Go enrichment analysis of the up-regulated proteins and down-regulated proteins. Up: up-regulated proteins in red (FC ≥ 1.50, p < 0.05); Down: down-regulated proteins in green (FC ≤ 0.67, p < 0.05).