

Original Article

Estrogen/estrogen receptor promotes the proliferation of endometrial carcinoma cells by enhancing hMOF expression

Yue Qi^{1,†}, Mingzi Tan^{1,2,†}, Mingjun Zheng¹, Shan Jin¹, Huimin Wang^{1,2}, Juanjuan Liu¹, Peiyao Wang¹, Xin Nie¹, Lingling Gao¹, and Bei Lin^{1,*}

¹Department of Obstetrics and Gynecology, Shengjing Hospital Affiliated to China Medical University, Shenyang, Liaoning, China, and ²Department of Gynecology, Liaoning Cancer Hospital & Institute, Shenyang, Liaoning, China

*For reprints and all correspondence: Bei Lin, Department of Obstetrics and Gynecology, Shengjing Hospital Affiliated to China Medical University, Sanhao Street, Shenyang, Liaoning, 110004, China. E-mail: linbei88@hotmail.com

†Co-first author

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Abstract

Background: This study aims to analyse the expression of human MOF in endometrial carcinoma cells and its relationship with estrogen and estrogen receptor and to investigate the effect of estrogen–human MOF on the malignant biological behaviours of endometrial carcinoma cells.

Methods: The expression of human MOF was detected in different endometrial tissues by immunohistochemistry. The effects of human MOF, human MOF combined with estrogen stimulation and estrogen plus anti-human MOF antibody blocking on the proliferation of endometrial carcinoma cells were evaluated by western blotting, real-time polymerase chain reaction, cell proliferation assay and cell cycle distribution. Bioinformatics was used to identify the correlations of human MOF and estrogen and involved pathways.

Results: The expression levels of human MOF in endometrial carcinoma tissues were significantly higher than that in atypical hyperplasia and normal endometrial tissues. High expression of human MOF was associated with late-stage cancer, lymph node metastasis and short survival time, and it was also an independent prognostic risk factor for endometrial carcinoma. After human MOF knockdown, the proliferation, migration and invasive capacity of Ishikawa cells decreased and cell apoptosis increased. After stimulation with estrogen, the PI3K/Akt and Ras–Raf–MEK–ERK signalling pathways were activated, and the expression of the human MOF protein was increased. human MOF (KAT8) expression showed a positive correlation with ESR1 expression, and KAT8-associated genes were enriched in the cell cycle pathways and splicing pathways.

Conclusion: Human MOF was highly expressed in endometrial carcinoma and associated with proliferation. Estrogen/estrogen receptor enhanced human MOF expression; promoted the proliferation, migration and invasion of Ishikawa cells; and inhibited cell apoptosis by activating the PI3K/Akt and Ras–Raf–MEK–ERK signalling pathways.

Key words: hMOF, estrogen, estrogen receptor, proliferation, endometrial carcinoma

Introduction

Endometrial carcinoma is a malignant tumour that originates from the endometrial epithelium (1). It is well-known that the development of endometrial carcinoma is associated with stimulation of local low-dose estrogen stimulation without the antagonism of progesterone (2). Estrogen enhances malignant biological behaviours of endometrial carcinoma through mediation by estrogen receptor (3). According to the pathogenic mechanism and biological behaviour characteristics, endometrial carcinoma was divided into estrogen-dependent type (type I) and estrogen-independent type (type II). Type I is mainly composed of endometrioid carcinoma (80–90%), and type II comprises non-endometrioid subtypes such as serous carcinoma and clear cell carcinoma (4). Histone acetylation modification is one of the important epigenetic modifications, which can induce transcription of related genes by changing the binding of specific histones to certain regions of DNA (5). Histone acetylation modification is regulated by both histone acetyltransferases (HATs) and histone deacetylases (HDACs) (6). A growing number of studies have suggested that histone deacetylase is closely associated with lung inflammation and neurodegenerative lesions (7) and may be an important treatment target for some cancers. Human MOF (hMOF) is an important member of the MYST family, which is one of the three major histone deacetylase families (GNAT family (Gcn5-related *N*-acetyltransferase family) and P300 (E1B-associated protein of 300 kDa)/CBP (CREB-binding protein) and the MYST family). hMOF is involved in the maintenance of chromosomal stability and DNA damage repair (8). Studies have shown that blastocyst implantation was incomplete in hMOF-knockout mice due to changes in chromosome conformation. Abnormal expression of hMOF also affects the occurrence, development and prognosis of malignant tumours (9, 10). In breast cancer tissue, low expression of hMOF is related to late TNM stages, and downregulation expression of hMOF is an adverse prognosis factor for breast cancer patients (11). Our team found that hMOF expression was low in ovarian carcinoma tissues, which was an unfavourable risk factor for ovarian carcinoma patients (12). However, it is worth noting that the expression level of hMOF in ovarian endometrioid carcinoma is significantly higher than that in ovarian clear cell carcinoma (12), thus suggesting that hMOF expression is histology-specific. Ovarian endometrioid carcinoma is similar to endometrial carcinoma in terms of molecular pathological mechanism; however, the expression of hMOF in endometrial carcinoma has not been reported. Type I endometrial carcinoma is an estrogen-dependent cancer. Previous studies have shown that estrogen can promote high expression of HBO1, another member of the MYST family, in estrogen receptor-positive breast cancer, and this effect is mediated by estrogen receptor (13, 14). However, there are no related reports regarding the regulation of hMOF expression by estrogen and its receptor.

The present study aimed to detect the expression of hMOF in endometrial carcinoma tissues and cells and to relate its expression to clinicopathological features and prognosis of endometrial carcinoma. Effects of hMOF expression on the malignant biological behaviours of endometrial carcinoma cells were analysed, and the correlation between estrogen and hMOF expression and the mechanisms by which estrogen regulates hMOF expression were investigated. The study also investigated the role of histone acetylase hMOF in the development of endometrial carcinoma and the related mechanisms and provided a theoretical basis for the application of histone acetylation as a new target in the treatment of endometrial carcinoma.

Materials and methods

Specimen sources

Paraffin specimens of surgical resection from 111 cases of endometrial cancer (63 of endometrioid carcinoma, 22 of serous carcinoma, 18 of clear cell carcinoma, 8 of other types carcinoma), 41 atypical hyperplasia, 25 proliferative endometrium and 25 secretory endometrium tissue paraffin specimens (normal endometrium tissues were from hysterectomized patients with CIN III), all of which were from the Department of Obstetrics and Gynecology, Shengjing Hospital of China Medical University (Shenyang, China), from 2004 to 2013, were used in this study.

The age range of all the patients was 23–79 years; median age was 54 years. The age range of the endometrial cancer group was 33–79 years; median age was 58.24 years. The age range of the endometrial atypical hyperplasia group was 33–69 years; median age was 51 years. The age range of the proliferative endometrium group was 38–53 years; median age was 43 years; and the age range of the secretory endometrium group was 23–54 years; median age was 46 years. There was no statistically significant age difference among endometrial cancer, endometrial atypical hyperplasia and normal endometrium patients ($P > 0.05$). In the endometrial carcinoma group, according to histopathological grading, there were 23 cases of high differentiation, 39 cases of medium differentiation and 49 cases of low differentiation. The classification of cancer stage was according to the International Federation of Gynaecology and Obstetrics (FIGO, 2009). Among the 111 patients with EC, 74 cases were FIGO stages I–II, and 37 cases were FIGO stages III–IV. There were 12 cases without lymph node dissection and 99 cases with lymph node dissection. And there were 27 cases with lymphatic metastasis and 72 cases without lymphatic metastasis. All endometrial cancer patients were primary endometrial cancer and have not underwent chemotherapy or radiotherapy before the surgery. And pathology sections were confirmed by experts. This study was approved by the ethics committee of Shengjing Hospital affiliated to China Medical University (No. 2019PS522K).

Methods

Immunohistochemistry

Expression of hMOF was detected using strept avidin–biotin complex (SABC) immunohistochemistry staining, using mouse anti-human hMOF antibody (1:500, GeneTex). All other reagents were provided by the central laboratory of Shengjing Hospital of China Medical University (Shenyang, China). Each experimental trial included endometrioid carcinoma, serous carcinoma, clear cell carcinoma and normal ovarian tissue sections, as well as both positive and negative control sections. Staining protocols were pursuant to the manufacturer's instructions for the reagent kit. The positive control sections for hMOF were non-small-cell carcinoma tissues. For negative controls, PBS was substituted for primary antibodies.

Immunohistochemistry results were interpreted as previously described (12). The sections were marked positive when the cell nucleus appeared brown or yellow. The sections were separated into high expression (++/+++) and low expression (-/+) groups according to levels of hMOF staining. Each section was independently assessed by two observers to control for error.

Cell culture and hMOF gene transfection

Ishikawa (ER+) and HEC1A (ER-) endometrial carcinoma cell lines were purchased from Cell Culture Collection of Shanghai. Cells were cultured in RPMI 1640 media with 10% FBS and antibiotics (penicillin-streptomycin, amphotericin B and tetracycline). Transfection was carried out using liposomes with a vector transfection kit according to the instructions. Ishikawa cells were transfected with hMOF siRNA (Zimmer AG) and plasmid vectors using lipofectamine 3000 (Invitrogen).

Western blotting

Western blotting was prepared as previously described (12). Antibodies used were mouse anti-human hMOF (1:100, GeneTex), mouse anti-human GAPDH (1:3000, ZSGB-BIO, Beijing, China), rabbit anti-human p-Akt, rabbit anti-human Akt, rabbit anti-human p-ERK and rabbit anti-human ERK (1:500, Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China).

Real-time PCR

Real-time PCR was performed using SYBR Premix Ex Taq™ II. The empirical procedure was performed based on the manufacturer's instructions. The quantity of hMOF transcripts in each sample was standardized to that of a housekeeping gene, GAPDH. hMOF was amplified with the primer pair, 5'-GACACTGTACTTTGACGTGGA GC-3' and 5'-CACTGTGATGGGTGGTTTCTT-3'. GAPDH cDNA was amplified using the primer pair, 5'-CCTTCATTG ACCTCCACTAC-3' and 5'-GTTGTCATACTTCTCATGGTTC-3'. The relative mRNA expression was quantified through a comparison of the cycle threshold (Ct) values. The experimental data were processed using the $2^{-\Delta\Delta Ct}$ method. Each experimental group was repeated three times.

Cell proliferation assay

Primary Ishikawa cells were seeded onto 96-well plates at a density of 600 cells/well, respectively and cultured until 50 or 70% confluent for E2 or hMOF antibody treatments, respectively; cells were synchronized in serum-free media for 24 h and treated in triplicate with various concentrations of E2 (10^{-10} ~ 10^{-4} mol/l) or E2 (10^{-10} mol/l) + hMOF antibody (1:100) for the times indicated. Cell proliferation was determined by MTT assay. Optical density (OD) was measured at the 0 point and then after 12, 24, 48, and 72 h. At the end of each experiment, 10 μ l of MTT solution (5 μ g/ml) was added into each well, and the plates were incubated for an additional 4 h at 37°C. Then, 150 μ l of dimethyl sulphoxide (DMSO) was added into each well to dissolve formazan by agitation for 10 min. The absorbance values of cells were measured at a wavelength of 490 nm using a microplate reader. The experiments were performed in five wells and were repeated at least three times. The data were summarized as a percentage of the control using the formula: (OD_{treated}/OD_{control}) \times 100%.

Cell cycle distribution

For cell cycle analysis, cells were harvested and fixed in 70% ethanol overnight at 4°C. After being washed three times in cold PBS, the cells were incubated with RNase and stained with PI. ModFit LT software was used for analysis of cell cycle. All assays were carried out independently in triplicate. The experiment was repeated three times.

In vitro scratch assay

Fully grown, plated cells of Ishikawa were scratched and then photographed at 0 and 24 h, as previously described (12). The experiment was repeated three times.

Transwell invasion experiment

The ECM gel was diluted by 1:8 with serum-free medium, Matrigel 60 μ l was added into the upper chambers; then, they were placed in an incubator at 37°C overnight. Ishikawa cells were cultured in zero serum medium. For each cell line, 200 μ l was added to the upper chamber of transwells (Corning, Tewksbury, MA, USA), and 500 μ l of foetal bovine serum (FBS) was added to the lower chamber. The transwells were then cultured for 36 h and processed as previously described (12). Data for statistical analysis were collected by counting five horizon cells under a 100X microscope lens. The experiment was repeated three times.

Bioinformatics analysis

Data collection from TCGA database. Endometrial cancer data were downloaded and prescreened, and 586 tumour samples were included in this study. Samples were sorted according to the expression level of KAT8, from low to high, and equally aliquoted into four parts: the first 25% of the samples were the KAT8 low expression group. The last 25% of samples were the high expression group.

GSEA. GSEA 3.0 was used to analyse data 13. C2.cp.kegg.v6.1.symbols.gmt data cluster was downloaded from the Molecular Signatures Database data bank on the GSEA website. Enrichment analysis was performed on the sorted samples using default weighted enrichment statistics. Random assortment times were set to 1000.

Analysis of copy number variation. The endometrial cancer copy number variation data were downloaded from xena (<https://xenabrowser.net/datapages/>), and the samples were divided into four groups according to copy number: shallow deletion, diploid, gain and amplification. t-test was used to compare the expression levels of KAT8.

Biological function enrichment analysis. A list of top 500 genes with the highest co-expression correlation with KAT8 in the cBioPortal was submitted to DAVID Bioinformatics Resources 6.7 (<http://david.abcc.ncifcrf.gov>) for Gene Ontology (GO) enrichment analysis (15,16). $P < 0.05$ was set as the cutoff criterion.

PPI network establishment. STRING is designed to evaluate protein-protein interaction (PPI) network information. To detect the potential relationships among genes correlated with KAT8, PPI was constructed using the Search Tool for the Retrieval of Interacting Genes (STRING; <http://string.embl.de/>) database and subsequently was visualized using Cytoscape (17,18). A confidence score ≥ 0.4 was set as the cut-off criterion.

Statistical analysis

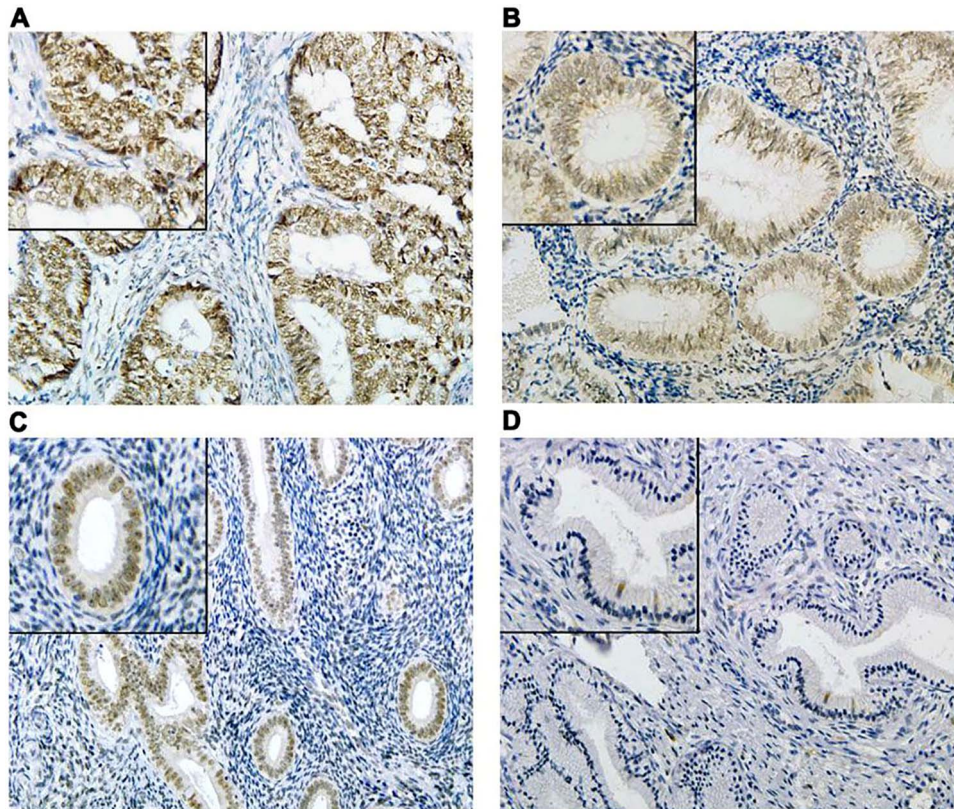
The SPSS20.0 software system (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. χ^2 analysis, variance analysis and t-test were employed. The Cox regression model was used for analysis of risk factors. The Kaplan-Meier and log-rank methods were used to analyse and compare survival curves. In addition, Spearman

Table 1. The expression of hMOF in endometrial tissues

Group	n	hMOF expression		P
		High n (%)	Low n (%)	
Malignant	111	78 (70.3)	33 (29.7)	0.001*
Atypical hyperplasia	41	15 (36.6)	26 (63.4)	<0.001**
Normal	50	22 (44.0)	28 (56.0)	
Proliferative phase	25	15 (60.0)	10 (40.0)	0.845**
Secretory phase	25	7 (28.0)	18 (72.0)	<0.001**

*compared with normal groups.

**compared with malignant groups.

**Figure 1.** Human MOF (hMOF) expression in endometrial endometrioid carcinomas (A). Atypical hyperplasia (B), normal tissue: proliferative (C), secretory (D), original magnification $\times 200$ and the upper left corner $\times 400$.

correlation analysis was used to analyse the correlation between the two proteins. A bilateral $P < 0.05$ was considered statistically significant.

Results

hMOF expression in endometrial tissues of different pathological groups

hMOF protein is mainly localized in the nucleus of endometrial glands, but is also expressed in the cytoplasm. Its high expression ratio (70.3%) in endometrial carcinoma was significantly higher than that in the group atypical hyperplasia and normal endometrium (36.6 and 44.0%, $P < 0.05$). In normal endometrium, the expression rate of hMOF at proliferative phase (60.0%) was significantly higher

than secretory phase (28.0%) ($P < 0.05$), and there is no significant difference in the expression of hMOF between proliferative phase endometrium and endometrial cancer tissue ($P > 0.05$) (Table 1; Fig. 1).

The relationship between the expression of hMOF and clinicopathological parameters of endometrial carcinoma

The expression of hMOF varied with pathological subtypes of endometrial carcinoma. The expression level of hMOF in endometrial carcinoma cells was significantly higher than that in non-endometrial carcinoma cells. Moreover, hMOF expression was higher in endometrioid adenocarcinoma than in papillary serous carcinoma. These findings suggested that hMOF expression played

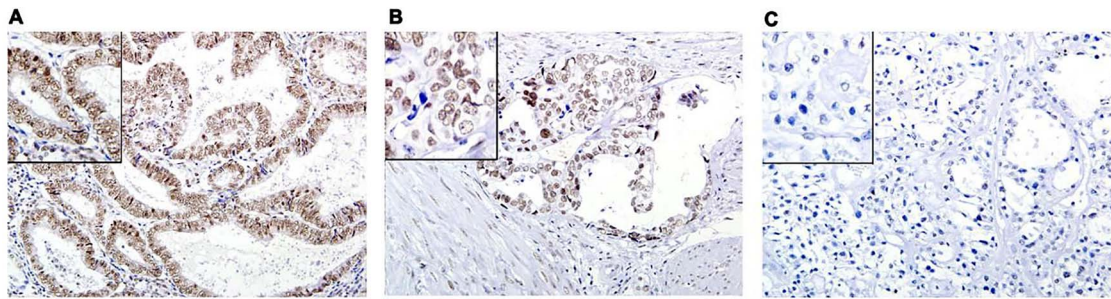


Figure 2. hMOF expression in different pathological types of endometrial carcinoma. Endometrioid (A), serous (B), clear cell (C). Original magnification $\times 200$ and the upper left corner $\times 400$.

Table 2. The relationship between the expression of hMOF and the clinicopathological parameters for endometrial cancer

Features	Cases	hMOF expression		P
		High n (%)	Low n (%)	
Pathological type				
Endometrioid	63	50 (79.4)	13 (20.6)	0.024*
Serous	22	12 (54.5)	10 (45.5)	
Clear cell	18	12 (66.7)	6 (33.3)	
Others	8	4 (50.0)	4 (50.0)	
FIGO stage				
I-II	74	47 (63.5)	27 (36.5)	0.028
III-IV	37	31 (83.8)	6 (16.2)	
FIGO stage (endometrioid)				
I-II	40	28 (70.0)	12 (30.0)	0.036
III-IV	23	22 (95.6)	1 (0.04)	
Invasive depth				
<1/2	64	41 (64.1)	23 (35.9)	0.095
$\geq 1/2$	47	37 (78.7)	10 (21.3)	
Lymphatic metastasis				
No	72	48 (66.7)	24 (33.3)	0.027
Yes	27	24 (88.9)	3 (11.1)	
ER				
Positive	40	34 (85.0)	6 (15.0)	0.041
Negative	60	40 (66.7)	20 (33.3)	
PR				
Positive	48	39 (81.2)	9 (18.8)	0.112
Negative	52	35 (67.3)	17 (32.7)	

*compared between endometrioid and serous types.

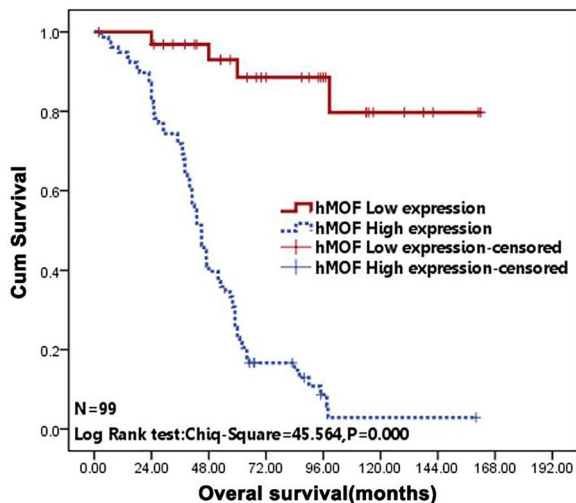
an important role in the development of endometrial carcinoma (Fig. 2).

Further comparisons of hMOF expression in endometrioid adenocarcinoma and non-endometrioid carcinoma showed that the expression rate of hMOF in endometrioid carcinoma (79.4%) was significantly higher than that in non-endometrioid carcinoma (58.3%) ($P = 0.016$), suggesting that hMOF expression was closely associated with endometrioid carcinoma. Further analysis showed that together with an increase in FIGO staging, hMOF expression rate significantly increased, with the expression rate in III-IV endometrial carcinoma (83.8%, 63.5%, $P = 0.028$) significantly

higher than that in I-II endometrial carcinoma (83.8%, 63.5%, $P = 0.028$). Correlation analysis indicated that the expression rate of hMOF in the lymph node metastasis group (88.9%, 66.7%, $P = 0.027$) was significantly higher than that in the group without lymph node metastasis (88.9%, 66.7%, $P = 0.027$). Correlation analysis of hMOF expression and estrogen and progesterone receptors showed that the expression rate of hMOF in estrogen receptor positive patients (85%) was significantly higher than that in estrogen receptor-negative patients (66.7%) ($P = 0.041$). Further correlation analysis showed that estrogen receptor positivity was correlated with high expression of hMOF ($r = 0.205$, $P < 0.05$) (Table 2).

Table 3. Multivariate analysis of the prognosis of patients with endometrial carcinoma

	<i>P</i>	Exp(B)	95.0% CI for Exp(B)
FIGO stage (I/II vs. III/IV)	0.001	0.449	0.28–0.72
ER (negative vs. positive)	0.039	2.867	1.052–7.815
hMOF (low vs. high)	0.000	0.064	0.022–0.178

**Figure 3.** Kaplan–Meier survival analysis of endometrial carcinoma. Number, *P* value, log-rank test.

High expression of hMOF is an independent risk factor for prognosis in patients with endometrial carcinoma

111 cases of endometrial cancer were followed up, a total of 99 patients had complete follow-up and, 12 patients were lost to follow-up. The longest follow-up period was 138 months, and the shortest was 28 months (median follow-up time was 45 months). Twenty-seven patients died. Kaplan–Meier survival analysis of 99 patients suggested that the survival time of patients with low hMOF expression (mean = 123 months) was significantly higher than that of patients with high hMOF expression (mean = 72 months), $P = 0.045$ (Fig. 3).

Multivariate regression analysis including variables of estrogen receptor expression, FIGO stage, hMOF expression, pathological subtype, degree of differentiation and depth of invasion showed that stages III–IV, negative estrogen receptor expression and high hMOF expression were independent risk factors for endometrial carcinoma (Table 3).

The high expression of hMOF promotes proliferation, migration and invasion, but inhibits apoptosis of endometrial carcinoma cell

We detected the expression of hMOF in Ishikawa (ER+) and HEC1A (ER-) cell lines. The results showed that hMOF was expressed in Ishikawa cells, but almost not expressed in HEC1A cells. Therefore, we chose Ishikawa cell line for subsequent experiments. We observed the changes in biological behaviours of Ishikawa cells before and after transient transfection of hMOF. After hMOF expression knocked down, the proliferative ability of Ishikawa cells decreased significantly, and both early and late apoptoses increased; in addition,

migration and invasive capacity of the cells significantly decreased compared with that of the control group (Fig. 4).

Low concentration of estrogen enhanced the expression of hMOF in endometrial carcinoma (ER+) cells and promoted the proliferation of the cells

Endometrial carcinoma cell lines with different estrogen receptor (ER) status, including Ishikawa (ER+) and HEC1A (ER-), were treated with gradient concentrations of estrogen (10^{-10} , 10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} mol/l). The MTT assay was performed at 24, 48 and 72 h after treatment. Results showed that the proliferation of Ishikawa cells treated with 10^{-9} and 10^{-8} mol/l of estrogen for 72 h was significantly higher than that of the control group ($P = 0.003$), while HEC1A cells treated with the same concentrations of estrogen and assessed at the same time points did not show significant differences in cell proliferation when compared with the control group ($P > 0.05$). Thus, estrogen affected only estrogen receptor-positive cells such as Ishikawa cells, but did not affect estrogen receptor-negative cells (Fig. 5A).

Ishikawa cells were further stimulated by gradient concentrations of estrogen and the estrogen receptor antagonist ICI182780, and the expression of hMOF mRNA and protein was then detected. The results showed that low concentration of estrogen promoted hMOF protein expression in Ishikawa cells, but high concentration of estrogen inhibited hMOF protein expression. Furthermore, hMOF protein expression increased accordingly with the increase in the concentration of the estrogen receptor antagonist. In HEC1A cells with low hMOF protein level compared with that in Ishikawa cells, hMOF protein expression did not show a significant change after estrogen stimulation. It is worth noting that the hMOF mRNA level was not significantly different between the two cell lines, and there was no significant change in hMOF mRNA expression after estrogen stimulation (Fig. 5B).

Blocking hMOF by a monoclonal antibody inhibited the pro-proliferative effect of estrogen on Ishikawa cells

To further investigate the effect of hMOF on endometrial carcinoma proliferation, we observed the changes in the proliferation of endometrial carcinoma cells before and after the addition of estrogen and blocking anti-hMOF antibody. Flow cytometry analysis indicated that the number of Ishikawa cells in the G1 phase was significantly decreased after stimulation with estrogen at 10^{-8} mol/l concentration and the number of cells in the S phase was significantly increased, indicating the entry of the cells into the cell cycle mode ($P < 0.01$). Thus, this finding show that estrogen promoted the proliferation of Ishikawa cells. However, estrogen treatment combined with blocking with anti-hMOF antibody significantly decreased cell proliferation. The number of cells in the S phase decreased, and most of the cells remained in the G1 phase (Fig. 6).

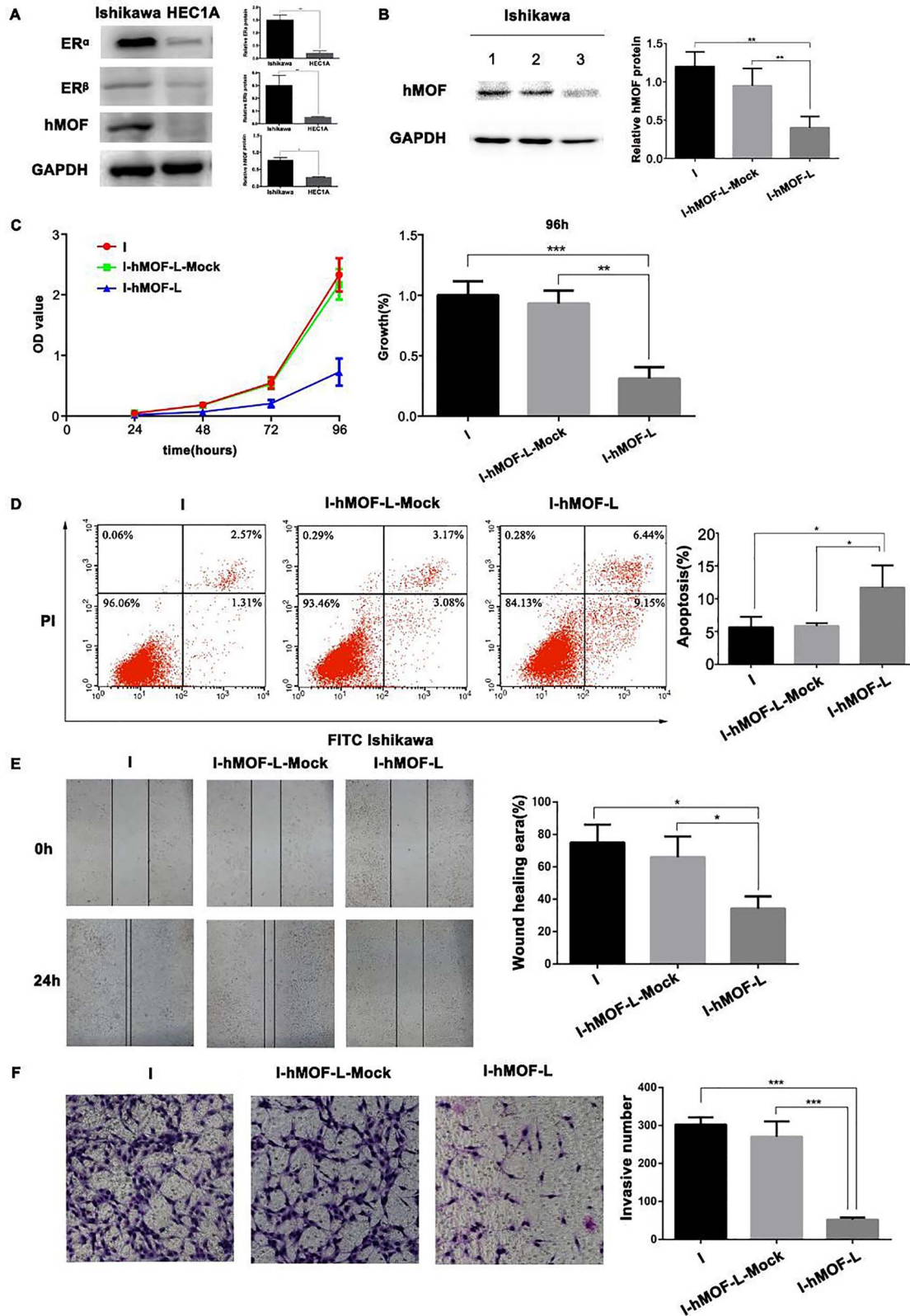


Figure 4. High expression of hMOF promotes proliferation, migration and invasion, but inhibits apoptosis of endometrial carcinoma cell. A. The expression of ER α , ER β and hMOF protein in Ishikawa and HEC1A cell ($n = 3$, means \pm SD; * $P < 0.01$ vs. HEC-1A). B. hMOF protein levels in Ishikawa before and after transfection of *sihMOF*. C: Changes in cell proliferation rate before and after transfection of *sihMOF*. D: Apoptosis changes before and after transfection of *sihMOF*. E: Changes in cell migration before and after transfection of *sihMOF*. F: Changes in cell invasive abilities before and after transfection of *sihMOF*.

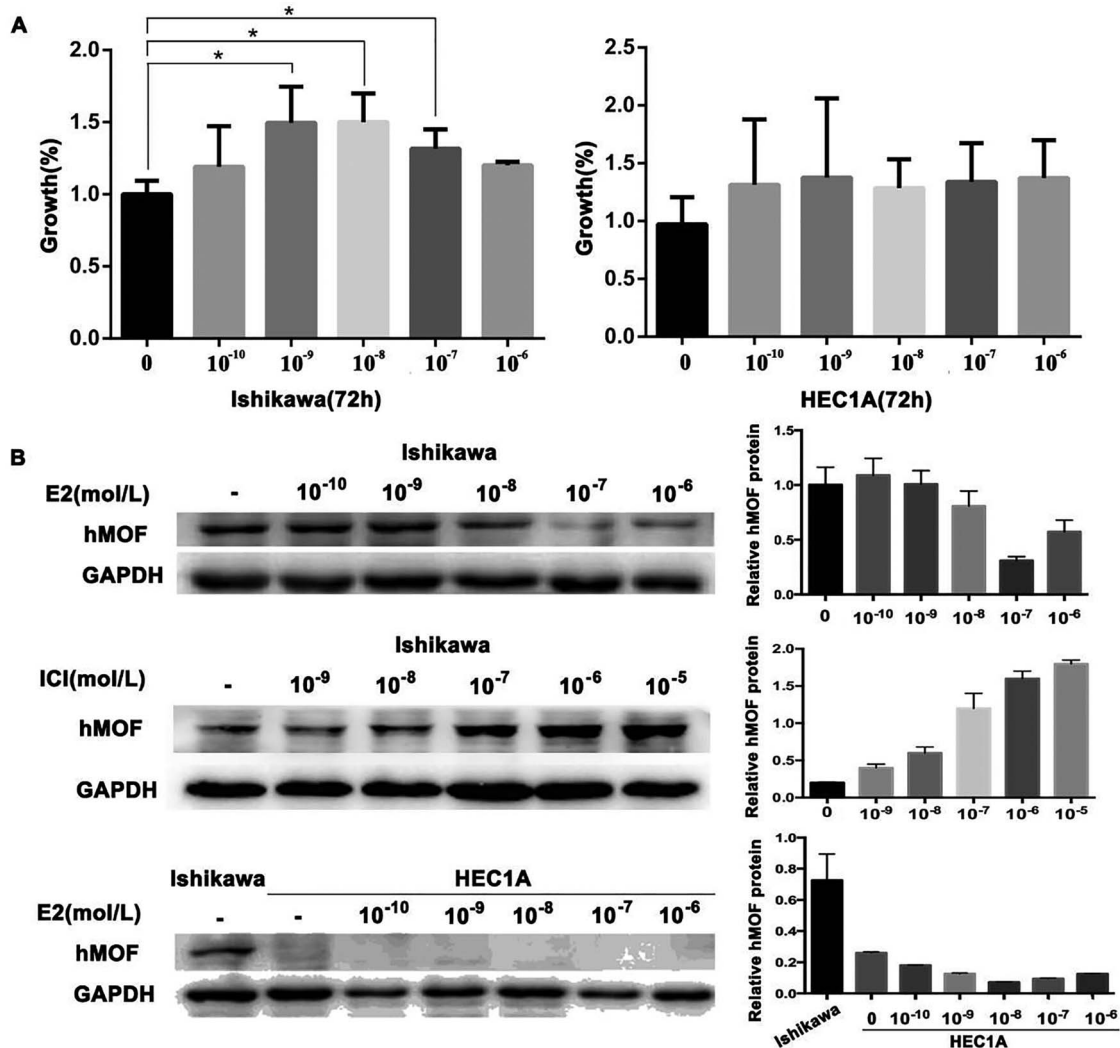


Figure 5. Low concentration of estrogen enhanced the expression of hMOF in Ishikawa cells and promoted its proliferation. A: Changes in cell proliferation rate before and after treatment with gradient concentrations of estrogen (10^{-10} , 10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} mol/l). B: hMOF protein levels in Ishikawa and HEC1A cells: E2 dose response, ICI dose response.

Estrogen activated the PI3K/Akt and Ras-Raf-MEK-ERK signalling pathways to promote hMOF expression

Western blotting analysis showed that the phosphorylation levels of Akt and ERK in Ishikawa cells were significantly increased after estrogen stimulation ($P < 0.05$) (Fig. 7A). The hMOF protein level was significantly increased after estrogen treatment alone; however, the addition of LY294002, a specific inhibitor of the PI3K pathway, or inhibitor of the MEK pathway, significantly reversed the effect of estrogen on increasing hMOF expression ($P < 0.05$) (Fig. 7B) ($P = 0.0334, P = 0.0447$).

Gene enrichment analysis

To further investigate pathways regulated by KAT8, gene enrichment analysis of KAT8 was performed. The results indicated that the cell cycle pathways and spliceosome-related gene clusters were enriched in KAT8 high expression samples (Fig. 8).

Relationship of KAT8 expression with copy number variation and ESR1

To investigate the high expression of KAT8 in endometrial cancer, a correlation analysis of copy number and methylation level was

conducted. There were 61 samples underwent copy number amplification and gain in the TCGA endometrial cancer data (Supplementary material 1). Correspondingly, the expression of the samples with copy number amplification was also significantly increased, indicating that the high expression of KAT8 in endometrial cancer was partly caused by copy number amplification and gain (Fig. 9A). KAT8 expression and ESR1 correlation analysis showed a positive correlation; combined with the literature, it is suggested that the expression of ESR1 promotes the expression of KAT8 to a certain extent (Fig. 9B) (Supplementary material 2).

GO function and PPI network construction

GO function enrichment analysis of related genes was applied using DAVID (Supplementary material 3). The bubble map was drawn using the ggplot2.R package for the significant biological processes based on P value. GO analysis results showed that these related genes were particularly enriched in GO:0006412, translation; GO:0006364, rRNA processing; GO:0019083, viral transcription; and GO:0000398, mRNA splicing, via spliceosome and so on (Fig. 10A) (Supplementary material 4). On the basis of information from the STRING database, we constructed the PPI

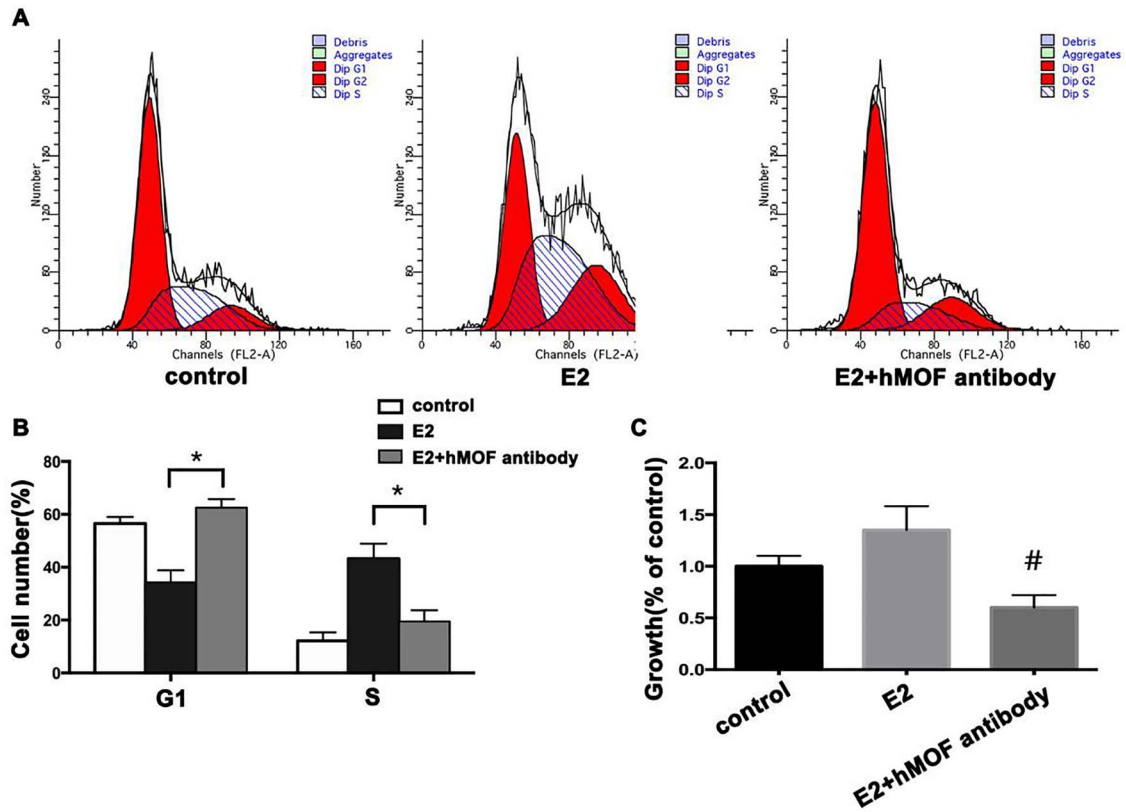


Figure 6. hMOF monoclonal antibody inhibited the proliferation of Ishikawa cells induced by estrogen. A: Cell cycle changes of Ishikawa cells treated with estrogen and estrogen plus hMOF antibody. B: Quantitative analysis of cell cycle in G0/G1 phase and S phase ($n=3$, means \pm SD; * $P < 0.01$ vs. control group). C: Treated with estrogen and estrogen plus hMOF antibody, and cell viability was estimated using MTT assay ($n=3$, means \pm SD; # $P < 0.01$ vs. E2 group).

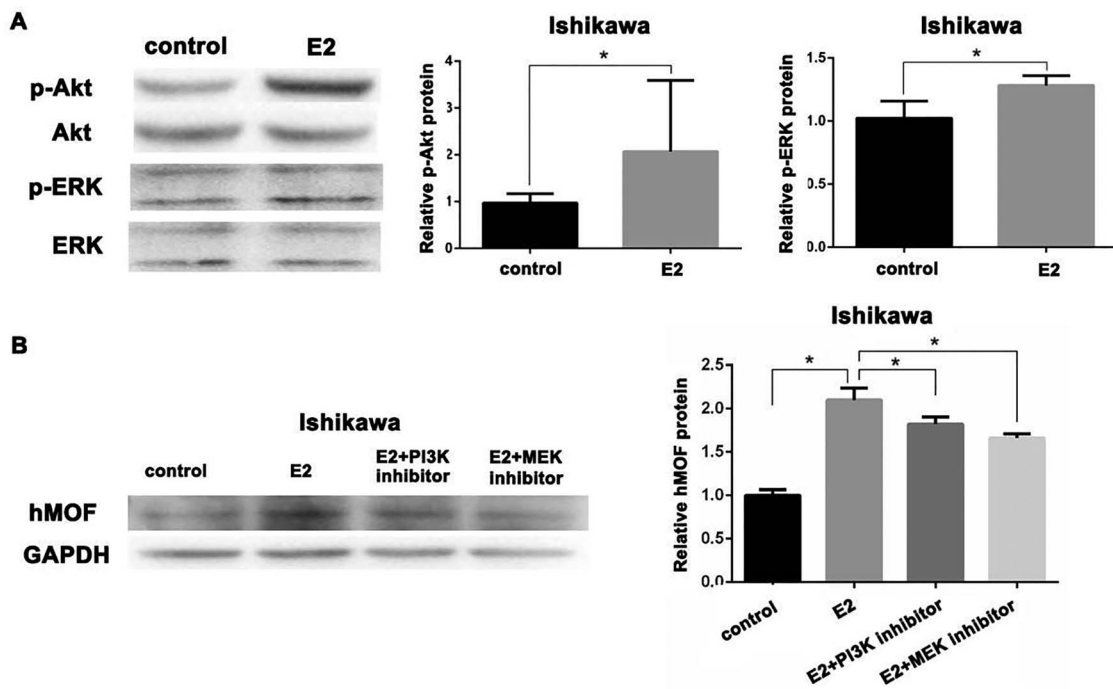


Figure 7. Estrogen activated the PI3K/Akt and Ras-Raf-MEK-ERK pathways to promote hMOF expression. A: The expression of p-AKT; p-ERK treated with estrogen. B: Treated with estrogen, PI3K inhibitor and MEK inhibitor.

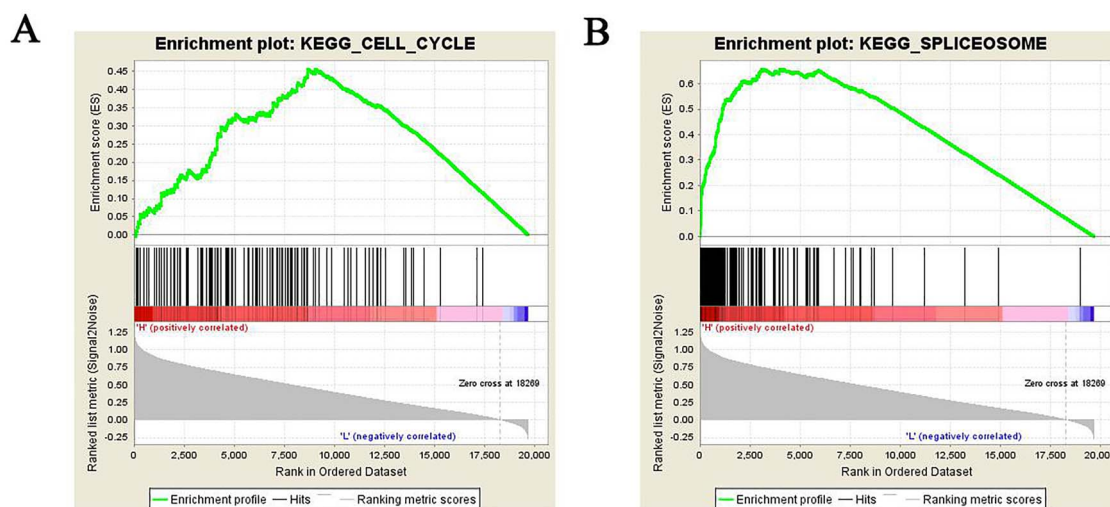


Figure 8. KAT8 correlated enrichment gene analysis with GSEA. A: Cell cycle (P value = 0.000; FDR = 0.035; enrichment score = 0.456). B: Spliceosome (P value = 0.000; FDR = 0.000; enrichment score = 0.658).

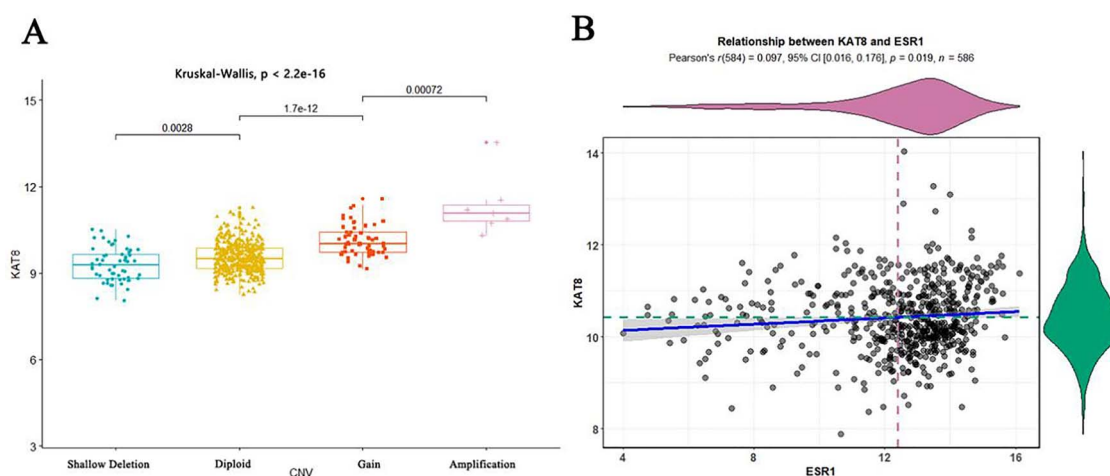


Figure 9. Correlation of KAT8 expression with copy number variation and ESR1. A: Correlation analysis between KAT8 expression and copy number variation. B: Correlation analysis between KAT8 expression and ESR1.

network of the gene expression product in endometrial cancer. After removing the separated and partially connected nodes, a complex DEG network was constructed. The final PPI network had 291 nodes and 1283 edges; the average aggregation coefficient was 0.555, and the enrichment P value was $< 1.0e-16$ (Fig. 10B).

Conclusion

Endometrial cancer is mainly hormone dependent; estrogen can induce histone acetylation (19) and play an important role in the pathogenesis of endometrial carcinoma (20). Studies have shown that histone acetylation levels in mouse mammary tissue were significantly increased after long-term stimulation with high-dose estrogen and obvious pathological changes were observed (21), suggesting that estrogen may also cause changes in the acetylation levels of endometrial carcinoma tissue. By regulating histone acetyltransferase p300/CBP, estrogen induces H4 hyperacetylation and transcription activation of target genes in the breast cancer cell line MCF-7,

which promote the proliferation of MCF-7 cells (22), suggesting that estrogen session of the estrogen receptor is a key mediator in the impact of estrogen on the occurrence and development of endometrial cancer (23). Therefore, it is speculated that estrogen regulates the histone acetylation level by regulating the expression of histone deacetylase through estrogen receptors.

hMOF is an important member of MYST family, which is one of the three main acetylase families. Abnormal expression of hMOF is closely associated with the occurrence, development and prognosis of various tumours, although its expression varies with tumour types. Our previous studies also showed that hMOF expression is low in ovarian cancer tissue but high in endometrial epithelial ovarian cancer tissue (12). We further detected hMOF expression in endometrial carcinoma and found that compared with normal endometrial tissue (including proliferative and secretory phase), hMOF expression in endometrial carcinoma tissue was significantly increased. High expression of hMOF was positively correlated with FIGO stage and lymphatic metastasis and was an independent risk factor for the prognosis of patients with endometrial carcinoma. We

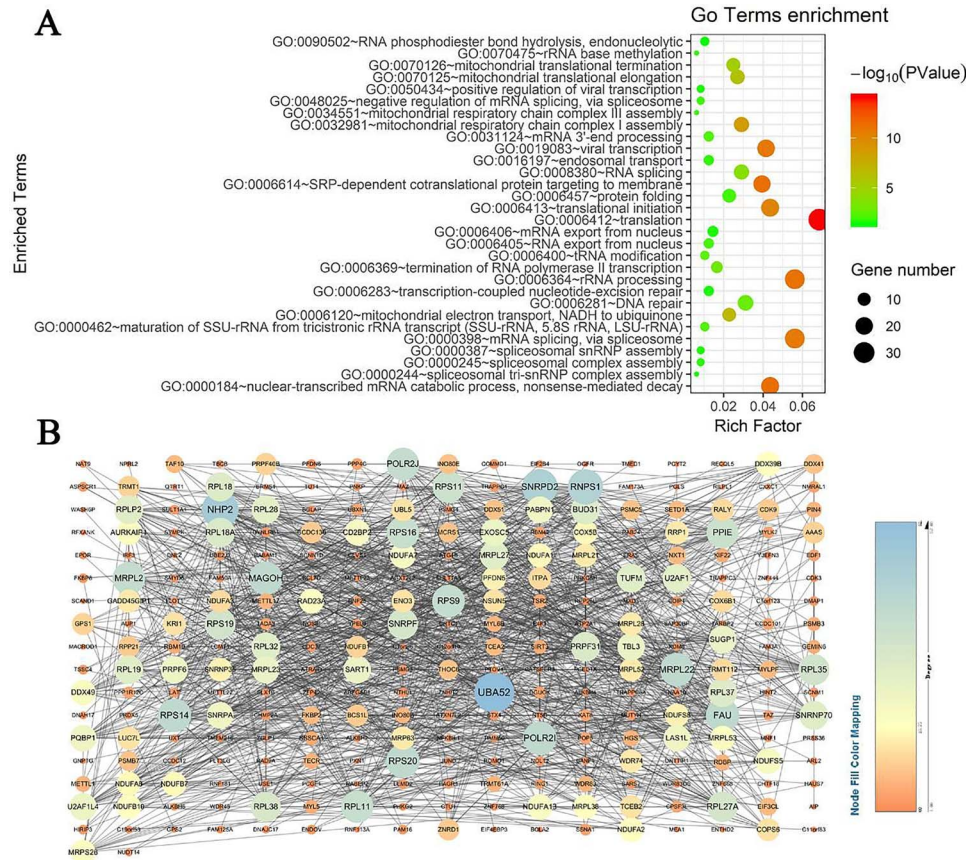


Figure 10. Biological processes enriched in gene ontology terms and protein–protein interaction network based on correlated genes with KAT8. A: Enriched terms of biological processes. B: Each node in the figure represents a protein, and the edge between the nodes represents the interaction between the two proteins. Different thicknesses of the edges represent the strengths of the relationships. The larger the degree, the larger the node.

also found that hMOF was highly expressed in the endometrial tissue during the normal proliferative phase without a significant difference compared with the expression level in the cancer tissue. The development of endometrial carcinoma is associated with long-term estrogen stimulation. The proliferation of the endometrium is mainly regulated by estrogen. Thus, the common mechanism suggested that hMOF expression may be affected by estrogen. By analysing the correlation between hMOF and the clinicopathological features of endometrial carcinoma, we found that high expression of hMOF was positively correlated with estrogen receptor; furthermore, the expression level of hMOF in endometrioid (estrogen dependent) was significantly higher than that in serous carcinoma and clear cell carcinoma (estrogen independent). Further analysis of endometrial serous cancers revealed that eight were estrogen receptor-positive (8/22,36.4%), and among these eight cases, seven had high expression of hMOF. Therefore, we hypothesized that the abnormal expression of hMOF in different pathological types of endometrial carcinoma was closely associated with the expression of estrogen receptor. By *in vitro* experiments, we found that Ishikawa cells (estrogen receptor-positive) expressed hMOF and HEC1A cells (estrogen receptor-negative) showed negligible hMOF expression. We then knocked down hMOF expression by transiently transfecting interfering RNA of hMOF in Ishikawa cells. Subsequently, the proliferative ability of Ishikawa cells significantly decreased, together with its migration and invasion ability; moreover, both early and late apoptotic rates of the cells increased. Therefore, hMOF had

the potential to promote Ishikawa cell proliferation, migration and invasion and inhibit apoptosis. By blocking hMOF with anti-hMOF monoclonal antibody, we found that compared with endometrial carcinoma cells treated with estrogen alone, the cells treated with estrogen plus antibody blocking were more arrested in the G1 phase. This finding suggested that inhibiting hMOF expression could inhibit Ishikawa cell proliferation stimulated by estrogen, thus highlighting the important role of hMOF in estrogen-regulated endometrial carcinoma proliferation. To determine the reason of the high expression of hMOF in endometrial carcinoma cells, we analysed the copy number variation of hMOF in endometrial carcinoma cells and found the amplification of hMOF partially contributed to its high expression in endometrial carcinoma; moreover, hMOF expression was positively correlated with ESR1 (estrogen receptor gene) expression, thus suggesting that ESR1 promotes hMOF expression to a certain extent. We therefore concluded that estrogen regulated hMOF expression in endometrial carcinoma cells mediated by estrogen receptor to affect the malignant biological behaviours of endometrial carcinoma cells.

Interestingly, we found that a low concentration of estrogen promoted the expression of hMOF protein but a high concentration of estrogen inhibited its expression. The concentration range used in our experiments was 10^{-10} – 10^{-8} mol/l, which was close to the concentration of estrogen in the endometrial carcinoma tissue (24). Previous studies have shown that the optimal concentration of estrogen to stimulate Ishikawa cell proliferation is 10^{-9} – 10^{-8} mol/l, and

high concentration of estrogen inhibits Ishikawa cell proliferation. Estrogen at a concentration of 10^{-7} mol/l or higher could induce Ishikawa cell apoptosis, which may be due to the inhibition of the hMOF protein by the high concentration of estrogen; this finding also supported the important function of hMOF in estrogen-stimulated Ishikawa cell proliferation.

Next, we searched the KEGG database to find the pathways related to estrogen function in order to investigate the mechanisms by which estrogen regulates hMOF. We found that the PI3K/Akt and Ras-Raf-MEK-ERK signalling pathway was critical for estrogen function. The PI3K/Akt pathway is one of the most important signalling pathways that is closely related to cell proliferation (25). The Ras-Raf-MEK-ERK pathway is also important for cell survival, proliferation, invasion and migration (26). We detected the expression of Akt and ERK, the important signalling molecules in the two abovementioned pathways, in Ishikawa cells after treatment with estrogen and found that the phosphorylation levels of Akt and ERK were significantly increased after estrogen stimulation; further, hMOF protein expression was also increased. The addition of LY294002, a specific inhibitor of the PI3K pathway, or a specific inhibitor of the MEK pathway decreased hMOF protein expression. This finding confirmed that estrogen could activate the PI3K/Akt and Ras-Raf-MEK-ERK signalling pathway to regulate hMOF expression.

hMOF is encoded by the KAT8 gene. We found that KAT8-related genes were enriched in the cell cycle pathways and gene splicing pathways; this finding was in line with our previous results that the increase in hMOF expression promotes cell cycle progression of cancer cells.

Our study found that estrogen enhanced the expression of hMOF in endometrial carcinoma cells through estrogen receptor, thereby promoting cell proliferation, migration and invasion and inhibiting apoptosis of these cells. The high expression of hMOF in the endometrial carcinoma tissue was associated with late-stage cancer and poor differentiation and was an independent risk factor for poor prognosis of patients with endometrial carcinoma. Estrogen enhanced hMOF expression by regulating the PI3K/Akt and Ras-Raf-MEK-ERK signalling pathways, further affecting the acetylation levels of the histones associated with genes involved in transcription, mRNA splicing or viral transcription. Consequently, the balance of the expression of oncogenes and tumour-suppressor genes as well as genes related to cell proliferation and apoptosis is impaired, and endometrial carcinoma develops. Further studies are needed to elucidate the specific mechanisms by which hMOF affects the biological behaviours of endometrial carcinoma cells. Our study revealed that like histone deacetylase, hMOF is a potential target for endometrial carcinoma treatment.

Supplementary data

Supplementary data are available at *JJCOJ* online.

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Conflict of interest statement

The authors declare that they have no conflict of interests.

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