Pathology & Oncology Research (2020) 26:1757–1766
https://doi.org/10.1007/s12253-019-00718-w

ORIGINAL ARTICLE

LncRNA HEIH Enhances Paclitaxel-Tolerance of Endometrial Cancer Cells via Activation of MAPK Signaling Pathway

Jun-Liang Guo[1] · Tian Tang[1] · Jin-Hong Li[1] · Yi-Hong Yang[1] · Long Zhang[1] · Yi Quan[1]

Received: 30 January 2019 / Accepted: 13 August 2019 / Published online: 24 October 2019

Abstract

This study aimed to investigate the function of lncRNA HEIH on promoting endometrial cancer cells' tolerance of paclitaxel (PTX). LncRNA HEIH expression was measured by QRT-PCR in endometrial cancer tissues, human healthy tissues and cell lines. The PTX-resistant endometrial cancer cells (Ishikawa-RE and HHUA-RE) were intermittently exposed to increase concentrations of PTX and were constructed as evidenced by MTT assay. Besides, the specific siRNA of HEIH (siHEIH) and pcDNA3.1-HEIH plasmid transfection were utilized to alter the expression of HEIH in the cells and investigate the effects of HEIH on resistance to PTX in endometrial cancer cells. Moreover, MTT, colony formation and apoptosis analysis were taken advantage to evaluate cell viability and proliferation when treated with PTX. Then, differential genes in PTX-resistant and HEIH-knock-down PTX-resistant endometrial cancer cells were screened out by microarray analysis. Finally, gene-set enrichment analysis was used to predict the promising signaling pathway of HEIH and western blotting analysis were performed to verify the relevant genes expression of MAPK signaling pathway. LncRNA HEIH, the dysregulation of which involved in production of drug-resistance, was overexpressed in PTX-resistant endometrial cancer cells. Up-regulating HEIH would activate MAPK pathway, promote chemo-resistance of endometrial cancer cells and enhance cell proliferation and viability, whereas silencing HEIH depressed the MAPK signaling pathway, contributed to restoring chemo-sensitivity to PTX and repressed cell physiological process. Down-regulating lncRNA HEIH expression reversed the PTX-resistance of endometrial cancer cells through MAPK signaling pathway.

Keywords Endometrial cancer · HEIH · Paclitaxel resistance · MAPK signaling pathway

Introduction

Endometrial cancer is one of the eight most common causes of cancer-related death among females [1]. It is also the leading cause of gynecologic malignancy [2]. There were more than 5000 women diagnosed ever year and approximate 8000 death in America, furthermore with the incidence of the disease being in increasing steadily [3]. By the age of 75, the risk of developing endometrial cancer for female was about 0.6% in developing nations, which was doubled than developed nations [4]. In the early period, for over 80% of the disease located within the uterus, primary surgery along with radiation treatment was curative enough. However, when it came to advanced phase, chemotherapy displayed an irreplaceable role under treatment [5]. So, uncovering the novel treatment strategy to relieve this issue had far-reaching significance.

For endometrial cancer, paclitaxel (PTX) was a single-agent with response rates of more than 20% [6]. For example, synergy of PTX and trichostatin A was reported to inhibit the cell growth and induce apoptosis in human endometrial carcinoma cells [7]. Single-agent PTX was proved to be as effective as the combination of PTX and carboplatin, and more effective than single carboplatin, in inhibiting proliferation of endometrial cancer cells [8]. However, the therapeutic effect was not fairly effective resulting from the development of resistance to chemotherapy [1]. Nevertheless, investigation focused on the underlying molecular mechanism of chemo-

Jun-Liang Guo and Tian Tang contributed equally to this work.

✉ Yi Quan
quanyi516@163.com

[1] Center for Reproductive Medicine, Department of Gynecology and Obstetrics, West China Second University Hospital, Key Laboratory of Birth Defects and Related Diseases of Women and Children, Ministry of Education, Sichuan University, No.20 Section 3, Renmin South Road, Chengdu City 610041, Sichuan, People's Republic of China

resistance of endometrial cancer to the PTX was poor and might provide a comprehensive insight into developing novel therapeutic strategy.

Long non-coding RNAs (lncRNAs, ~200 nt) were a class of RNAs that didn't encode proteins [9]. Before it was found to play an essential part in many cell life activities, researchers believed this material without any biological function [10]. It was understood that a large amount of non-coding RNAs, both long and short, played critical roles in physiological and pathological process that were essential for life [11, 12]. Furthermore, some researchers reported that some lncRNAs, such as lnc-PRKCQ-1 and lnc-MTRNR2L1-2, which were significantly overexpressed or down-regulated in proteasome inhibitors-resistant myeloma cell lines [13]. Besides, overexpression of lncRNA FER1L4 in ovarian cancer cells suppressed PTX resistance via regulating MAPK pathways [14]. Therefore, some lncRNAs might play a critical role in the chemosensitivity of endometrial cancer and should be considered into more researches.

LncRNA-HEIH, locating in chromosome 5, was one member of lncRNA family, which was first identified and investigated in hepatocellular carcinoma [15], finding its high-expression in the patients with hepatocellular carcinoma [16]. Moreover, investigators considered HEIH might be a cancer-promoting factor resulting from its inhibition for cell differentiation in G0/G1 [17]. For example, HEIH promoted melanoma cell proliferation and migration [15]. Besides, the differential expression of HEIH was uncovered in HPV-negative head and neck squamous cell carcinoma [18]. However, the biological roles and effects of HEIH on drug-resistance, in endometrial cancer, were elusive and deserved our deeper investigation.

In the present study, we aim to investigate the function of lncRNA HEIH, via regulating HEIH expression in the cell models, in promoting the resistance of two kinds of endometrial cancer cells to PTX. These results demonstrated that the expression of HEIH involved in influencing the PTX resistance of cancer cells. The investigation was conducted to develop promising treatment strategies and improve the effectiveness of drug combination.

Materials and Methods

Clinical Tissue Sample

We obtained 45 endometrial tumor tissues and their adjacent tissues from patients in West China Second University Hospital. All specimens were collected from the uterus and placed in liquid nitrogen immediately after resection before putting them in the refrigerator at −80 °C. Preoperative chemotherapy and radiotherapy were not used to treat the above patients, and all of them gave informed consent before experiments. The study has been approved by the ethics committee of West China Second University Hospital.

Cell Culture and Construction of Resistance Cell Model

Human endometrial cancer cell lines (Ishikawa, HHUA) and hESC (Human endometrial stromal cells) were purchased from BeNa Culture Collection (Beijing, China) and cultured based on the specification of the company. To begin with, Ishikawa and HHUA cells at log-growth phase were treated with 0.1 μM PTX for 24 h. PBS was then used to remove fresh medium containing PTX for continuous culture. After cells were passed for three times, concentration of PTX was gradually elevated 0.2, 0.5, 1.0 and 2.0 μM until these cells could maintain stable growth at 2.0 μM PTX to establish drug resistant cell strain. Then Ishikawa, Ishikawa-RE, HHUA-RE and HHUA cells were treated with 0, 0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 μM PTX to verify the successful construction of drug-resistance cell lines. Moreover, all cells were cultured under the condition containing 5% CO_2 at 37 °C and collected in the logarithmic phase.

Cell Transfection

The pcDNA3.1-HEIH and specific siRNA against HEIH, named as siHEIH, were purchased from Shanghai GenePharma (GenePharma, Shanghai, China). Every cell line was placed in 6-well plates to a confluence of 70–90% in the previous day before transfection, and then was transfected with vector or siRNA using Lipofectamine 2000 Reagent (Life technologies) according to the manufacturer's instructions. The grouping information is as follows: Ishikawa, Ishikawa+pcDNA3.1-HEIH (Ishikawa+HEIH), Ishikawa-RE, Ishikawa-RE + siHEIH, HHUA, HHUA+pcDNA3.1-HEIH (HHUA+HEIH), HHUA-RE and HHUA-RE + siHEIH. Cells were incubated at 37 °C in a humidified incubator with 5% CO_2. After incubation for 48 h, cells were harvested.

MTT Assay

The transfected cells were seeded in 96-well plates which contained 2×10^3 cells per well. The cells in all groups were treated with PTX after reaching 70%–80% and incubated for 48 h. Next, MTT was added to those wells to reach the final concentration of 0.5 mg/ml. After 4 h, add DMSO to the well to dissolve the formazan. Finally, an enzyme-linked immunosorbent assay reader was used to measure absorbance at 490 nm.

Quantitative PCR Analysis

The Trizol reagent (Invitrogen, CA, USA) was utilized to isolate RNA from cell lysates. After quantification by NanoDrop 2000 (Thermo Fisier Scientific Inc., USA), Random Primers (Invitrogen) were used to generate cDNA according to the manufacturer's protocol. Real time system was utilized for quantitative reverse transcriptase–PCR (qRT–PCR) reactions. A relative quantification method was used to analyze the qRT-PCR data that were depicted as average fold change versus the control group (β-actin was utilized as internal control). The primers used in qRT-PCR were shown as follows: HEIH forward 5'-AAGAACTCTTCGCT CCAGCC-3′, HEIH reverse 5'-ACAAAAGCAGACTA GGGCGG-3′; β-actin forward 5'-GGACTTCGAGCAAG AGATGG-3′, β-actin reverse 5'-AGCACTGTGTTGGC GTACAG-3′. The relative expression levels were analyzed using the eq. $2^{-\Delta\Delta Ct}$ method.

Apoptosis Analysis

To investigate the effect of HEIH on cell apoptosis when treated with PTX, flow cytometry (FCM) assay was performed. We detached cells by trypsinization and collected them by centrifugation for 5 min. Then those cells were assayed by staining with 50 μl Annexin V binding buffer and 10 μl freshly prepared Annexin V–propidium iodide (PI) in dark for 30 min. Finally, a FACSC alibur flow cytometry (BD Biosciences, San Jose, CA, USA) was used to detect cell apoptosis.

Colony Forming Assay

Cells in all groups were seeded on 12-well cell culture dishes at 400 cells/well. On day 1 (day post-seeding), cells were treated with 2.5 μM PTX. All conditions were performed in quadruplicate. Cells were maintained for 2 weeks at which point they fixed with 4% paraformaldehyde for 15 min, to stationary phase. Next, appropriate amount of GIMSA was used to dye the cells for 10–30 min. Furthermore, colonies counted using the GelCount (Oxford Optronix, United Kingdom).

Bioinformatics Analysis

Microarray analysis was used to screen out differentially expressed genes in PTX-resistant endometrial cancer cells and HEIH-knock-down PTX-resistant endometrial cancer cells. Differential genes were selected based on their fold change and adjusted *p*-values, which were generated by R package. The inclusion criteria were set as follows: FDRPadj <0.05 and |log2(fold change)| > 1. Gene-set enrichment analysis (GSEA) of the differential genes was conducted to elucidate the regulation of HEIH on MAPK signaling pathway.

Western Blot Assay

Total protein lysates were obtained using Protein Extraction Kit (QIAGEN, USA), and quantified by Bradford Protein Assay Kit (Beyotime, Beijing, China). 20 μg of total protein lysate was loaded in each lane and samples were separated by SDS-PAGE. Proteins were then transferred to a polyvinylidene fluoride (PVDF) membrane, first blocked with Tris-buffered saline (TBS) with skim milk at room temperature for 60 min. Subsequently the membrane was incubated with primary antibodies: p-p38 MAPK and total p38 MAPK (Cell Signaling Technology, Boston, MA, USA), p-AKT1 (phospho S473) (ab81283, Abcam, Cambridge, MA, USA), total AKT1 (ab28422, Abcam, Cambridge, MA, USA), c-Fos (ab190289, Abcam, Cambridge, MA, USA), c-Jun (ab32137, Abcam, Cambridge, MA, USA) and β-actin (sc-1616, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The incubation with primary antibodies was carried out at 4 °C overnight followed by an one-hour incubation of the secondary antibody at room temperature. The immunoreactive protein bands were visualized by ECL Kit (Pierce, Thermo Fisher Scientific, IL, USA). The experiment was performed three separate times.

Statistical Analysis

All data were analyzed by Graphpad Software, version 6.0 (La Jolla, CA, USA) and represented as the mean the mean ± standard deviation (SD). The all results of two different groups was evaluated by Pearson's correlation analysis. All experiments were repeated at least thrice, and if $P < 0.05$, the data was considered to indicate statistical significance.

Results

High-Expression of HEIH in Endometrial Cancer Tissues and Construction of PTX-Resistant Cell Model

To investigate the expression of HEIH in endometrial cancer tissues, QRT-PCR was employed. HEIH was up-regulated in tumor tissues compared with adjacent tissues (Fig. 1a, $P < 0.01$). Furthermore, two endometrial cancer cell lines, Ishikawa and HHUA, were involved in further study. QRT-PCR analysis demonstrated that HEIH was up-regulated in endometrial cancer cells, Ishikawa and HHUA, compared with hESC (Fig. 1b, $P < 0.05$). MTT assay was utilized to determine cell viability when cells were treated with PTX. The results showed the construction of PTX-resistance cell lines were successful (Fig. 1c & d, $P < 0.05$). Besides, MTT assay demonstrated that higher cell viability was observed in Ishikawa-RE and HHUA-RE group cells when treated with paclitaxel compared with the matched control groups (Fig. 1e, $P < 0.01$). This result suggested successful construction of PTX-resistance cell models.

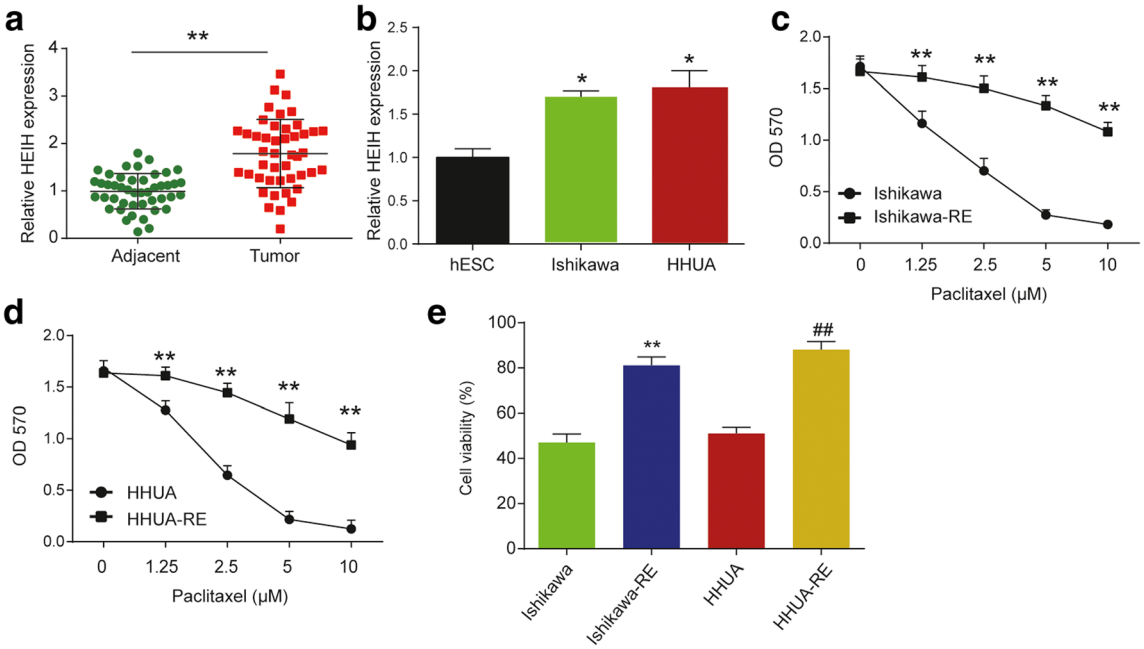

Fig. 1 High-expression of HEIH in endometrial cancer tissues and construction of PTX-resistance cell model. a QRT-PCR detected the expression of HEIH in adjacent and tumor tissues. $^{**}P < 0.01$ vs adjacent tissues. **b** Q RT-PCR was utilized to evaluate HEIH expression level in three cell lines, hESC, Ishikawa and HHUA. $^{*}P < 0.05$ vs hESC group. **c** Cell viability of Ishikawa and Ishikawa-RE wrer determined when treated with different concentrations of PTX via MTT assay. $^{**}P < 0.01$ vs Ishikawa group. **d** Cell viability of HHUA and HHUA-RE were determined when treated with different concentrations of PTX via MTT assay. $^{**}P < 0.01$ vs HHUA group. (E) MTT assay was taken advantage to assess cell viability with 2.5 μM PTX treatment. $^{**}P < 0.01$ vs Ishikawa group and $^{\#\#}P < 0.01$ vs HHUA group

HEIH Was Overexpressed in Drug-Resistance Cells

To evaluate the role of HEIH in paclitaxel-resistance, we firstly detected the expression of HEIH in PTX-resistance cells and endometrial cancer cells. HEIH was up-regulated in Ishikawa-RE and HHUA-RE group cells compared with endometrial cancer cell lines (Fig. 2a & b, $P < 0.01$). Moreover, siHEIH was performed to silence HEIH in Ishikawa-RE and HHUA-RE cells and pcDNA-3.1-HEIH plasmid was performed to up-regulate HEIH in Ishikawa and HHUA cells respectively. The high-expression level of HEIH was demonstrated in pcDNA-3.1 HEIH transfection group, named Ishikawa+HEIH and HHUA+HEIH, whereas HEIH expression was down-regulated in siRNA transfection group, named Ishikawa-RE + siHEIH and HHUA-RE + siHEIH (Fig. 2c & d, $P < 0.01$).

The Function of HEIH on Cell Proliferation and Viability

After altering HEIH expression, the effects of HEIH on cell viability were investigated by MTT analysis. Figure 3a & b showed that drug-resistance cell lines, Ishikawa-RE and HHUA-RE, presented higher cell viability compared with Ishikawa and HHUA cell lines when treated with PTX. Up-regulating HEIH expression in HHUA and Ishikawa cells enhanced cell viability under PTX treatment. Knocking down HEIH expression via siRNA restored the sensitivity of Ishikawa-RE and HHUA-RE cells to PTX ($P < 0.01$). Moreover, colony forming assay was used to evaluate cell proliferation ability after transfecting. As shown in Fig. 3c & d, Ishikawa-RE and HHUA-RE cells presented stronger cell proliferation compared with Ishikawa or HHUA cells. HEIH overexpression enhanced PTX-resistance of Ishikawa and HHUA cells, while si-HEIH suppressed PTX-resistance of Ishikawa-RE and HHUA-RE cells (all $P < 0.05$). These results proved that HEIH overexpression could promote drug-resistance ability of endometrial cancer cells to PTX and knocking down HEIH expression partly recovered the sensitivity of Ishikawa-RE and HHUA-RE to PTX.

HEIH Overexpression Depressed Cell Apoptosis in Drug-Resistance Cells

Before FCM assay being exerted to determine cell apoptosis ratio, pcDNA-3.1 HEIH or siRNA HEIH was transfected into endometrial cancer cells and PTX-resistance cells along with PTX treatment respectively. According to Fig. 4a, overexpression of HEIH in Ishikawa could significantly attenuate cell apoptosis induced by PTX compared with non-transfection Ishikawa, and suppressing HEIH expression in Ishikawa-RE cells contributed to higher cell apoptosis ratio compared with Ishikawa-RE cells ($P < 0.01$). Besides, similar trends were also observed in HHUA and HHUA-RE cells (Fig. 4b, $P < 0.01$). In conclusion, HEIH could hinder cell apoptosis to enhance the resistance of endometrial cancer cells to PTX.

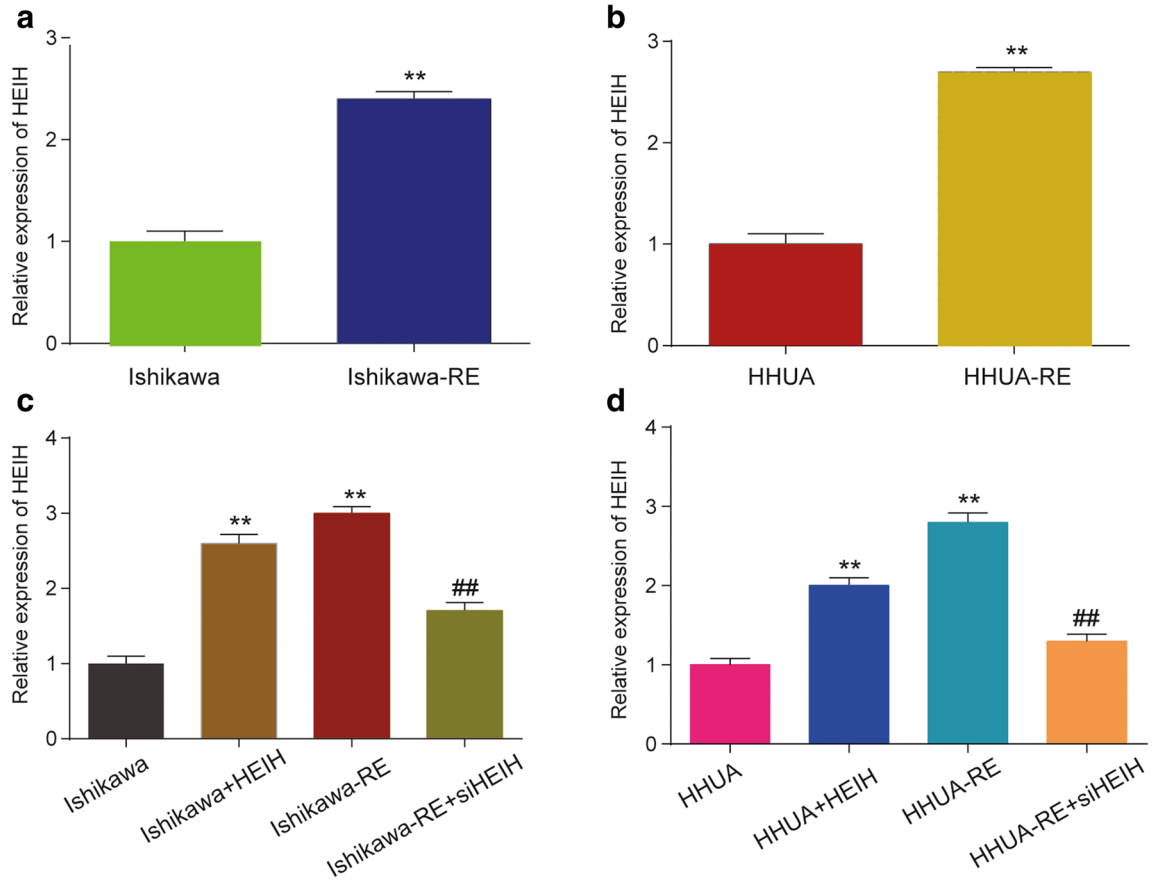

Fig. 2 Relative expressions of HEIH in endometrial cancer cells and PTX-resistance cells. a-b QRT-PCR was employed to evaluate relative expression level of HEIH in Ishikawa-RE, HHUA-RE, Ishikawa and HHUA. $^{**}P < 0.01$ vs the matched control group. **c-d** QRT-PCR was performed to assess expression level of HEIH in Ishikawa, HHUA, Ishikawa-RE and HHUA-RE after transfecting with pcDNA3.1-HEIH or siHEIH, respectively. $^{**}P < 0.01$ vs Ishikawa or HHUA group. $^{\#\#}P < 0.01$ vs Ishikawa-RE or HHUA-RE group

HEIH Knocking Down Inhibited MAPK Pathway in Drug-Resistance Cells

To explore the underlying molecular mechanisms of HEIH on PTX resistance, microarray analysis was performed to screen out differentially expressed genes in PTX-resistant endometrial cancer cells and HEIH-knock-down PTX-resistant endometrial cancer cells. Total 1458 differential gene were screened out and top 10 upregulated and downregulated gene were displayed in Fig. 5a. GSEA results displayed that MAPK signaling pathway was suppressed after knocking down HEIH in endometrial cancer cells (Fig. 5b). Figure 5c showed differential genes in MAPK signaling pathway. Among these differential genes, AKT1, Fos and Jun were low-expressed in siHEIH transfection group (Fig. 5c). The relative protein levels of p-p38 were also detected to investigate on MAPK signaling pathway. The high-expression levels of p-p38, p-AKT1, c-Fos and c-Jun were demonstrated in Ishikawa+HEIH and HHUA+HEIH groups compared with Ishikawa and HHUA groups, whereas corresponding expressions were down-regulated in Ishikawa-RE + siHEIH and HHUA-RE + siHEIH groups compared with Ishikawa-RE and HHUA-RE groups respectively. These results suggest that HEIH may perform its functions by activating MAPK signaling pathway in PTX-resistance cancer cells (Fig. 5d & e, $P < 0.01$).

Discussion

In the present study, chemo-resistance cell models were constructed to further study, which uncovered that dysregulation of lncRNA HEIH, in endometrial cancer cells, contributed to the forming of resistance to PTX and influenced cell viability, proliferation and apoptosis.

As showed by previous investigation, lncRNA HEIH was overexpressed in colorectal cancer cells, which would promote tumorigenesis via regulating Bcl-xL expression [19]. Moreover, there was research indicating that lncRNA HEIH, which was up-regulated in liver cells, promoted cells proliferation and even played a crucial role in regulating the invasion ability of liver cancer cells [20]. Besides, lncRNA HEIH was

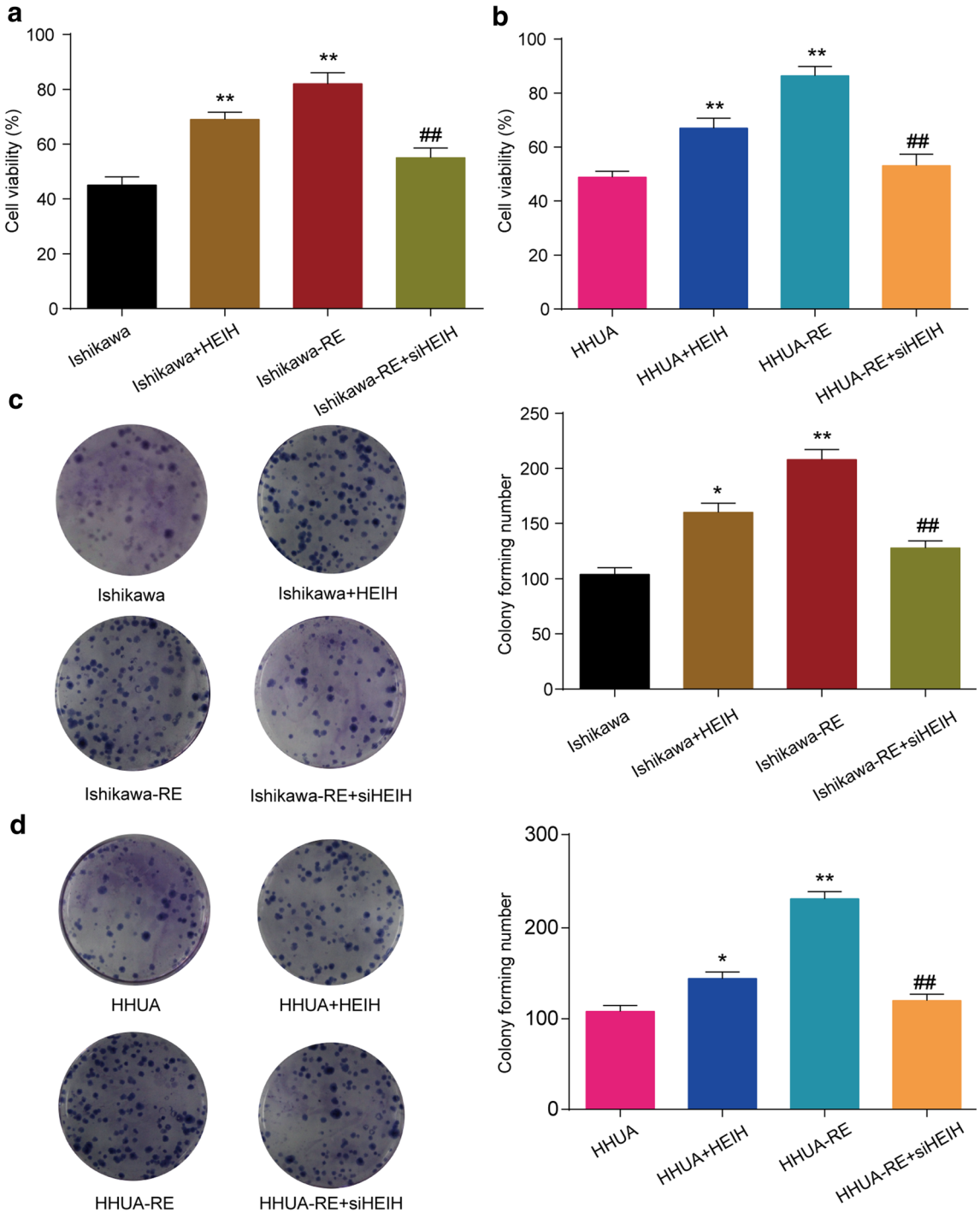

Fig. 3 Dysregulation of HEIH influences cell viability and proliferation. a Cell viability was determined by MTT assay for Ishikawa, Ishikawa+HEIH, Ishikawa-RE and Ishikawa-RE + siHEIH with PTX treatment. **b** Cell viability was determined by MTT assay for HHUA, HHUA+HEIH, HHUA-RE and HHUA-RE + siHEIH with PTX treatment. **c** The representative images of colony forming assay and colony forming cell number of different groups with PTX treatment. **d** The representative images of colony forming assay and colony forming cell number of different groups with PTX treatment. $^{*}P < 0.01$ and $^{**}P < 0.01$ vs Ishikawa or HHUA group. $^{\#\#}P < 0.01$ vs Ishikawa-RE or HHUA-RE group

up-regulated in hepatocellular carcinoma cells and interacted with zeste homolog 2, which could promote cell proliferation and metastasis via repressing zeste homolog 2 target genes [16]. In addition, Bawa et al. reported that HEIH was a kind of lncRNAs upregulated in prostate cancer via utilizing multiple RNA-seq datasets to take an integrative analysis [11]. However, investigation focused on function of HEIH in the

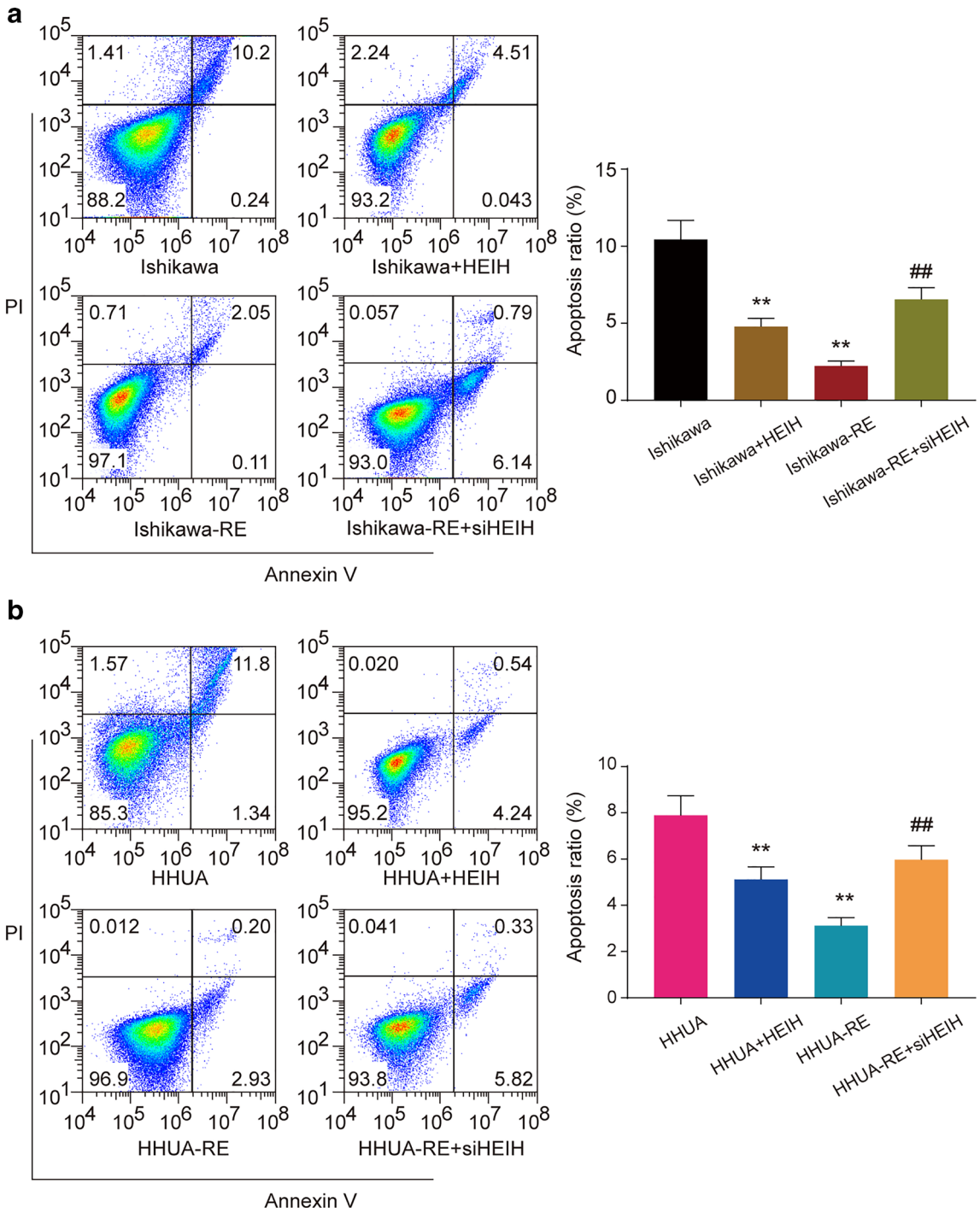

Fig. 4 HEIH affect PTX-resistance through regulating cell apoptosis. **a** FCM assay was utilized to determine cell apoptosis ratio in different groups, Ishikawa, Ishikawa+HEIH, Ishikawa-RE and Ishikawa-RE + siHEIH after PTX treatment. **b** FCM assay was utilized to determine cell apoptosis ratio in different groups, HHUA, HHUA+HEIH, HHUA-RE and HHUA-RE + siHEIH after PTX treatment. $^{**}P < 0.01$ vs Ishikawa or HHUA group. $^{\#\#}P < 0.01$ vs Ishikawa-RE or HHUA-RE group

tumorigenesis of endometrial cancer and chemotherapy was rare. In the study herein, the overexpression of lncRNA HEIH in endometrial cancer tissues and cells was illustrated through QRT-PCR assay.

A kind of anticancer compound, PTX, which was extracted from the Pacific yew tree, could stabilize microtubules and then exerted defects in the mitotic spindle assembly to involve in cellular fission [21]. However, Li et al. pointed out that efficacy could be reduced according to lower sensitivity to PTX in cancer cells [22]. Some reporters focused on the effects of lncRNA on chemo-resistance in certain cancers, indicating the important role of lncRNA in this filed. For example,

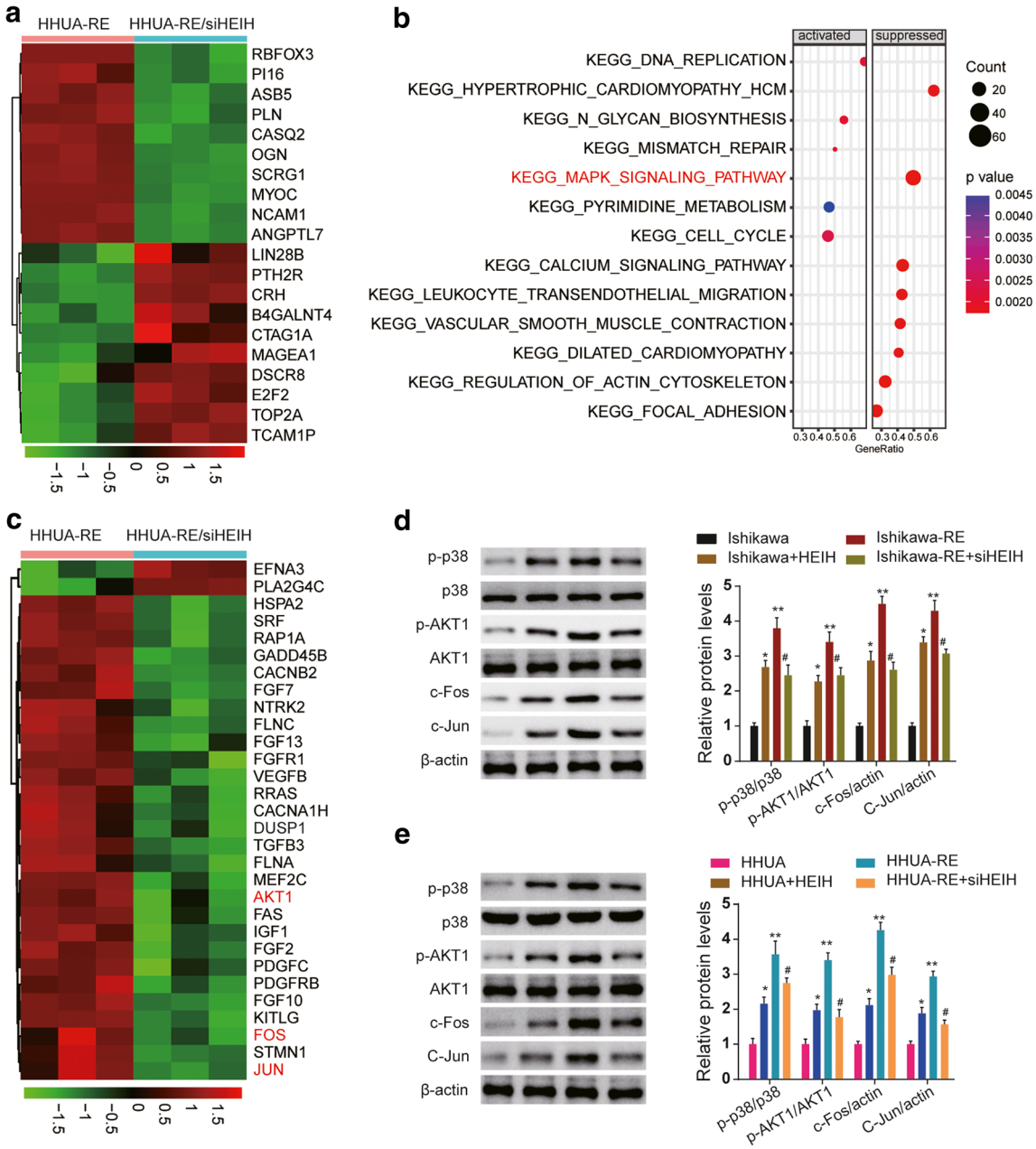

Fig. 5 Knocking down HEIH resulted in suppression of MAPK signaling pathway. a Heatmap for differential genes in PTX-resistant HHUA-RE and HHUA-RE/si-HEIH cells. **b** Gene-set enrichment analysis of differential genes showed that MAPK signaling pathway was suppressed after knocking down HEIH in PTX-resistant endometrial cancer cells. **c** Heatmap for differential genes in MAPK signaling pathway among HHUA-RE and HHUA-RE/si-HEIH cells. **d** Western blot was conducted to detect relative protein levels involved in MAPK pathway in 4-group, Ishikawa cells, Ishikawa, Ishikawa+HEIH, Ishikawa-RE and Ishikawa-RE + siHEIH after PTX treatment. **e** Western blot was conducted to detect relative protein levels involved in MAPK pathway in 4-group, HHUA cells, HHUA, HHUA+HEIH, HHUA-RE and HHUA-RE + siHEIH after PTX treatment. $^{*}P < 0.05$, $^{**}P < 0.01$ vs Ishikawa or HHUA group. $^{\#}P < 0.05$ vs Ishikawa-RE or HHUA-RE group

Liu et al. suggested that upregulation of lncRNA HOTAIR involved in the cisplatin resistance of lung adenocarcinoma cells [23]. Besides, lncRNA CCAT1 modulating the sensitivity of PTX in nasopharynx cancers cells via miR-181a/CPEB2 axis was reported by Wang et al. [24]. Similarly, in our study, drug-resistance cell models were applied to demonstrate lncRNA HEIH contributed to PTX resistance in endometrial carcinoma.

Mitogen-activated protein kinase (MAPK) is an important signaling pathway in cells, mainly involving c-Jun NH2-

terminal kinase/stress-activated protein kinase, extracellular signal regulated protein kinase and p38-MAPK [25]. The p38-MAPK signaling pathway has complex effects on tumors [26]. c-jun and c-fos are two transcriptions factors known to be regulated by MAPK cascades. ERK1/2, one of MAPK kinases, was indicated to promote the development of cervical neoplasm cells via regulating the expression of c-FOS and c-JUN proteins [27], while folate receptor alpha was proved to regulate the growth of cervical cancer cells by activating ERK1/2/c-Fos/c-Jun [28]. Additionally, the function of MAPK pathway on drug-resistance in cancer cells has long been investigated. For instance, activation of MAPK signaling was proved to result in resistance to saracatinib (AZD0530) in ovarian cancer [29]. LncRNA SNHG12 was found to cause multidrug resistance through activating the MAPK/Slug pathway in non-small cell lung cancer [30]. However, the role of MAPK pathway on PTX resistance in endometrial cancer was unclear and deserved deeper investigation. Consequently, our research focused on this area.

In conclusion, these results demonstrated that silencing lncRNA HEIH played a critical role in restoring chemo-sensitivity, suppressing cell proliferation and promoting cell apoptosis via MAPK pathway when treated with PTX. Therefore, this research exerted far-reaching significance in developing promising therapy for patients with endometrial cancer.

Author Contributions JG, TT and JL: conception and design, analysis and interpretation of data; YY and LZ: drafting the article; YQ: revising it critically for important intellectual content. YQ is the guarantor.

Funding Information This study was funded by New Bud Science Foundation(KX104), West China Second University Hospital, Sichuan University.

Compliance with Ethical Standards

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the committee of West China Second University Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Dong L, Zhou Q, Zhang Z, Zhu Y, Duan T, Feng Y (2012) Metformin sensitizes endometrial cancer cells to chemotherapy by repressing glyoxalase I expression. J Obstet Gynaecol Res 38(8): 1077–1085. https://doi.org/10.1111/j.1447-0756.2011.01839.x
2. Kharma B, Baba T, Mandai M, Matsumura N, Murphy SK, Kang HS, Yamanoi K, Hamanishi J, Yamaguchi K, Yoshioka Y, Konishi I (2013) Utilization of genomic signatures to identify high-efficacy candidate drugs for chemorefractory endometrial cancers. Int J Cancer 133(9):2234–2244. https://doi.org/10.1002/ijc.28220
3. Viswanathan AN, Moughan J, Miller BE, Xiao Y, Jhingran A, Portelance L, Bosch WR, Matulonis UA, Horowitz NS, Mannel RS, Souhami L, Erickson BA, Winter KA, Small W Jr, Gaffney DK (2015) NRG oncology/RTOG 0921: a phase 2 study of post-operative intensity-modulated radiotherapy with concurrent cisplatin and bevacizumab followed by carboplatin and paclitaxel for patients with endometrial cancer. Cancer 121(13):2156–2163. https://doi.org/10.1002/cncr.29337
4. Galaal K, Al Moundhri M, Bryant A, Lopes AD, Lawrie TA (2014) Adjuvant chemotherapy for advanced endometrial cancer. Cochrane Database Syst Rev 5:CD010681. https://doi.org/10.1002/14651858.CD010681.pub2
5. Reyes HD, Miecznikowski J, Gonzalez-Bosquet J, Devor EJ, Zhang Y, Thiel KW, Samuelson MI, McDonald M, Stephan JM, Hanjani P, Guntupalli S, Tewari KS, Backes F, Ramirez N, Fleming GF, Filiaci V, Birrer MJ, Leslie KK (2017) High stathmin expression is a marker for poor clinical outcome in endometrial cancer: an NRG oncology group/gynecologic oncology group study. Gynecol Oncol 146(2):247–253. https://doi.org/10.1016/j.ygyno.2017.05.017
6. Altundag O, Dursun P, Ayhan A (2010) Emerging drugs in endometrial cancers. Expert Opin Emerg Drugs 15(4):557–568. https://doi.org/10.1517/14728214.2010.517521
7. Jiang SJ, Zhang S, Mu XY, Li W, Wang Y (2008) Effects of trichostatin a and paclitaxel on apoptosis and microtubule stabilization in endometrial carcinoma cells: an in vitro research. Zhonghua Yi Xue Za Zhi 88(34):2427–2431
8. Kuittinen T, Rovio P, Staff S, Luukkaala T, Kallioniemi A, Grenman S, Laurila M, Maenpaa J (2017) Paclitaxel, carboplatin and 1,25-D3 inhibit proliferation of endometrial cancer cells in vitro. Anticancer Res 37(12):6575–6581. https://doi.org/10.21873/anticanres.12114
9. Liz J, Esteller M (2016) lncRNAs and microRNAs with a role in cancer development. Biochim Biophys Acta 1859(1):169–176. https://doi.org/10.1016/j.bbagrm.2015.06.015
10. Philippen LE, Dirkx E, da Costa-Martins PA, De Windt LJ (2015) Non-coding RNA in control of gene regulatory programs in cardiac development and disease. J Mol Cell Cardiol 89(Pt A):51–58. https://doi.org/10.1016/j.yjmcc.2015.03.014
11. Bawa P, Zackaria S, Verma M, Gupta S, Srivatsan R, Chaudhary B, Srinivasan S (2015) Integrative analysis of Normal long intergenic non-coding RNAs in prostate Cancer. PLoS One 10(5):e0122143. https://doi.org/10.1371/journal.pone.0122143
12. Szafranski K, Abraham KJ, Mekhail K (2015) Non-coding RNA in neural function, disease, and aging. Front Genet 6:87. https://doi.org/10.3389/fgene.2015.00087
13. Malek E, Kim BG, Driscoll JJ (2016) Identification of long non-coding RNAs deregulated in multiple myeloma cells resistant to proteasome inhibitors. Genes (Basel) 7(10). https://doi.org/10.3390/genes7100084
14. Liu S, Zou B, Tian T, Luo X, Mao B, Zhang X, Lei H (2018) Overexpression of the lncRNA FER1L4 inhibits paclitaxel tolerance of ovarian cancer cells via the regulation of the MAPK signaling pathway. J Cell Biochem. https://doi.org/10.1002/jcb.28032
15. Zhao H, Xing G, Wang Y, Luo Z, Liu G, Meng H (2017) Long noncoding RNA HEIH promotes melanoma cell proliferation, migration and invasion via inhibition of miR-200b/a/429. Biosci Rep 37(3). https://doi.org/10.1042/BSR20170682

16. Yang F, Zhang L, Huo XS, Yuan JH, Xu D, Yuan SX, Zhu N, Zhou WP, Yang GS, Wang YZ, Shang JL, Gao CF, Zhang FR, Wang F, Sun SH (2011) Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. Hepatology 54(5):1679–1689. https://doi.org/10.1002/hep.24563
17. He Y, Meng XM, Huang C, Wu BM, Zhang L, Lv XW, Li J (2014) Long noncoding RNAs: novel insights into hepatocelluar carcinoma. Cancer Lett 344(1):20–27. https://doi.org/10.1016/j.canlet.2013.10.021
18. Haque SU, Niu L, Kuhnell D, Hendershot J, Biesiada J, Niu W, Hagan MC, Kelsey KT, Casper KA, Wise-Draper TM, Medvedovic M, Langevin SM (2018) Differential expression and prognostic value of long non-coding RNA in HPV-negative head and neck squamous cell carcinoma. Head Neck 40(7):1555–1564. https://doi.org/10.1002/hed.25136
19. Cui C, Zhai D, Cai L, Duan Q, Xie L, Yu J (2018) Long noncoding RNA HEIH promotes colorectal cancer tumorigenesis via counteracting miR-939Mediated transcriptional repression of Bcl-xL. Cancer Res Treat 50(3):992–1008. https://doi.org/10.4143/crt.2017.226
20. Zhang Y, Li Z, Zhang Y, Zhong Q, Chen Q, Zhang L (2015) Molecular mechanism of HEIH and HULC in the proliferation and invasion of hepatoma cells. Int J Clin Exp Med 8(8):12956–12962
21. Tanaka T, Toujima S, Tanaka J (2012) Differential sensitivity to paclitaxel-induced apoptosis and growth suppression in paclitaxel-resistant cell lines established from HEC-1 human endometrial adenocarcinoma cells. Int J Oncol 41(5):1837–1844. https://doi.org/10.3892/ijo.2012.1600
22. Li L, Shou H, Wang Q, Liu S (2019) Investigation of the potential theranostic role of KDM5B/miR-29c signaling axis in paclitaxel resistant endometrial carcinoma. Gene. https://doi.org/10.1016/j.gene.2018.12.076
23. Liu Z, Sun M, Lu K, Liu J, Zhang M, Wu W, De W, Wang Z, Wang R (2013) The long noncoding RNA HOTAIR contributes to cisplatin resistance of human lung adenocarcinoma cells via downregualtion of p21(WAF1/CIP1) expression. PLoS One 8(10):e77293. https://doi.org/10.1371/journal.pone.0077293
24. Wang Q, Zhang W, Hao S (2017) LncRNA CCAT1 modulates the sensitivity of paclitaxel in nasopharynx cancers cells via miR-181a/CPEB2 axis. Cell Cycle 16(8):795–801. https://doi.org/10.1080/15384101.2017.1301334
25. Gaundar SS, Bendall LJ (2010) The potential and limitations of p38MAPK as a drug target for the treatment of hematological malignancies. Curr Drug Targets 11(7):823–833
26. Noel JK, Crean S, Claflin JE, Ranganathan G, Linz H, Lahn M (2008) Systematic review to establish the safety profiles for direct and indirect inhibitors of p38 mitogen-activated protein kinases for treatment of cancer. A systematic review of the literature. Med Oncol 25(3):323–330. https://doi.org/10.1007/s12032-008-9039-1
27. Bai L, Mao R, Wang J, Ding L, Jiang S, Gao C, Kang H, Chen X, Sun X, Xu J (2015) ERK1/2 promoted proliferation and inhibited apoptosis of human cervical cancer cells and regulated the expression of c-Fos and c-Jun proteins. Med Oncol 32(3):57. https://doi.org/10.1007/s12032-015-0490-5
28. Liu C, Ding L, Bai L, Chen X, Kang H, Hou L, Wang J (2017) Folate receptor alpha is associated with cervical carcinogenesis and regulates cervical cancer cells growth by activating ERK1/2/c-Fos/c-Jun. Biochem Biophys Res Commun 491(4):1083–1091. https://doi.org/10.1016/j.bbrc.2017.08.015
29. McGivern N, El-Helali A, Mullan P, McNeish IA, Paul Harkin D, Kennedy RD, McCabe N (2018) Activation of MAPK signalling results in resistance to saracatinib (AZD0530) in ovarian cancer. Oncotarget 9(4):4722–4736. https://doi.org/10.18632/oncotarget.23524
30. Wang P, Chen D, Ma H, Li Y (2017) LncRNA SNHG12 contributes to multidrug resistance through activating the MAPK/slug pathway by sponging miR-181a in non-small cell lung cancer. Oncotarget 8(48):84086–84101. https://doi.org/10.18632/oncotarget.20475