British Journal of Cancer www.nature.com/bjc

ARTICLE

Check for updates

Cellular and Molecular Biology

TSLP enhances progestin response in endometrial cancer via androgen receptor signal pathway

Mu Lv[1,10], Yuan Xu[2,10], Peiqin Chen[3], Jingjie Li[2], Zuoshu Qin[1], Baozhu Huang[2], Yong Liu[4], Xiang Tao[5], Jun Xiang[1], Yanqiu Wang[1], Youji Feng[2], Wenxin Zheng[6,7], Zhenbo Zhang[1]✉, Linxia Li[8]✉ and Hong Liao[9]✉

BACKGROUND: The enriched proteins within in vitro fertilisation (IVF)-generated human embryonic microenvironment could reverse progestin resistance in endometrial cancer (EC).
METHODS: The expression of thymic stromal lymphopoietin (TSLP) in EC was evaluated by immunoblot and IHC analysis. Transcriptome sequencing screened out the downstream pathway regulated by TSLP. The role of TSLP, androgen receptor (AR) and KANK1 in regulating the sensitivity of EC to progestin was verified through a series of in vitro and in vivo experiments.
RESULTS: TSLP facilitates the formation of a BMP4/BMP7 heterodimer, resulting in activation of Smad5, augmenting AR signalling. AR in turn sensitises EC cells to progestin via KANK1. Downregulation of TSLP, loss of AR and KANK1 in EC patients are associated with tumour malignant progress. Moreover, exogenous TSLP could rescue the anti-tumour effect of progestin on mouse in vivo xenograft tumour.
CONCLUSIONS: Our findings suggest that TSLP enhances the sensitivity of EC to progestin through the BMP4/Smad5/AR/KANK1 axis, and provide a link between embryo development and cancer progress, paving the way for the establishment of novel strategy overcoming progestin resistance using embryo original factors.

British Journal of Cancer (2024) 130:585–596; https://doi.org/10.1038/s41416-023-02545-y

INTRODUCTION

Endometrial cancer (EC) is one of the most common types of gynaecologic cancers in women worldwide [1]. Long-term, high-dose progestin therapy has historically been the most commonly used strategy in conservative treatment for endometrial cancer and hyperplasia [2], providing considerable relief to young EC patients with infertility. However, not all patients respond well to progestin and the failure rate of progestin therapy is still as high as 30% [3]. Once progestin resistance occurs, the time of surgery is delayed appropriately, which commonly results in a poor prognosis. Therefore, preventing progestin resistance is a long-sought goal in conservative therapy for EC.

Substantial progress has been made in overcoming progestin resistance in recent decades. However, a suitable method to sensitise EC cells to progestin is still lacking. An embryonic microenvironment may be able to reverse the metastatic behaviour of cancer cells [4]. Mintz and Illmensee demonstrated that teratocarcinoma cells' tumorigenic phenotype could be suppressed in a mouse embryonic blastocyst microenvironment, and the developmental adaptability of these tumour cells was manifested in their ability to participate in generating normal tissue [4]. Testing of numerous cancer cell lines revealed the tumour-suppressive characteristics of embryonic microenvironments, supporting this theory [5, 6]. In our previous study, we discovered that the in vitro fertilisation (IVF)-generated human embryonic microenvironment can reverse progestin resistance in EC by enhancing cancer stem cell differentiation [7]. This observation suggests that the components, including proteins, RNA and DNA, of the human embryonic microenvironment might play an essential role in the suppression of EC cell growth. Importantly, these components are derived from human embryos, and are thus safe if they were developed to overcome human EC progestin resistance. Another intriguing thing is that embryonic cells share some similar properties with cancer cells, such as the morphology, multipotency and differentiation of naïve cells. This prompted us to investigate which factor in the embryonic

[1]Reproductive Medicine Center, Department of Obstetrics and Gynecology, Tongji Hospital, School of Medicine, Tongji University, 200065 Shanghai, China. [2]Department of Obstetrics and Gynecology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, 200080 Shanghai, China. [3]Department of Obstetrics and Gynecology, The International Peace Maternity & Child Health Hospital of China Welfare Institute, Shanghai Jiao Tong University School of Medicine, 200030 Shanghai, China. [4]Department of Radiation Oncology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, 200080 Shanghai, China. [5]Department of Pathology, Obstetrics and Gynecology Hospital of Fudan University, 200090 Shanghai, China. [6]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA. [7]Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA. [8]Department of Obstetrics and Gynecology, Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine, 358 Datong Road, 200137 Shanghai, China. [9]Department of Clinical Laboratory Medicine, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, 200040 Shanghai, China. [10]These authors contributed equally: Mu Lv, Yuan Xu.
✉email: zhangzhenbozzb@aliyun.com; lilinxia@hosno7.com; liaohonglp@aliyun.com

Received: 14 June 2023 Revised: 5 December 2023 Accepted: 7 December 2023
Published online: 3 January 2024

microenvironment is indispensable in facilitating progestin response and to explore the function of the candidate factor in EC.

Thymic stromal lymphopoietin (TSLP) is found in the IVF-generated human embryonic microenvironment [7]. Whether TSLP plays a role in overcoming progestin resistance is unknown. The inflammatory cytokine TSLP is secreted mostly by epithelial cells. TSLP has been shown to regulate the occurrence and progression of tumours [8–10]. Overexpression of TSLP in the early stages of breast and pancreatic cancer can activate Th2 cells and inhibit tumour progression [11]. Furthermore, TSLP inhibits cancer progression by stimulating apoptosis and suppressing cell growth [12]. Demehri et al. noted that increased TSLP levels in the epidermis can stimulate the formation of an antitumor environment [13]. However, the expression profile and role of TSLP in EC have not been fully elucidated.

Since the 1990s, increasing evidence has shown that the androgen receptor (AR) is involved in EC occurrence and development [14, 15]. As we all know, progesterone receptor (PR) signalling antagonises oestrogen-driven tumorigenesis. AR and PR have a certain functional overlap, which prompts us to investigate whether AR performs similar functions in endometrial tumorigenesis. Mechanistically, both receptors can bind to the same cis-responsive elements and regulate similar downstream genes [16]. Functionally, androgen and its ligand are responsible for the suppression of EC growth [17, 18]. This conclusion is supported by the finding that using testosterone to treat women who underwent denaturation can lessen endometrial gland and stromal cell proliferation, and enhance AR expression in the matrix [19]. Functional studies have also revealed that treatment of human primary endometrial stromal cells with dihydrotestosterone (DHT) in vitro can significantly diminish cell proliferation, migration, and AR-dependent apoptosis [20]. Accumulating evidence clearly illustrates that AR signalling is crucially involved in EC therapy.

Bone morphogenetic protein (BMP) is the most critical member of the TGF-β superfamily, a group of cell growth factors secreted from the bone matrix and highly effective in inducing bone, cartilage, and tissue formation [21]. The R-Smads, which comprise Smad1/5/8, are the main BMP receptor signal transducers. The ligand-bound BMP type I receptor phosphorylates the C-terminal of R-Smads, allowing them to interact with Smad4 and translocate to the nucleus where they control the transcription of target genes [22]. In DU145 prostate cancer cells, Smad3 can function as a positive coregulator of AR [20]. Thus, because EC is a sex hormone-dependent cancer, it would be interesting to investigate the interrelationship between BMP/Smad signalling and the AR axis in EC. Since AR is also a transcription factor, it generally regulates downstream gene expression by binding to the androgen response element (ARE) in the promoter region of a target gene [19]. KANK1 was defined as an anti-oncogene for the first time in renal cell carcinoma [23]. This gene is downregulated in a variety of cancers, including renal cell carcinoma, nasopharyngeal carcinoma, gastric cancer, and others [23–25]. By regulating RhoA activity via PI3K/Akt signalling, KANK1 can regulate actin polymerisation, and inhibit cell migration [26]. Our earlier findings indicated that the PI3K/Akt pathway was closely related to progestin resistance [27]. Therefore, we speculated that KANK1 may be involved in progestin sensitivity.

Our goal in this study is to investigate: (1) the role of TSLP in reversing progestin resistance or facilitating progestin sensitivity; (2) how AR signalling contributes to TSLP-mediated progestin sensitivity; and (3) the relationship between TSLP-AR-KANK1 signalling and EC clinical outcomes. These findings will be beneficial for the development of new therapeutic strategies for the conservative treatment of EC.

MATERIALS AND METHODS

Cell lines and cell culture

All cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The human EC cell lines ECC1 (RRID: CVCL_7260) and SPEC2 (RRID: CVCL_A679) were cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10% foetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA). The human endometrial adenocarcinoma cancer cell lines Ishikawa (RRID: CVCL_2529), HEC1B (RRID: CVCL_0294), AN3CA (RRID: CVCL_0028) and normal human endometrial stromal cell (HESC) (RRID: CVCL_C464) were cultured in DMEM medium (Gibco, USA) supplemented with 10% foetal bovine serum, and 1% penicillin/streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C. All cell lines were authenticated in the past 3 years by short tandem repeat identification, and all experiments were performed with mycoplasma-free cells.

Protein microarray analysis of IVF-generated fluids

In case it forms the ice crystal during vitrification of the embryos selected for clinical use at the Reproductive Medicine Center, high-quality blastocysts were routinely treated with laser and then the blastocyst fluid flowed out and has been collected as described previously [7]. We used commercial G2 blastocyst medium without cultured embryos as a control, and chose Raybiotech's AAH-BLM-1000 ProteinChip to test in accordance with standard operating procedures. Informed consent was obtained from all subjects. The study was approved by the Medical Ethics Committee of Shanghai General Hospital, Shanghai Jiao Tong University, with a project licence number of 2021KY130.

Transient transfection of plasmids and construction of stable cell lines

The TSLP, TXK, APEX1, ADAMTSL2, TNK1, LALBA, RYK and AR overexpression plasmids were constructed in the pCDNA3.1 vector and maintained in our laboratory. The KANK1, Smad5 overexpression plasmids, and the shRNA-KANK1 plasmid were purchased from Shandong WZ Biosciences Inc. (Shandong, China). The plasmids Flag-BMP4, HA-BMP4, and Flag-BMP7 were purchased from Shanghai Generay Biotech Co., Ltd (Shanghai, China). The above plasmids were transfected into EC cells using Lipofectamin™ 3000 (Invitrogen, USA) according to the manufacturer's instructions. Stably transfected cell lines overexpressing Flag-TSLP were produced using retrovirus as previously described [28].

Immunoblot analysis

Immunoblot analysis was performed as previously described [7]. Detailed methods are provided in the Supplementary Methods. The primary antibodies used for western blot included anti-TSLP, anti-Cyclin D1, anti-AR, anti-PR and anti-β-actin (all from Cell Signaling Technology), anti-α-Tubulin, anti-BMP4, anti-BMP6, anti-BMP7, anti-Smad1, anti-Smad5, anti-Smad9, anti-phospho-Smad5 and anti-Lamin B1 (all from Abcam), and anti-KANK1 (Santa Cruz Biotechnology).

RNA extraction and transcriptome sequencing

According to the manufacturer's protocol, total RNA was extracted from Ishikawa and Ishikawa-TSLP cell lines using the TRIzol reagent (TAKARA, Japan). The cDNA library was created by Personalbio (Shanghai Personal Biotechnology, Co., Ltd., Shanghai, China) using the TruSeq RNA Sample Prep Kit (Illumina). Quantification was performed using the Quantifluor-ST fluorometer (Promega, E6090) and the Quant-iTPicoGreen dsDNA Assay Kit (Invitrogen, P7589), and qualified by using Agilent 2100 Bioanalyzer and High Sensitivity DNA Kit (5067-4626, Agilent Technologies, USA). Following that, 100-bp paired-end reads were used for paired-end sequencing on an Illumina Hiseq 2000 platform at Personalbio.

Quantitative real-time PCR (qPCR)

TRIzol reagent was used for RNA extraction, which was followed by reverse transcription with a reverse transcriptase kit (TAKARA, Japan) according to the manufacturer's instructions. The ABI QuantStudio6 system was used to perform qPCR. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method using GAPDH as a loading control. The PCR primers are listed in Supplementary Table 1.

Hormone treatment and cell proliferation

A total of 3000 cells were inoculated in each well of a 96-well plate. Medroxyprogesterone acetate (MPA) was applied to treat EC cells for the given time and at the given dose. Then, 10 μL of CCK-8 reagent (Dojindo, Japan) was added directly to each well of culture medium at the indicated times. The cells were then incubated in a 37 °C incubator for 2 h and the

optional density (OD) was measured at 450 nm using a microplate reader (BioTek Instruments, USA).

Dual-luciferase reporter assay

The AR-Luc reporter plasmid was purchased from Shanghai Genomeditech (Shanghai, China). The ARE sequences in the KANK1 promotor and the corresponding mutant sequences were designed and inserted into the pGL4.74 vector, with the resulting luciferase reporter plasmids termed KANK1-AREs, and AREs-mut, respectively. The Dual-Luciferase Assay Kit (Promega) was used to evaluate the relative luciferase activity. The pGL4.27 plasmid was transfected as a negative control. The ARE sequences in the KANK1 promotor and the corresponding mutant sequences were as follows:

#1:AAAAGACTGTGTTCT Mutant #1: AAAGGACTGTGGGAT
#2: GTAAGTGTTTGTTCT Mutant #2: GTAGGTGTTTGGGAT
#3: AACAGTTTATGTTCT Mutant #3: AACGGTTTATGGGAT
#4: GGTTCAAGCAGTTCT Mutant #4: GGTGCAAGCAAGGCT.

Electrophoretic mobility shift assay (EMSA)

The extraction method of cellular nuclear proteins was based on previous reports [29]. The biotin-labelled oligonucleotides utilised as probes were purified using Sephadex G50 spin columns. The labelled probe was incubated at 50,000 c.p.m. with nuclear extracts (20 mg) [29]. The mixture was incubated for 20 min at room temperature in the presence or absence of the unlabelled competitive oligonucleotide. Then the whole reaction mixture was subjected to electrophoresis on a 6% polyacrylamide gel for 3 h at 150 volts. The sample was then transferred to a nylon membrane at 380 mA (~100 V) for 30–60 min using 0.5×TBE solution as the transfer solution. Next, the nylon membrane was placed in a UV crosslinker, selecting 254 nm UV wavelength, and crosslinked at 120 mJ/cm^2 for 45–60 s. Then the nylon membrane was put into the sealing solution for sealing. Finally, the biotin-labelled probes were detected by chemiluminescence.

Co-immunoprecipitation (Co-IP)

Ishikawa cells were co-transfected with HA-tag BMP4 plasmid, Flag-tag BMP4 plasmid, and Flag-tag BMP7 plasmid, and then treated with 100 ng/ml rhTSLP for 48 h or left untreated. Cells were lysed with NP-40 lysis buffer. Cell lysates were incubated with an anti-HA polyclonal antibody and protein G-agarose beads (Roche) overnight at 4 °C. The agarose beads were then washed three times with weak protein lysate. Finally, the agarose beads-antigen-antibody complexes were collected and boiled at 100 °C for 10 min as IP samples. The samples were centrifuged at 2000 rpm for 3 min and the supernatants were collected to verify protein binding by western blot.

Chromatin immunoprecipitation (ChIP)

The ChIP assay was carried out using a ChIP Assay Kit (Beyotime). Cells were fixed and harvested using the manufacturer's protocol. DNA was sonicated and was then incubated with the indicated antibodies. The quantity of bound DNA was determined using qPCR. The primer sequences utilised were as follows: 5′-TAACTGGCCGGTACCG-3′ and 3′-TAAGCTTCTGCAGATC-5′. The process of qPCR was as follows: Denaturing at 95 °C for 10 s, Annealing at 60 °C for 30 s, 40 cycles. The value of enrichment was calculated based on the relative amount of input and the ratio of IgG.

Immunofluorescence

Immunofluorescence was performed as previously described [7]. An anti-phospho-Smad5 antibody (Beyotime) was used for immunofluorescence analysis. Pictures were taken under a fluorescence microscope (Leica, Munich, Germany).

Clinical specimens and immunohistochemical (IHC) analysis

A total of 135 samples were collected from the Department of Obstetrics and Gynaecologist at Shanghai General Hospital in China, comprising 117 samples of EC and 18 samples of the proliferative phase. The experiments were undertaken with the understanding and written consent of each subject. The study methodologies conformed to the standards set by the Declaration of Helsinki. Tissues were obtained in accordance with the Medical College's Institutional Review Board's rules from Shanghai Jiao Tong University in China, with a project licence number of 2022KY053. Samples were stained for TSLP (Abcam, ab188766, 1:400), AR (Cell Signaling Technology, #5153, 1:400) and KANK1 (Santa Cruz, sc-517629, 1:200). IHC staining and scoring were carried out exactly as previously demonstrated [30, 31].

In vivo xenograft experiments

The animal experiments in this study were authorised by Shanghai General Hospital's Animal Ethics Committee, with a project licence number of 2020AW121. Ishikawa or Ishikawa-TSLP endometrial cancer cells were subcutaneously implanted into female BALB/c nude mice (1×10^6 cells, respectively, $n = 5$). The mice were then randomly divided into groups of five mice each. After the tumours were successfully implanted, the mice were injected intraperitoneally with MPA at a dose of 100 mg/kg every 2 days according to their body weight, and the mice were weighed. The control group was injected with an equal volume of saline. Tumour volume was measured with callipers three times a week. Tumour volume was calculated as (length × width^2)/2. The tumours from these animals were harvested for IHC experiments and weighed as previously reported [32].

Statistical analysis

Sample size and statistical tests are described in the figure legends. Every series of data are shown as mean ± SEM. Data analysis was conducted using SPSS 19.0. The Kolmogorov–Smirnov test was used for the normality test. The Student's *t* test (two groups), or one-way analysis of variance (ANOVA) (more than two groups) was used to compare the data that were normally distributed. Mann–Whitney *U* test was used when data were not normally distributed. The Kruskal–Wallis test was performed to compare quantitative factors between three groups. Pearson correlation test was used to examine the association between the IHC staining of the TSLP, AR and KANK1 proteins. $P < 0.05$ indicates a significant difference compared with the control group.

RESULTS

TSLP facilitates the sensitivity of EC cells to progestin

To identify the critical factors within the embryonic microenvironment facilitating the response to MPA, we screened the IVF-generated human embryonic sac-derived fluid and all proteins with expression differences are listed in Supplementary Table 2. The top 20 upregulated factors are shown in Fig. 1a, with blank G2 blastocyst culture medium as a control. TSLP is included, with an ~12-fold increase. Seven candidate molecules (TSLP, TXK, APEX1, ADAMTSL2, TNK1, LALBA and RYK) were selected for determination of their roles in EC cell growth according to their function annotations. TSLP exhibited the maximal inhibitory effect on cell proliferation among these factors in both Ishikawa and ECC1 cell lines (Fig. 1b). It has been demonstrated that TSLP is secreted mainly by primary epithelial cells in response to some microbial products, inflammatory cytokines, or physical injury [33]. This prompted us to investigate the expression profile and roles of TSLP in the glandular epithelium of EC. We hypothesised that aberrant or a lack of TSLP expression in EC cells results in uncontrollable proliferation. As expected, compared with the normal HESC cells, EC cells showed a relatively lower level of TSLP expression, including the Ishikawa, ECC1, HEC1B, SPEC2, and AN3CA cell lines (Fig. 1c). Moreover, TSLP was highly expressed in the normal endometrium in the TCGA database (Supplementary Fig. 1a). TSLP suppressed EC cell proliferation in a dose-dependent manner (Fig. 1d), which paralleled the decline in the Cyclin D1 level (Fig. 1e). Most importantly, TSLP overexpression reinforced the effect of MPA on proliferation arrest (Fig. 1f). These findings suggest that TSLP expression declines in EC cells and that TSLP downregulation might contribute to blunted response to MPA. In addition, the inhibitory effect of transfected TSLP plasmid on the invasion and migration of EC cells was observed (Supplementary Fig. 1b–d).

Augmented AR signalling attributes to intensive progestin response

To explore the underlying mechanisms by which TSLP promotes the progestin response, gene expression profiles were

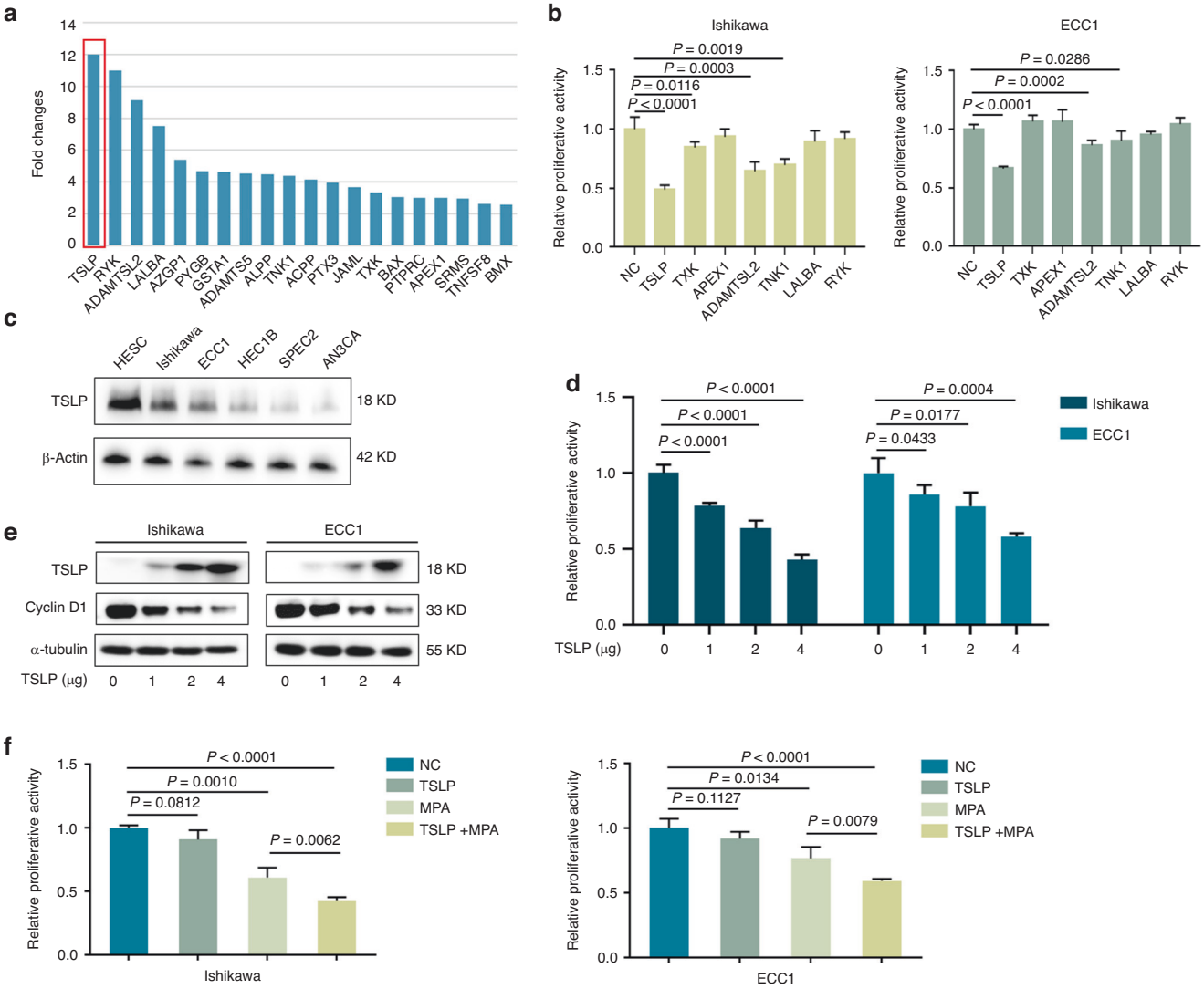

Fig. 1 TSLP facilitates the sensitivity of EC cells to progestin. a The top 20 highly expressed factors in human embryo sac-derived fluid produced by IVF. **b** The impact of factors with remarkable fold changes (TSLP, TXK, APEX1, ADAMTSL2, TNK1, LALBA and RYK) from the embryonic microenvironment on Ishikawa and ECC1 cell proliferation. **c** Western blot assay showing the expression of TSLP in EC cell lines and normal HESC cells. **d** CCK-8 assay for measuring EC cell proliferative activity in response to elevated TSLP gradients **e** Western blot analysis of Cyclin D1 after transfection with increasing doses of TSLP plasmid. The results shown represent three (**c**, **e)** independent experiments. **f** The proliferative activity of EC cells was detected with the CCK-8 assay after transfection of TSLP (0.5 μg) alone or plus MPA (40 μM). Data are mean ± SD of three independent experiments (**b, d, f**). NC negative control. *P* values are from one-way ANOVA test (**b**, **d**, **f**).

quantitatively analysed by RNA-seq in Ishikawa and Ishikawa-TSLP cells. The regulation of cell proliferation, PR, and AR signalling pathways were shown to be involved in TLSP-mediated regulation by GO enrichment analysis (Fig. 2b). Immunoblotting revealed that TSLP had no effect on PR expression but enhanced AR expression in a dose-dependent manner (Fig. 2c). Given that PR and AR share similar biofunctions and parts of downstream genes [16], we analysed the correlation between their expression levels in EC. A positive association was found based on the TCGA dataset in GEPIA (Supplementary Fig. 2a). A high expression level of AR was also positively correlated with the overall survival rate of EC patients (Supplementary Fig. 2b), and AR was highly expressed in normal endometrium compared with carcinoma tissue in the TCGA database (Supplementary Fig. 2c), suggesting a potential role of AR expression in predicting a good clinical outcome. Thus, there is a need to determine whether AR contributes to TSLP-driven progestin sensitivity. It has been found that TSLP upregulated AR in both mRNA and protein levels (Fig. 2c, d). Furthermore, TSLP overexpression mediated enhanced AR transcriptional activity in a dose-dependent manner by dual-luciferase reporter assay (Fig. 2e). The increase in AR expression significantly suppressed EC cell growth (Fig. 2f) and was accompanied by decreased Cyclin D1 expression (Fig. 2g). AR overexpression plus MPA treatment arrested cell proliferation activity compared with AR transfection or MPA treatment alone, and the change in the proliferation profile upon the indicated treatment was negatively correlated with the change in the AR expression pattern (Fig. 2h). To determine the underlying mechanism by which TSLP enhanced the progestin response, the AR expression pattern in cells with the indicated treatment was detected. As shown in Fig. 2i, overexpression of TSLP combined with MPA treatment increased AR expression. Conversely, knockdown of AR attenuated TSLP overexpression-induced proliferation arrest (Fig. 2j). Immunoblot confirmed the effect of siRNA in knocking down AR protein (Fig. 2j). Together, these data demonstrate that TSLP triggers AR signalling activation and in turn sensitises EC cells to progestin treatment.

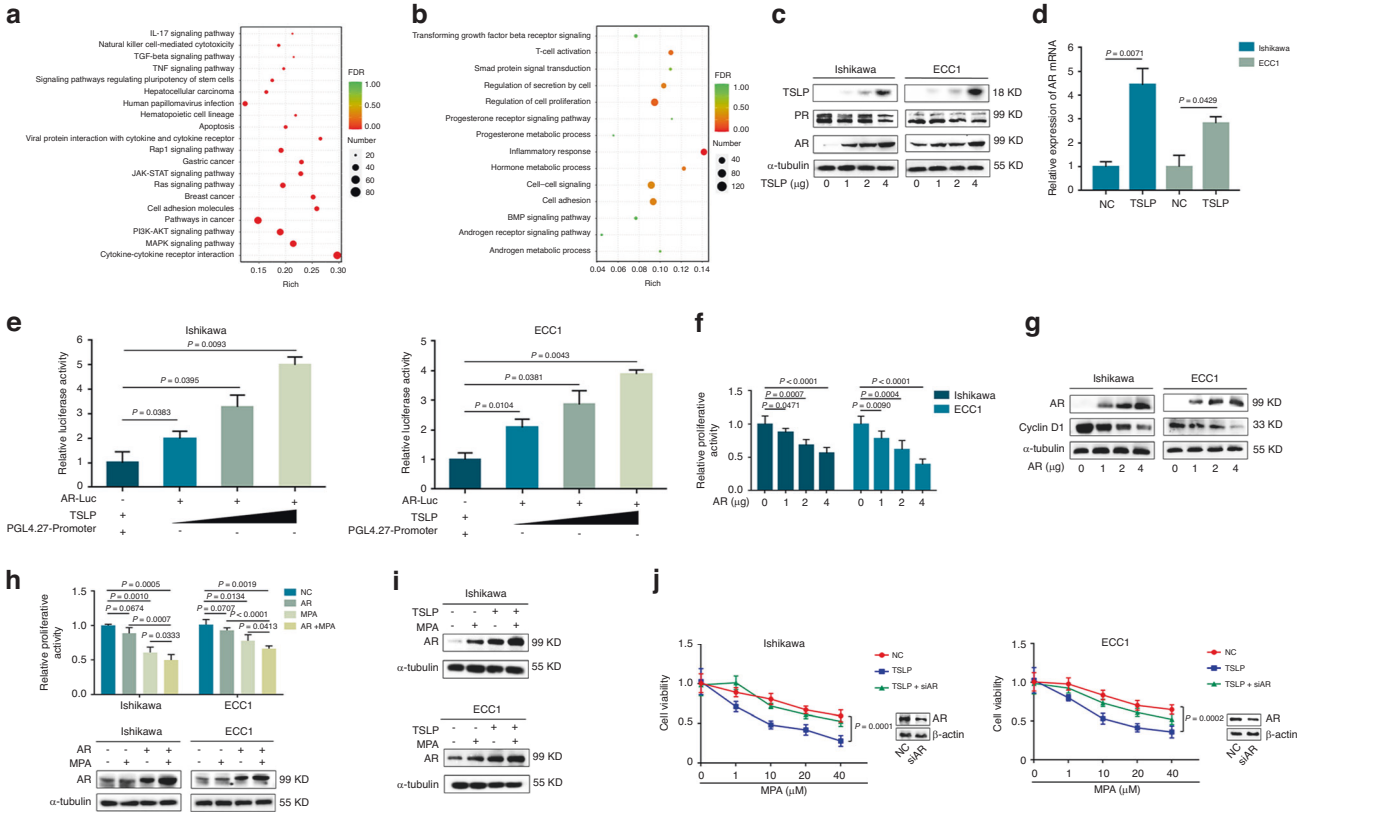

Fig. 2 Augmented AR signalling attributes to intensive progestin response. a KEGG pathway-rich analysis of the RNA-seq assay. **b** GO analysis of the RNA-seq assay. **c** Western blot was performed to determine the PR and AR expression after TSLP overexpression in EC cells. **d** The expression of AR mRNA level after TSLP overexpression in EC cells were detected with Real-time PCR, respectively. **e** The luciferase activities was detected after co-transfection with the AR-Luc reporter plasmid, TSLP plasmid, or pGL4.27 plasmid, respectively. The dose of TSLP plasmid is 1 μg, 2 μg, and 4 μg respectively. **f** CCK-8 assay was performed to determine the cellular proliferation after transfection with an increasing dose of AR plasmid. **g** Western blot analysis of AR and Cyclin D1 after transfection with increasing doses of AR plasmid. **h** EC cells underwent indicated treatments. The proliferation profile (upper panels) and AR expression pattern (lower panels) were examined with CCK-8 assay and western blot assays, respectively. **i** EC cells were transfected with the TSLP plasmid, then treated with MPA (40 μM) for another 48 h. Western blot was used to measure the protein level of AR. **j** AR siRNA was transfected in TSLP stably transfected EC cells, reducing sensitivity to MPA treatment. CCK-8 assay was used to determine cell viability. Western blot was used to measure the knockdown efficiency of AR. All lanes were loaded with the same amount of total protein (**c**, **g**, **h**, **i**, **j**). Results shown represent three independent experiments (**c**, **g**, **h**, **i**, **j**). Data are mean ± SD of three independent experiments (**d**–**f**, **h**, **j**). *P* values are from one-way ANOVA (**e**, **f**, **h**, **j**) or two-sided unpaired Student's *t* tests (**d**).

TSLP upregulates AR through the BMP4/Smad5 pathway

The mechanism by which TSLP regulates AR remains unclear. KEGG pathway (Fig. 2a) and GO enrichment analyses (Fig. 2b) of the RNA-seq data showed that "TGF-β signalling pathway", "BMP signalling pathway" and "Smad protein signal transduction" were involved in the regulation of EC proliferation by TSLP. According to these results as well as the associations among TGF-β, BMP and Smad previously reported [21], we assumed that TSLP regulates AR via the BMP/Smad pathway. We measured the protein levels of BMP4, BMP6, and BMP7 when TSLP was overexpressed. Among these BMP subtypes, induced expression of only BMP4 was observed, the rest have no significant changes (Fig. 3a). Higher levels of Smad-dependent signalling are triggered by hetero-dimeric BMPs than by homodimeric BMPs [34]. We further evaluated whether TSLP affects the dimerisation of BMP4 in EC cells after treatment with 100 ng/ml of rhTSLP, a concentration defined based on its dose-dependent effect on cell growth (Supplementary Fig. 3). The co-IP assay showed that TSLP mainly promoted the formation of the BMP4/BMP7 heterodimer, not the BMP4/BMP4 homodimer (Fig. 3b).

As Smad family genes are downstream genes regulated by BMPs, we attempted to determine which member of the Smad family is involved in the TSLP-mediated enhancement of AR expression. Knockdown of Smad5 blocked the TSLP-induced upregulation of AR mRNA expression, while silence other Smad members slightly attenuated TSLP-induced AR without significant difference (Fig. 3c). Immunoblot confirming the specificity of siRNA in knocking down Smad1, 5 and 8 protein is shown in Supplementary Fig. 4. As shown in Fig. 3d, e, TSLP was able to upregulate the protein and mRNA expression of Smad5. The expression of BMP4 and Smad5 were upregulated under rhTSLP treatment (Supplementary Fig. 5). TSLP also increased the p-Smad5 protein level with a dosed manner (Fig. 3e). These findings suggest that TSLP not only increases Smad5 expression at the transcriptional level, but also regulates Smad5 activity via the BMP4/7 heterodimer, which both ultimately increases the abundance of p-Smad5 and promotes downstream transcription. As shown in Fig. 3f, overexpression of Smad5 markedly enhanced AR transcriptional activity by dual-luciferase reporter assay. Furthermore, we used EMSA to determine whether Smad5 is recruited to the promoter of AR. As expected, Smad5 over-expression significantly increased protein-DNA association (Fig. 3g, Lane 2), which was efficiently competed by a 100-fold molar excess of unlabelled probe (Fig. 3g, Lane 3). This inhibition was no longer detectable when a mutated oligonucleotide was used (Fig. 3g, Lane 4). ChIP assays further confirmed this

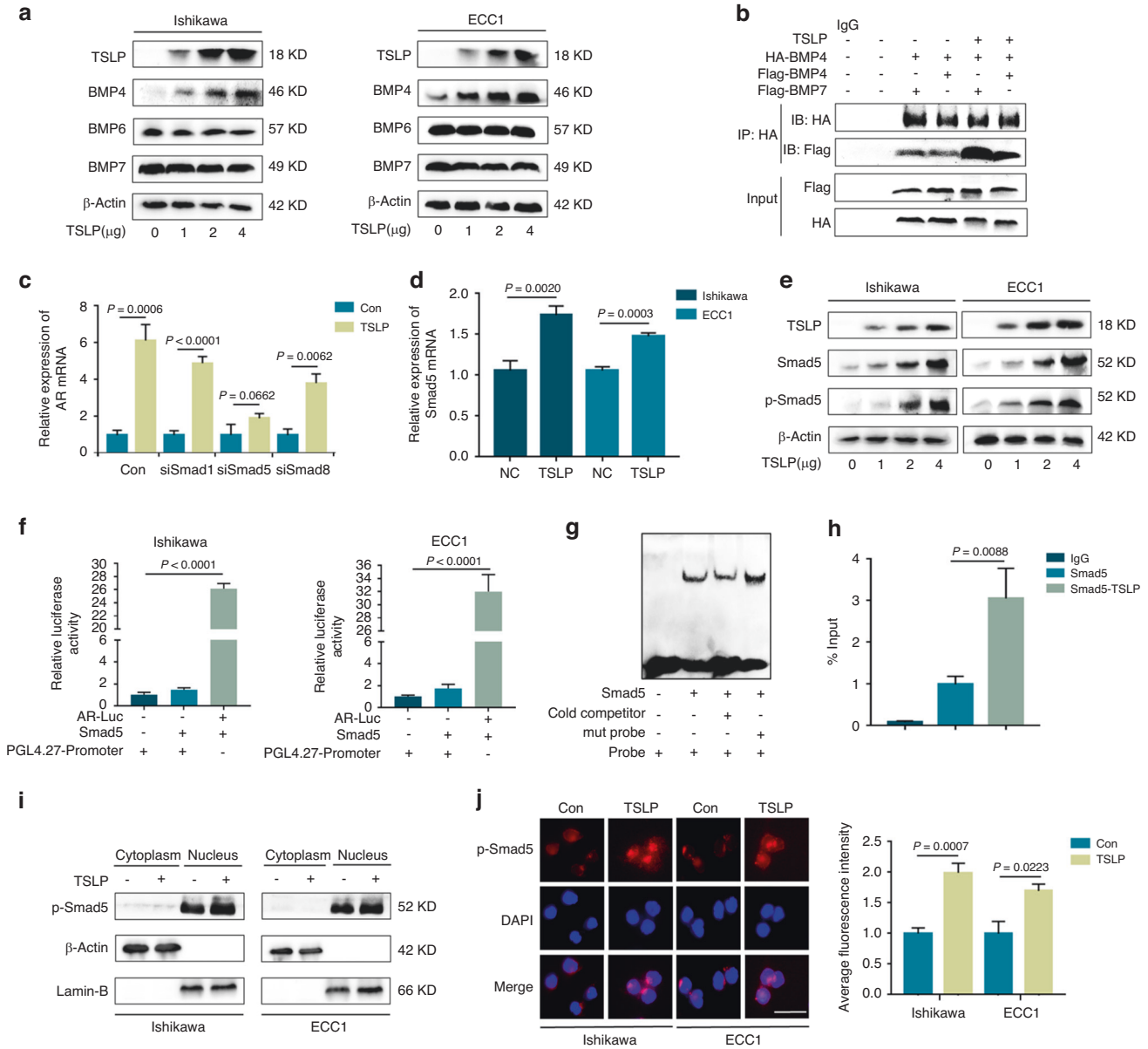

Fig. 3 TSLP upregulates AR through the BMP4/Smad5 pathway. a Western blot analysis of the expression changes of BMP family members after transfection of TSLP plasmid with indicated dose. **b** Co-IP experiments were performed to detect the binding status of HA-BMP4, Flag-BMP4 and Flag-BMP7. **c** When siRNA targeting each of the R-Smads was transfected into Ishikawa cells, TSLP-induced AR mRNA levels were detected. **d** qPCR assay of Smad5 after TSLP overexpression. **e** Western blot assay of Smad5 and p-Smad5 after transfection with increasing doses of TSLP plasmid. **f** The relative luciferase activity were measured in EC cells after co-transfection with the AR-Luc reporter plasmid, Smad5 plasmid, or pGL4.27 plasmid, respectively. **g** EMSA experiment for detecting the formation of Smad5 and AR complexes. Ishikawa cells were co-transfected with the respective plasmids, and the nucleus extracts were then incubated with an AR-specific consensus sequence probe that was tagged with 5′-biotin before being electrophoresed. In competition assays, a 100-fold molar excess of unlabelled probe (lane 3) or a 100-fold molar excess of unlabelled oligonucleotide harbouring a mutant probe (lane 4). Lane 1 contains the probe alone. **h** ChIP-qPCR was utilised to examine Smad5's interaction with the AR promoter and whether TSLP promotes this binding. Smad5 and non-specific IgG antibodies were used to immunoprecipitate the genomic DNA from Ishikawa cells, and this DNA was then utilised for qPCR. **i** After TSLP plasmid transfection, the p-Smad5 from the cytoplasmic and nuclear extractions was analysed by western blot. Results shown represent three independent experiments (**a**, **e**, **i**). **j** Ishikawa cells were either transfected with the TSLP plasmid or left untreated for 48 h. The cells were then stained with anti-phospho-Smad5 coupled to Alexa Fluro (red) and the nuclei with DAPI (blue). The scale bar represents 100 μm. Data are mean ± SD of three independent experiments (**c**, **d**, **f**, **h**, **j**). *P* values are from one-way ANOVA (**f**, **h**) or two-sided unpaired Student's *t* tests (**c**, **d**, **j**).

observation. As shown in Fig. 3h, approximate threefold increase of Smad5 recruited to AR promoter. As nuclear translocation is the critical step for p-Smad5 activation, the translocation phenomenon has been observed. TSLP treatment resulted in increasing of p-Smad5 level in the nucleus (Fig. 3i). Accumulation of the red fluorescence signal representing p-Smad5 was present in the nucleus after TSLP treatment (Fig. 3j). The findings indicated that TSLP regulates AR expression in EC cells by promoting the entry of p-Smad5 into the nucleus, where it binds to the promoter of AR.

KANK1 is required for TSLP-mediated enhancement of the progestin response

As a transcription factor, AR generally regulates downstream target gene expression by binding with an ARE in the target gene promoter region [19]. We predicted the potential targets of AR by using the CHEA Transcription Factor Targets dataset and CHIP-seq databases (GSM2235688 and GSM1909088). By intersecting the results of the three prediction tools, we identified 260 candidate genes for further validation (Fig. 4a). Then we performed GO analysis through the DAVID database (Fig. 4b) and scanned the promoter regions of the candidate genes. KANK1 was found to contain four ARE sequences (Fig. 4c). Further investigation

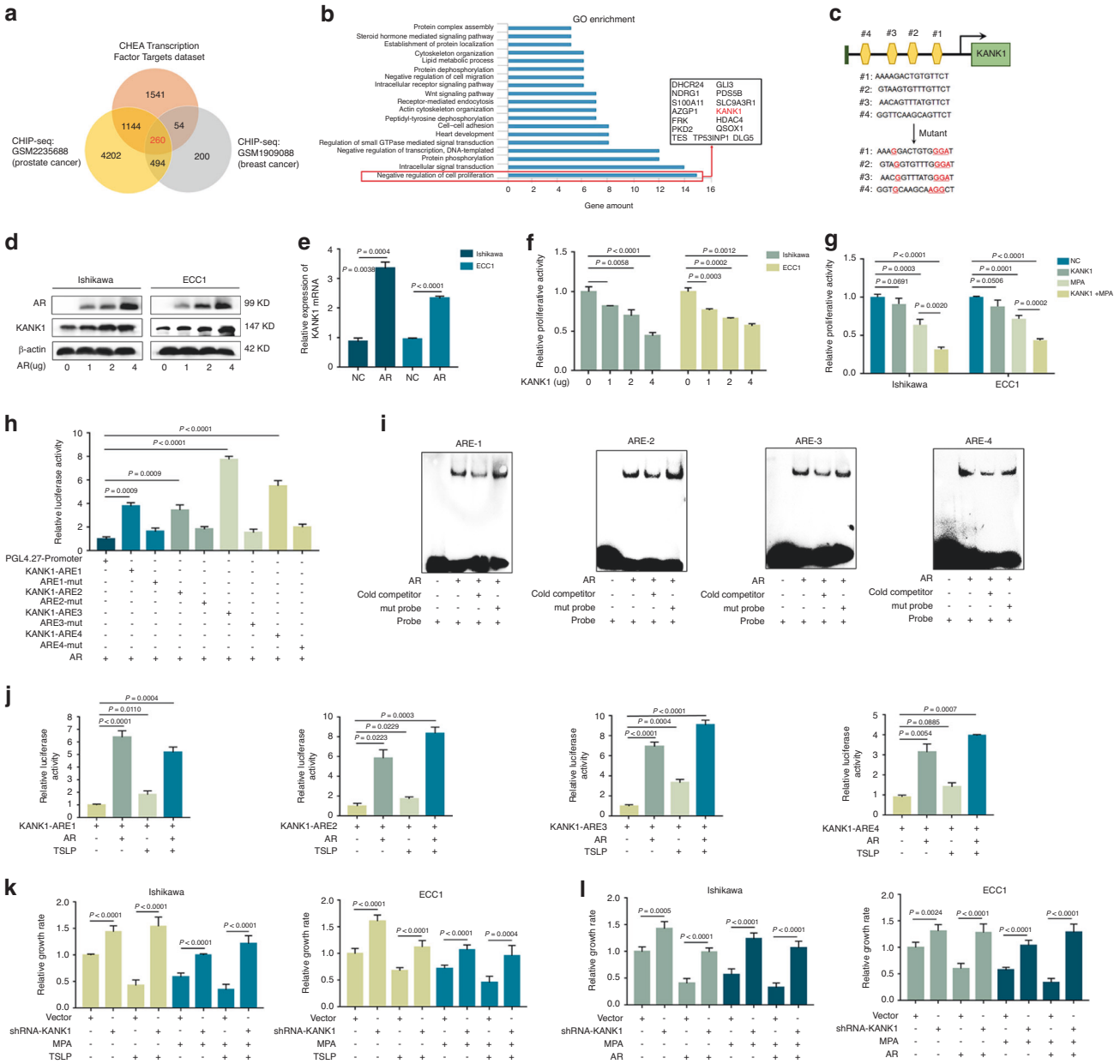

Fig. 4 KANK1 is required for TSLP-enhancing progestin response. **a** Schematic representation of the projected AR target genes' overlap predicted by the CHEA Transcription Factor Targets dataset and CHIP-seq (number: GSM2235688 and GSM1909088). **b** GO analysis of the projected AR target genes. **c** Schematic illustration of four AREs on the KANK1 promoter and their corresponding mutants. **d** Western blot assay of KANK1 after transfection with increasing doses of AR plasmid. The results shown represent three independent experiments. **e** qPCR assay of KANK1 after AR overexpression. **f** CCK-8 assay was used to determine the cellular proliferation after transfection with an increasing dose of KANK1 plasmid. **g** The relative proliferative activity of EC cells was detected with the CCK-8 assay after transfection of KANK1 (0.5 μg) alone or plus MPA (40 μM). **h** After co-transfection with four KANK1-ARE reporter plasmids, ARE-mut reporter plasmids, AR plasmids, or pGL4.27 plasmids, the relative luciferase activities were observed in Ishikawa cells. **i** EMSA experiment for detecting the bind of AR to the ARE region on the KANK1 promoter. Plasmids were co-transfected into Ishikawa cells in the manner shown and the nuclear extracts were incubated with four KANK1-ARE-specific consensus sequence probes labelled with 5′-biotin, respectively. **j** Ishikawa cells were co-transfected with the specified plasmids, followed by either a 48-h treatment with 100 ng/ml rhTSLP or left untreated. Then relative luciferase activities were detected. **k** In the presence of TSLP overexpression, CCK-8 assay was performed to detect the effect of KANK1 knockdown on the susceptibility of EC cells to progestin. **l** CCK-8 assay detected the effect of KANK1 knockdown on the susceptibility of EC cells to progestin in the presence of AR overexpression. Data are mean ± SD of three independent experiments (**d**–**f**, **h**, **j**, **k**, **l**). *P* values are from one-way ANOVA (**e**, **f**, **h**, **j**, **k**, **l**) or two-sided unpaired Student's *t* tests (**d**).

revealed that AR upregulated KANK1 at both the protein (Fig. 4d) and mRNA (Fig. 4e) levels. The expression of AR and KANK1 were upregulated under rhTSLP treatment (Supplementary Fig. 5). According to the CCK-8 assay, KANK1 overexpression drastically reduced the viability of EC cell proliferation with a dose manner (Fig. 4f). We also observed that the inhibitory effect of progestin on cell proliferation was enhanced by KANK1 overexpression (Fig. 4g). Therefore, overexpression of KANK1 may play a critical role in enhancing the sensitivity of EC cells to progestin.

To identify which ARE is the responsive element, we constructed four luciferase reporter plasmids (named KANK1-ARE-1, KANK1-ARE2, KANK1-ARE3, and KANK1-ARE4), each containing one of the ARE sequences. Transfection of AR markedly increased the luciferase activity by approximately fourfold in KANK1-ARE-1- and KANK-ARE2-transfected cells, eightfold in KANK1-ARE3-transfected cells, and sixfold in KANK1-ARE4-transfected cells (Fig. 4h), while AR could not increase the luciferase activity in cells transfected with the mutant ARE plasmid (Fig. 4c, h). Although the response extent differed, the results suggest that all four ARE sequences were functional in the KANK1 gene in response to AR axis signalling.

We carried out EMSA on each of the four ARE sequences to further confirm the specificity of the putative ARE sites (Fig. 4i). For EMSA of the ARE-1 sequence, components present in the nuclear extracts of Ishikawa cells slowed the mobility of the putative ARE domains in a particular way (Fig. 4i, Lane 1). Notably, AR overexpression significantly increased the protein-DNA interaction (Fig. 4i, Lane 2), which was offset by a 100-fold molar excess of unlabelled probe (Fig. 4i, Lane 3). This inhibition was absent when a mutated putative ARE oligonucleotide was used (Fig. 4i, Lane 4). The trend of EMSA results for other ARE sequences was the same as ARE-1. Furthermore, in order to verify whether TSLP has an effect on the transcriptional regulation of KANK1, ARE luciferase reporter plasmids and AR plasmid were transfected into Ishikawa cells prior to TSLP treatment. The notable elevation of AR transcription activity has been obtained with AR transfection plus TSLP treatment (Fig. 4j). To further investigate the effect of KANK1 on the mechanism by which TSLP enhances progestin sensitivity, three shRNAs targeting KANK1 (shKANK1) were constructed. shKANK1#1 was used for the subsequent experiments owing to its strong inhibitory effect (Supplementary Fig. 6). We found that under progestin treatment, knockout of KANK1 significantly weakened the inhibitory effects of TSLP (Fig. 4k) and AR (Fig. 4l) on proliferation in both Ishikawa and ECC1 cells. These results indicate that KANK1 is the necessary effector molecule for TSLP-mediated enhancement of the progestin response. Since hormone withdrawal is known to cause endometrium 'breakdown', we tested whether MPA removal can change AR and KANK1

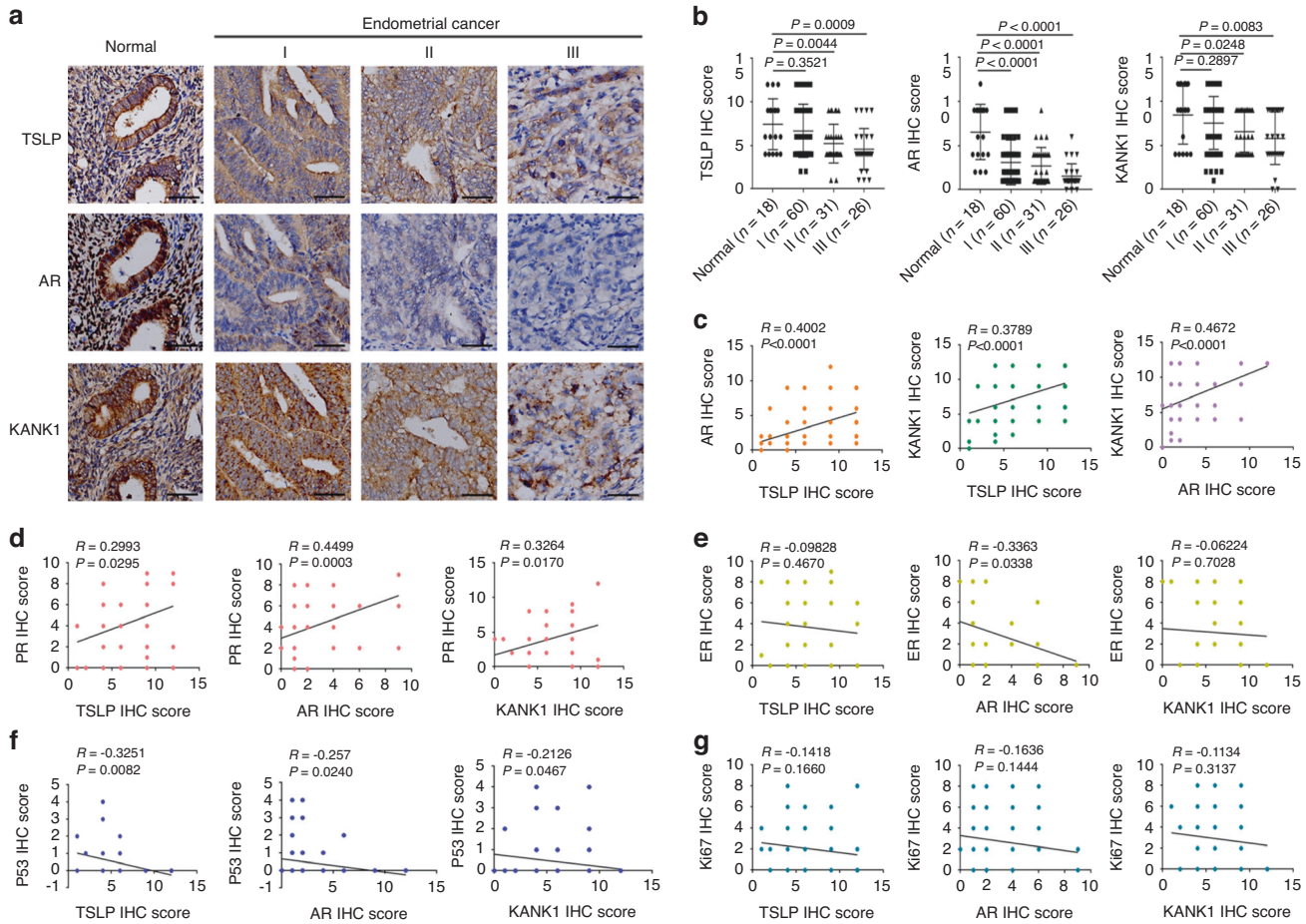

Fig. 5 Expression profiles of TSLP, AR and KANK1 in endometrial cancer and the clinical outcome. a IHC staining of TSLP, AR and KANK1 in consecutive sections of endometrial tissue samples, including normal endometrium ($n = 18$), phase I EC ($n = 60$), phase II EC ($n = 31$) and phase III EC ($n = 26$). Representative images were acquired at ×200 magnification. **b** IHC scores of TSLP, AR and KANK1 in consecutive sections of endometrial tissue samples. **c** Positive correlation of TSLP, AR and KANK1 expression levels in different pathological samples. **d**–**g** Correlation of TSLP, AR and KANK1 expression levels with PR, ER, P53, Ki67 expression levels in different pathological samples, respectively. Data are mean ± SD (**b**). *P* values are from one-way ANOVA (**b**) or Pearson correlation test (**c**–**g**). I: phase I EC; II: phase II EC; III: phase III EC.

expression, as well as cell growth. As indicated in Supplementary Fig. 7, AR and KANK1 expression was elevated 48 h after MPA removal, and this elevation became pronounced after 72 h. MPA removal resulted in a reduction in cell proliferation, consistent with the above findings. Notably, MPA withdrawal combined with TSLP overexpression further inhibited the proliferation of EC cells and upregulated the expression of AR and KANK1. This further suggests that TSLP treatment enhances EC sensitivity to progestin and that TSLP acts by modulating the AR/KANK1 signal pathway.

Expression profiles of TSLP, AR and KANK1 in endometrial cancer and the associations with clinical outcomes

To further investigate the link among TSLP, AR, and KANK1, we used IHC staining to identify TSLP, AR, and KANK1 expression patterns in serial sections of endometrial tissue samples with gradual malignant progression (Fig. 5a). The intensity of staining for these molecules was highest in normal endometrium and the expression of the aforementioned molecules was reduced as the lesions progressed from histological grade I to III (Fig. 5b and Table 1). In addition, we discovered comparable patterns of TSLP, AR and KANK1 staining levels across any pathologic endometrial period (Fig. 5c). PR and ER are common hormone receptors in EC, and we tested whether the expression of TSLP, AR and KANK1 is associated with that of either receptor. The expression of TSLP, AR and KANK1 was positively correlated with that of PR with statistical differences, consistent with the previously mentioned role of each of these three molecules in enhancing progestin sensitivity (Fig. 5d). However, the correlations of their expression with ER expression were not significant (Fig. 5e). We also found that the expression of these three molecules was negatively correlated with the expression of p53 and ki67, suggesting that these three molecules have a suppressive impact on EC growth (Fig. 5f, g). Furthermore, there was a statistically significant association between AR expression and the level of tumour infiltration (Table 1), suggesting that AR is a good prognostic marker in EC.

TSLP sensitised endometrial cancer to progestin treatment in vivo

Female BALB/c mice ($n = 5$) were implanted with Ishikawa and Ishikawa-TSLP cells in xenograft assays to establish the effect of TSLP on sensitising EC cells to progestin in vivo (Fig. 6a). After tumour implantation, mice were injected intraperitoneally with 100 mg/kg of MPA or normal saline every 2 days according to their body weight. The mice were observed for tumour growth and weighed in 20 days. MPA treatment dramatically reduced tumour development in nude mice in the Ishikawa-TSLP group, including average tumour volume (Fig. 6b, c) and weight (Fig. 6d). IHC assays also revealed that TSLP overexpression increased the expression of p-Smad5, BMP4, AR and KANK1 while decreasing the expression of PCNA in xenograft tumour tissues (Fig. 6e) and the changes have been summarised in Supplementary Fig. 8. All of the abovementioned findings suggest that TSLP as an anti-oncogene promotes the expression of AR through the BMP4/Smad5 pathway, following enhancement of KANK1 transcription, and then facilitates the EC cell response to progestin treatment (Fig. 6f).

DISCUSSION

In this study, we identified a novel molecule, TSLP, that could sensitise EC cells to progestin, which is secreted by IVF-generated blastocysts. TSLP-driven progestin sensitivity is mediated by the upregulation of AR via the BMP4/Smad5 pathway. Importantly, we first identified KANK1 as a novel AR target gene and an effector molecule controlling the progestin response via the TSLP/BMP4/Smad5/AR/KANK1 axis.

The role of TSLP in tumorigenesis is controversial [35]. TSLP was found to be produced by breast cancer cells, resulting in Th2

Table 1. Correlations of TSLP, AR, KANK1 and major clinical pathologic factors.

Factors	No. of patients	TSLP positive			TSLP negative	P value	AR positive			AR negative	P value	KANK1 positive			KANK1 negative	P value
		+	++	+++			+	++	+++			+	++	+++		
Age (year)						0.476[a]					0.411[a]					0.421[a]
<50	32	16	8	8	0		26	2	1	3		10	6	14	2	
≥50	85	52	13	20	0		62	5	8	10		32	5	46	2	
Histological grade						0.035[b]					0.039[b]					0.027[b]
Low (I)	60	27	10	23	0		44	5	6	5		20	7	31	2	
Intermediate (II)	31	15	10	6	0		25	1	3	2		12	11	8	0	
High (III)	26	19	4	3	0		19	1	0	6		15	4	5	2	
Clinical stage						0.135[a]					0.949[a]					0.406[a]
I	97	60	16	21	0		72	6	9	10		38	8	48	3	
II–IV	20	8	5	7	0		16	1	0	3		4	3	12	1	
Lymph node metastasis						0.657[a]					0.966[a]					0.842[a]
No	112	65	21	26	0		84	7	9	12		42	9	58	3	
Yes	5	3	0	2	0		4	0	0	1		0	2	2	1	
Invasion						0.789[a]					0.02[a]					0.557[a]
<1/2	81	47	15	19	0		62	5	9	5		30	7	43	1	
≥1/2	36	21	6	9	0		26	2	0	8		12	4	17	3	

[a]Mann–Whitney *U* test.
[b]Kruskal–Wallis test.

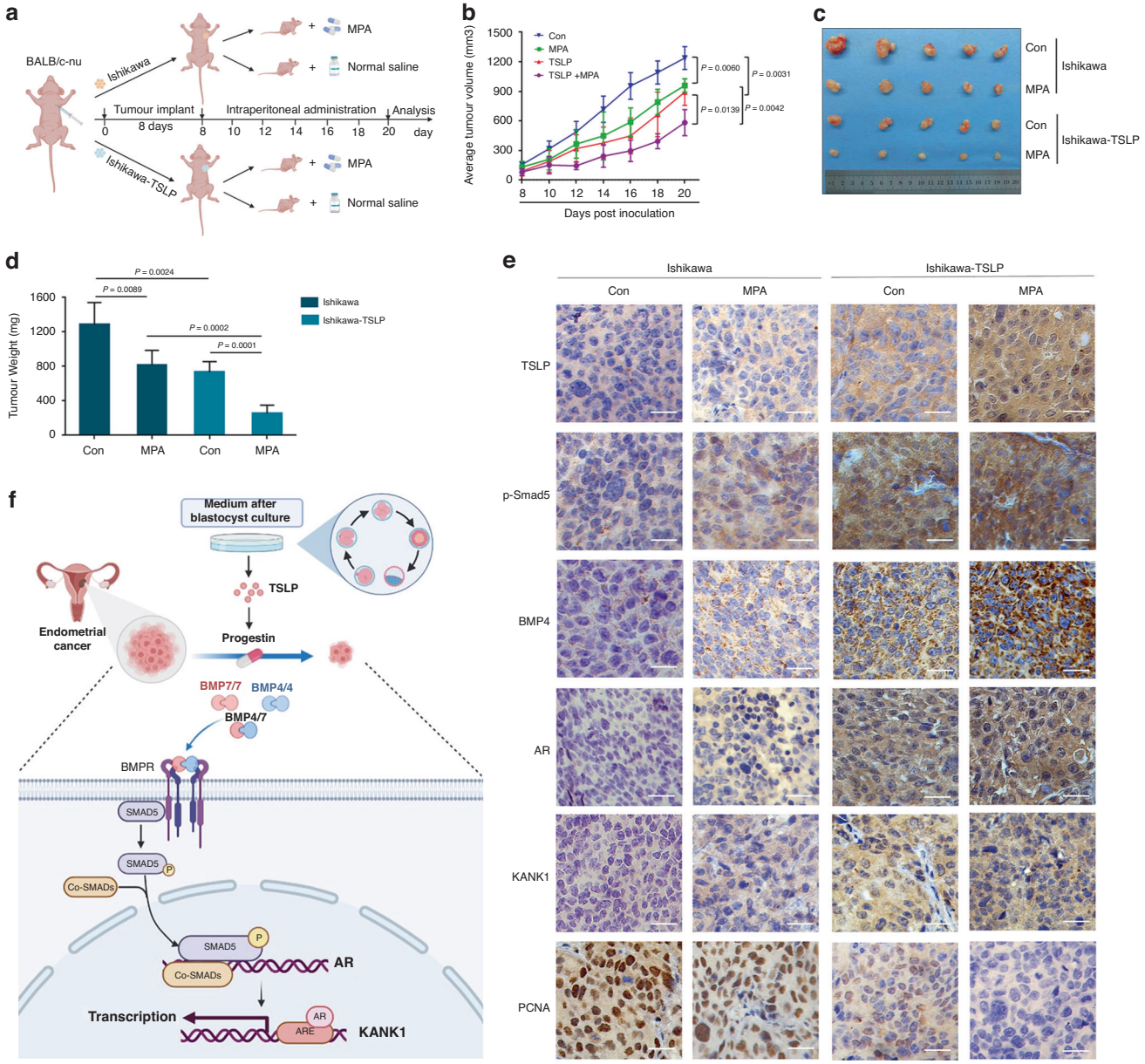

Fig. 6 TSLP sensitised endometrial cancer to progestin treatment in vivo. a The subcutaneous xenograft procedure and medication administration for nude mice. Subcutaneous tumour growth in female BALB/c mice inoculated with Ishikawa or Ishikawa-TSLP cells (1×10^6 cells, respectively, $n = 5$) and treated with MPA (100 mg/kg, ip) or normal saline in 20 days. **b** The xenograft tumour growth curve after being treated as recommended. **c** Typical image of xenograft tumours that were exposed to the indicated therapies. **d** The collected xenograft tumour weights. **e** Relative expression levels of TSLP, p-Smad5, BMP4, AR, KANK1, and PCNA were observed in subcutaneous tumour tissues by IHC. The white scale bar indicated 20μm. Magnification, ×400. **f** A proposed model illustrating that TSLP enhances progestin response in EC via the BMP4/Smad5/AR/KANK1 Axis. Data are mean ± SD (**b**, **d**). *P* values are from one-way ANOVA (**b**, **d**).

inflammation and promoting tumorigenesis [36]. Conversely, in breast cancer mouse models, TSLP-mediated inflammation has been shown to decrease tumour development [11]. In addition, TSLP could establish an inflammatory microenvironment to protect against skin tumorigenesis [13]. In a randomised controlled study, the synergistic suppressive effects of a TSLP inducer and 5-FU treatment in skin cancer were well confirmed [37]. The later studies are consistent with our current finding that TSLP exerts an antitumor effect in EC (Fig. 1d). The extended protective role of TSLP in other gynaecological processes, such as pregnancy, embryo development, and implantation has also been observed [38]. Considering that TSLP is an epithelium-derived cytokine, we further hypothesised that the loss of TSLP in endometrial gland epithelial cells may contribute to EC development and attenuate the response to progestin treatment. Indeed, a decline in the expression of TSLP in EC was observed (Fig. 5a). The clinical investigation indicated that loss of TSLP is tightly associated with malignant progression of EC (Table 1).

TSLP was the most highly expressed protein in the fluid obtained from IVF-generated blastocysts (Fig. 1a), indicating that TSLP may be related to embryonic development. Therefore, we hypothesised that TSLP may regulate embryonic development-related signalling pathways. Through KEGG and GO enrichment analyses, we identified the BMP/Smad pathway (Fig. 2a, b). In mature BMP protein complexes, two monomers are covalently linked by disulfide bonds [39]. BMP-target genes can be activated by heterodimeric BMPs to result in considerably higher levels of Smad-dependent signalling than those induced by homodimeric

BMPs [40, 41]. Cross-talk between TGF-β and androgen-signalling pathways has previously been reported in prostate cancer [42]. However, the association between AR and Smads in EC has not been reported. In this study, we first found that TSLP mainly promoted the formation of the BMP4/BMP7 heterodimer (Fig. 3b) and thus promoted Smad5 binding to AR (Fig. 3f, g).

An increasing number of studies have demonstrated that AR plays a protective role in EC. AR protein levels have been shown to decrease as EC progresses from a well-differentiated to a poorly differentiated tumour [43]. In mammalian cells expressing exogenous or endogenous AR, MPA exerts substantial agonistic effects on androgen. AR transcriptional activity was significantly increased in the COS-1 cell line after transient AR expression and MPA administration [44]. By upregulating AR signalling, progestin may also decrease the stimulatory effects of oestrogen signalling on the endometrium [14, 45]. In this study, we discovered that AR inhibited the proliferation of EC cells (Fig. 2f, g) and was primarily responsible for increasing progestin sensitivity in EC cells (Fig. 2h–l). However, the mechanisms underlying the suppression of EC cell proliferation by activated AR remain unknown. We first identified KANK1 as an AR target gene because it contains four AREs, and each ARE is required for AR-mediated regulation of KANK1 transcriptional activity. KANK1 has been identified as a tumour suppressor [46, 47]. The roles of KANK1 in tumours mainly include affecting tumour cell apoptosis and cell cycle. KANK1 has been reported to play a role in promoting cell apoptosis in lung cancer cells by regulating the Bcl-2/Bax signalling pathway, thereby acting as anti-oncogene in lung cancer [46]. Through enhanced apoptosis, KANK1 overexpression made cells more susceptible to cisplatin [48, 49]. Moreover, CircDDX17 inhibits carcinogenesis and lowers 5-fluorouracil resistance in colorectal cancer by modulating KANK1 expression [50]. In addition, KANK1 inhibits tumour growth by regulating the cell cycle. In lung cancer, KANK1 overexpression caused tumour cells to arrest in the G0/G1 phase and significantly inhibited the proliferation of lung cancer cells [46]. Functionally, overexpression of KANK1 increased the effectiveness of progestin in inhibiting EC proliferation and TSLP enhanced progestin sensitivity by upregulating KANK1 expression (Fig. 4g, l).

One of the primary causes of progestin resistance is believed to be the downregulation of PR during long-term continuous progestin therapy [51, 52]. Our previous findings showed that the PR level might be effectively increased following MPA withdrawal, resulting in increased progestin sensitivity [32]. In this study, if TSLP was added externally during intermittent administration, progestin resistance could be prevented more effectively (Supplementary Fig. 7), suggesting that treatment with TSLP plus MPA may be more effective than MPA alone in reducing the possibility of progestin resistance.

CONCLUSIONS

In summary, we have first demonstrated that TSLP, as an anti-oncogene in the IVF-originating human embryonic microenvironment, facilitates progestin sensitivity via the BMP4/Smad5/AR/KANK1 axis. In addition, our findings established a link between reproduction and cancer, which may provide a new strategy to overcome progestin resistance and benefit EC therapy.

DATA AVAILABILITY

The datasets used and/or analysed during this study are available from the corresponding author on reasonable request.

REFERENCES

1. Yang S, Thiel KW, Leslie KK. Progesterone: the ultimate endometrial tumor suppressor. Trends Endocrinol Metab TEM. 2011;22:145–52.
2. Zhang Z, Dong L, Sui L, Yang Y, Liu X, Yu Y, et al. Metformin reverses progestin resistance in endometrial cancer cells by downregulating GloI expression. Int J Gynecol Cancer. 2011;21:213–21.
3. Kim MK, Seong SJ, Kang SB, Bae DS, Kim JW, Nam JH, et al. Six months response rate of combined oral medroxyprogesterone/levonorgestrel-intrauterine system for early-stage endometrial cancer in young women: a Korean Gynecologic-Oncology Group Study. J Gynecol Oncol. 2019;30:e47.
4. Mintz B, Illmensee K. Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc Natl Acad Sci USA. 1975;72:3585–9.
5. Pierce GB, Pantazis CG, Caldwell JE, Wells RS. Specificity of the control of tumor formation by the blastocyst. Cancer Res. 1982;42:1082–7.
6. Gerschenson M, Graves K, Carson SD, Wells RS, Pierce GB. Regulation of melanoma by the embryonic skin. Proc Natl Acad Sci USA. 1986;83:7307–10.
7. Sun D, Qin Z, Xu Y, Xiao Q, Xu Y, Bai M, et al. The IVF-generated human embryonic microenvironment reverses progestin resistance in endometrial cancer cells by inducing cancer stem cell differentiation. Cancer Lett. 2022;526:311–21.
8. De Monte L, Reni M, Tassi E, Clavenna D, Papa I, Recalde H, et al. Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer. J Exp Med. 2011;208:469–78.
9. Xie F, Meng YH, Liu LB, Chang KK, Li H, Li MQ, et al. Cervical carcinoma cells stimulate the angiogenesis through TSLP promoting growth and activation of vascular endothelial cells. Am J Reprod Immunol. 2013;70:69–79.
10. Li H, Zhao H, Yu J, Su Y, Cao S, An X, et al. Increased prevalence of regulatory T cells in the lung cancer microenvironment: a role of thymic stromal lymphopoietin. Cancer Immunol Immunother CII. 2011;60:1587–96.
11. Demehri S, Cunningham TJ, Manivasagam S, Ngo KH, Moradi Tuchayi S, Reddy R, et al. Thymic stromal lymphopoietin blocks early stages of breast carcinogenesis. J Clin Investig. 2016;126:1458–70.
12. Yue W, Lin Y, Yang X, Li B, Liu J, He R. Thymic stromal lymphopoietin (TSLP) inhibits human colon tumor growth by promoting apoptosis of tumor cells. Oncotarget. 2016;7:16840–54.
13. Demehri S, Turkoz A, Manivasagam S, Yockey LJ, Turkoz M, Kopan R. Elevated epidermal thymic stromal lymphopoietin levels establish an antitumor environment in the skin. Cancer Cell. 2012;22:494–505.
14. Hackenberg R, Beck S, Filmer A, Hushmand Nia A, Kunzmann R, Koch M, et al. Androgen responsiveness of the new human endometrial cancer cell line MFE-296. Int J Cancer. 1994;57:117–22.
15. Lovely LP, Appa Rao KB, Gui Y, Lessey BA. Characterization of androgen receptors in a well-differentiated endometrial adenocarcinoma cell line (Ishikawa). J Steroid Biochem Mol Biol. 2000;74:235–41.
16. Gibson DA, Simitsidellis I, Saunders PT. Regulation of androgen action during establishment of pregnancy. J Mol Endocrinol. 2016;57:R35–47.
17. Hackenberg R, Schulz KD. Androgen receptor mediated growth control of breast cancer and endometrial cancer modulated by antiandrogen- and androgen-like steroids. J Steroid Biochem Mol Biol. 1996;56:113–7.
18. Niwa K, Hashimoto M, Morishita S, Yokoyama Y, Lian Z, Tagami K, et al. Preventive effects of danazol on endometrial carcinogenesis in mice. Cancer Lett. 2000;158:133–9.
19. Gibson DA, Simitsidellis I, Cousins FL, Critchley HO, Saunders PT. Intracrine androgens enhance decidualization and modulate expression of human endometrial receptivity genes. Sci Rep. 2016;6:19970.
20. Marshall E, Lowrey J, MacPherson S, Maybin JA, Collins F, Critchley HO, et al. In silico analysis identifies a novel role for androgens in the regulation of human endometrial apoptosis. J Clin Endocrinol Metab. 2011;96:E1746–55.
21. Rahman MS, Akhtar N, Jamil HM, Banik RS, Asaduzzaman SM. TGF-β/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. Bone Res. 2015;3:15005.
22. Wan M, Cao X. BMP signaling in skeletal development. Biochem Biophys Res Commun. 2005;328:651–7.
23. Catic A, Kurtovic-Kozaric A, Johnson SH, Vasmatzis G, Pins MR, Kogan J. A novel cytogenetic and molecular characterization of renal metanephric adenoma: Identification of partner genes involved in translocation t(9;15)(p24;q24). Cancer Genet. 2017;214-215:9–15.
24. Luo FY, Xiao S, Liu ZH, Zhang PF, Xiao ZQ, Tang CE. Kank1 reexpression induced by 5-Aza-2′-deoxycytidine suppresses nasopharyngeal carcinoma cell proliferation and promotes apoptosis. Int J Clin Exp Pathol. 2015;8:1658–65.
25. Chen T, Wang K, Tong X. In vivo and in vitro inhibition of human gastric cancer progress by upregulating Kank1 gene. Oncol Rep. 2017;38:1663–9.
26. Kakinuma N, Roy BC, Zhu Y, Wang Y, Kiyama R. Kank regulates RhoA-dependent formation of actin stress fibers and cell migration via 14-3-3 in PI3K-Akt signaling. J Cell Biol. 2008;181:537–49.
27. Gu C, Zhang Z, Yu Y, Liu Y, Zhao F, Yin L, et al. Inhibiting the PI3K/Akt pathway reversed progestin resistance in endometrial cancer. Cancer Sci. 2011;102:557–64.

28. Jiang T, Chen N, Zhao F, Wang XJ, Kong B, Zheng W, et al. High levels of Nrf2 determine chemoresistance in type II endometrial cancer. Cancer Res. 2010;70:5486–96.
29. Catalano S, Giordano C, Rizza P, Gu G, Barone I, Bonofiglio D, et al. Evidence that leptin through STAT and CREB signaling enhances cyclin D1 expression and promotes human endometrial cancer proliferation. J Cell Physiol. 2009;218:490–500.
30. Wang Y, Wang Y, Zhang Z, Park JY, Guo D, Liao H, et al. Mechanism of progestin resistance in endometrial precancer/cancer through Nrf2-AKR1C1 pathway. Oncotarget. 2016;7:10363–72.
31. Chen J, Bai M, Ning C, Xie B, Zhang J, Liao H, et al. Gankyrin facilitates follicle-stimulating hormone-driven ovarian cancer cell proliferation through the PI3K/AKT/HIF-1α/cyclin D1 pathway. Oncogene. 2016;35:2506–17.
32. Chen X, Zhang Z, Feng Y, Fadare O, Wang J, Ai Z, et al. Aberrant survivin expression in endometrial hyperplasia: another mechanism of progestin resistance. Mod Pathol. 2009;22:699–708.
33. Bjerkan L, Schreurs O, Engen SA, Jahnsen FL, Baekkevold ES, Blix IJ, et al. The short form of TSLP is constitutively translated in human keratinocytes and has characteristics of an antimicrobial peptide. Mucosal Immunol. 2015;8:49–56.
34. Guo J, Wu G. The signaling and functions of heterodimeric bone morphogenetic proteins. Cytokine Growth Factor Rev. 2012;23:61–7.
35. Corren J, Ziegler SF. TSLP: from allergy to cancer. Nat Immunol. 2019;20:1603–9.
36. Pedroza-Gonzalez A, Xu K, Wu TC, Aspord C, Tindle S, Marches F, et al. Thymic stromal lymphopoietin fosters human breast tumor growth by promoting type 2 inflammation. J Exp Med. 2011;208:479–90.
37. Cunningham TJ, Tabacchi M, Eliane JP, Tuchayi SM, Manivasagam S, Mirzaalian H, et al. Randomized trial of calcipotriol combined with 5-fluorouracil for skin cancer precursor immunotherapy. J Clin Investig. 2017;127:106–16.
38. Zhang Y, Jin LP. Effects of TSLP on obstetrical and gynecological diseases. Am J Reprod Immunol. 2017;77:e12612.
39. Bessa PC, Casal M, Reis RL. Bone morphogenetic proteins in tissue engineering: the road from the laboratory to the clinic, part I (basic concepts). J Tissue Eng Regener Med. 2008;2:1–13.
40. Valera E, Isaacs MJ, Kawakami Y, Izpisúa Belmonte JC, Choe S. BMP-2/6 heterodimer is more effective than BMP-2 or BMP-6 homodimers as inductor of differentiation of human embryonic stem cells. PLoS ONE. 2010;5:e11167.
41. Koh JT, Zhao Z, Wang Z, Lewis IS, Krebsbach PH, Franceschi RT. Combinatorial gene therapy with BMP2/7 enhances cranial bone regeneration. J Dent Res. 2008;87:845–9.
42. Hayes SA, Zarnegar M, Sharma M, Yang F, Peehl DM, ten Dijke P, et al. SMAD3 represses androgen receptor-mediated transcription. Cancer Res. 2001;61:2112–8.
43. Kato J, Seto T. Correlation of androgen receptors with histological differentiation in human endometrial carcinomas. Acta Obstetricia et Gynecologica Scandinavica. 1985;64:209–12.
44. Sasagawa S, Shimizu Y, Kami H, Takeuchi T, Mita S, Imada K, et al. Dienogest is a selective progesterone receptor agonist in transactivation analysis with potent oral endometrial activity due to its efficient pharmacokinetic profile. Steroids. 2008;73:222–31.
45. Brenner RM, Slayden OD. Progesterone receptor antagonists and the endometrial antiproliferative effect. Semin Reprod Med. 2005;23:74–81.
46. Gu Y, Zhang M. Upregulation of the Kank1 gene inhibits human lung cancer progression in vitro and in vivo. Oncol Rep. 2018;40:1243–50.
47. Cui Z, Shen Y, Chen KH, Mittal SK, Yang JY, Zhang G. KANK1 inhibits cell growth by inducing apoptosis through regulating CXXC5 in human malignant peripheral nerve sheath tumors. Sci Rep. 2017;7:40325.
48. Wang J, Jia J, Zhou L. Long non-coding RNA CASC2 enhances cisplatin sensitivity in oral squamous cell cancer cells by the miR-31-5p/KANK1 axis. Neoplasma. 2020;67:1279–92.
49. Pu J, Shen J, Zhong Z, Yanling M, Gao J. KANK1 regulates paclitaxel resistance in lung adenocarcinoma A549 cells. Artif Cells Nanomed Biotechnol. 2020;48:639–47.
50. Ren TJ, Liu C, Hou JF, Shan FX. CircDDX17 reduces 5-fluorouracil resistance and hinders tumorigenesis in colorectal cancer by regulating miR-31-5p/KANK1 axis. Eur Rev Med Pharmacol Sci. 2020;24:1743–54.
51. Ai Z, Yin L, Zhou X, Zhu Y, Zhu D, Yu Y, et al. Inhibition of survivin reduces cell proliferation and induces apoptosis in human endometrial cancer. Cancer. 2006;107:746–56.
52. Murphy LC, Dotzlaw H, Wong MS, Miller T, Murphy LJ. Mechanisms involved in the evolution of progestin resistance in human breast cancer cells. Cancer Res. 1991;51:2051–7.

AUTHOR CONTRIBUTIONS

ZZ, LL and HL performed the study concept and designed the study. ML performed most of the experiments, analysed and interpreted the data, and wrote the manuscript. YX performed part of the experiments. PC, JL, ZQ and BH provided technical and material support. YL, XT, JX and YW conducted the data collection and analysis. YF and WZ revised the manuscript with comments from all authors. All authors read and approved the final manuscript.

FUNDING

This work was supported by grants from National Natural Science Foundation of China (grants 82371642), Shanghai Municipal Science and Technology Committee of Multi-center Clinical Study Project (20Z21900400), Shanghai Collaborative Innovation Center of Translational Medicine (TM202015), the Shanghai Municipal Public Health Bureau (202040078) for JX, Clinical Treatment Technology Standards for Assisted Reproductive Treatment in Patients with Crohn's Syndrome, Shanghai Science and Technology Commission (23DZ2202100), the start funding (RCQD2307) from Shanghai Tongji hospital for ZZ, Cross- and jointly project (2023-3-ZD-02) from Tongji University for ZZ, Health care project of Shanghai Pudong New Area Science and Technology Development Fund for Livelihood Research (PKJ2021-Y05) and Scientific research project of Shanghai Municipal Health Commission (202140279).

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The animal experiments in this study were authorised by Shanghai General Hospital's Animal Ethics Committee, with a project license number of 2020AW121.

CONSENT FOR PUBLICATION

Not applicable.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41416-023-02545-y.

Correspondence and requests for materials should be addressed to Zhenbo Zhang, Linxia Li or Hong Liao.