Ai-IncRNA *EGOT* enhancing autophagy sensitizes paclitaxel cytotoxicity via upregulation of ITPR1 expression by RNA-RNA and RNA-protein interactions in human cancer

Shouping Xu¹, Peiyuan Wang¹, Jian Zhang¹, Hao Wu¹, Shiyao Sui¹, Jinfeng Zhang¹, Qin Wang¹, Kun Qiao¹, Weiwei Yang², Hongbiao Xu¹ and Da Pang¹,³*

**Abstract**

**Background:** The biology function of antisense intronic long noncoding RNA (Ai-IncRNA) is still unknown. Meanwhile, cancer patients with paclitaxel resistance have limited therapeutic options in the clinic. However, the potential involvement of Ai-IncRNA in paclitaxel sensitivity remains unclear in human cancer.

**Methods:** Whole transcriptome sequencing of 33 breast specimens was performed to identify Ai-IncRNA *EGOT*. Next, the role of *EGOT* in regulation of paclitaxel sensitivity was investigated. Moreover, the mechanism of *EGOT* enhancing autophagy sensitizes paclitaxel cytotoxicity via upregulation of ITPR1 expression by RNA-RNA and RNA-protein interactions was investigated in detail. Furthermore, upstream transcriptional regulation of *EGOT* expression was also investigated by co-immunoprecipitation and chromatin immunoprecipitation. Finally, clinical breast specimens in our cohort, TCGA and ICGC were applied to validate the role of *EGOT* in enhancing of paclitaxel sensitivity.

**Results:** *EGOT* enhances autophagosome accumulation via the up-regulation of ITPR1 expression, thereby sensitizing cells to paclitaxel toxicity. Mechanistically, on one hand, *EGOT* upregulates ITPR1 levels via formation of a pre-ITPR1/*EGOT* dsRNA that induces pre-ITPR1 accumulation to increase ITPR1 protein expression in cis. On the other hand, *EGOT* recruits hnRNPH1 to enhance the alternative splicing of pre-ITPR1 in trans via two binding motifs in *EGOT* segment 2 (324–645 nucleotides) in exon 1. Moreover, *EGOT* is transcriptionally regulated by stress conditions. Finally, *EGOT* expression enhances paclitaxel sensitivity via assessment of cancer specimens.

**Conclusions:** These findings broaden comprehensive understanding of the biology function of Ai-IncRNAs. Proper regulation of *EGOT* may be a novel synergistic strategy for enhancing paclitaxel sensitivity in cancer therapy.

**Keywords:** Ai-IncRNA, *EGOT*, ITPR1, Paclitaxel, Autophagy, Cancer

* Correspondence: pangda@ems.hrbmu.edu.cn
† Shouping Xu and Peiyuan Wang contributed equally to this work.
1Department of Breast Surgery, Harbin Medical University Cancer Hospital, 150 Haping Road, Harbin 150081, China
3Heilongjiang Academy of Medical Sciences, 157 Baojian Road, Harbin 150086, China

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Background
The microtubule-disrupting agent paclitaxel, a plant alkaloid developed from the bark of the Pacific yew tree, Taxus brevifolia, is a first-line chemotherapeutic agent for solid tumors, such as lung, ovarian and breast cancer, and contributes to substantial improvement in patient survival [1]. However, clinical responses to paclitaxel have shown variable sensitivity in cancer patients [2]. Thus, elucidation of the underlying mechanisms of paclitaxel sensitivity and identification of reliable biomarkers that can predict the response to paclitaxel in human cancer are urgently required.

Autophagy is a homeostatic process for degrading cellular materials under cellular stress [3]. Accumulating evidence has shown that autophagy is involved in modulating paclitaxel sensitivity. Tumors expressing diminished levels of autophagy-initiating genes are resistant to paclitaxel therapy, whereas induction of autophagy improves the antitumoral efficiency of the microtubule-disrupting agent paclitaxel [4, 5]. Furthermore, several studies have proposed that autophagy-related genes/proteins could be investigated as possible prognostic or predictive markers of paclitaxel efficacy in the clinic [6]. However, it is not clear which autophagy-initiating genes can be used as a predictive factor for paclitaxel response.

Long noncoding RNAs (lncRNAs) are a heterogeneous class of transcripts with a minimum length of 200 bases and limited protein-coding potential [7]. LncRNAs exhibit a wide range of expression levels and distinct cellular localizations and are thus a large and diverse class of regulators [8]. LncRNAs can function in cis to regulate the expression of neighboring genes or in trans to perform many roles in various modes [9]. The landscape of lncRNAs dysregulated in autophagy and their molecular mechanisms in autophagy regulatory networks were summarized in our previous study in detail [10]. Studies have demonstrated that lncRNAs such as NBR2 and APF can regulate autophagy processes with the aid of certain important proteins [10]. Several lncRNAs have also recently been implicated in the modulation of drug resistance [11]. However, the relationship between lncRNA expression and paclitaxel insensitivity caused by abnormal autophagy remains largely unexplored.

Eosinophil granule ontogeny transcript (EGOT), as an antisense intronic long noncoding RNA (Ai-lncRNA), is expressed from ITPRI, which is a ligand-gated ion channel that mediates calcium release from intracellular stores [12, 13]. The biology function of Ai-lncRNAs including EGOT is still largely unknown. In our previous study, we first reported that downregulation of EGOT expression was correlated with advanced malignant status and worse prognosis in breast cancer [14]. Here, we describe a previously unrecognized role of EGOT in increasing sensitivity to paclitaxel via triggering autophagy. Mechanistically, EGOT enhances autophagosome accumulation via the upregulation of ITPRI expression in cis and in trans. On one hand, EGOT upregulates ITPRI levels via formation of a pre-ITPRI/EGOT dsRNA that induces pre-ITPRI accumulation to increase ITPRI protein expression in cis. On the other hand, EGOT recruits hnRNPH1 to enhance the alternative splicing of pre-ITPRI in trans via two binding motifs in EGOT segment 2 (324–645 nucleotides) in exon 1. We also uncover that hypoxia induces EGOT transcriptional expression, while estrogen suppresses its expression directly. Finally, EGOT is confirmed to be associated with a favorable prognosis and to enhance paclitaxel sensitivity in breast cancer patients and other human malignancies in our breast cancer cohort and public data cohorts. Given its significance in the autophagy signaling pathway, EGOT may be act as a promising predictive biomarker for paclitaxel response, and proper regulation of EGOT may be a novel synergistic strategy for enhancing paclitaxel sensitivity in human cancer.

Materials and methods
Breast specimens and clinical assessments
Eligible patients with a histological diagnosis of breast cancer who had received neither chemotherapy nor radiotherapy before surgical resection were recruited to the present study. In total, 258 breast cancer tissues and 258 normal tissues, along with 15 adjuvant chemotherapy regimens, were obtained from Harbin Medical University Cancer Hospital/Center in Harbin, China. All samples were frozen in liquid nitrogen immediately after surgical resection, and only tumors with > 80% tumor cells were selected for RNA extraction. Two independent senior pathologists confirmed the pathological diagnosis and molecular subtype of each cancer tissue. This study conformed to the clinical research guidelines and was approved by the research ethics committee of Harbin Medical University Cancer Hospital. We obtained written informed consent from all patients. For 184 patients, telephone follow-up was performed to verify survival status after primary treatment. Among them, 159 patients received paclitaxel treatment.

Cell culture and treatments
The human cervical cancer cell line HeLa and 10 breast cancer cell lines (MCF7, T47D, UACC-812, SK-BR-3, MDA-MB-453, MDA-MB-231, Hs578T, HCC70, BT549, and MDA-MB-468) were obtained from the Chinese Academy of Sciences Cell Bank and Cellbio (China), respectively. All cell lines were periodically authenticated (Cellbio). Unless specifically indicated, cells were cultured in DMEM (R10–017-CV, Corning, USA), RPMI-1640 (R10–040-CM, Corning) or Leibovitz’s L15 (PYG0038, Boster, China) medium supplemented with 10% fetal bovine serum (0500, ScienCell, USA) at 37 °C with 5% CO₂ or air and 95% humidity. In estrogen-related experiments,
MCF7 and T47D cells were washed three times with phenol red-free DMEM and subjected to hormone deprivation for up to 3 days with 10% charcoal-adsorbed fetal calf serum (FCS; P30–2302, PAN) before proceeding to the next steps. The reagents used were 17 β-oestradiol (E8875, Sigma, USA), the estrogen receptor antagonist tamoxifen (T5648, Sigma) and ICI 182780 (Asc-131, Ascent Scientific, USA). Autophagosome and LC3 experiments were performed as previously described [15] and were performed in one of two ways. Cells (10–20 x 10^4 cells/ml) were plated in medium containing 10% serum. After 24 h, the medium was changed to that containing 0.1% serum, and the cells were collected 48 h later with or without 100 mM CQ (ab142116, Abcam, USA) treatment for 1 h before collection. Alternatively, the cells were cultured with EBSS (E2888, Sigma) for the indicated durations before collection. For the ubiquitination assay, HeLa cells were treated with MG132 (10 μM/l) (ab141003, Abcam) for 40 min. Protein levels were detected by immunoblotting and quantified by densitometry. For the CHX chase assay, HeLa cells were incubated with 50 μg/ml CHX (Catalogue Number HY-12320, MCE, USA) for the indicated durations (0.15, 30, 60, 120, and 180 min) as previously described [16]. For transcriptional inhibition experiments, actinomycin D (6 μg/ml) (A1410, Sigma) was added to the cells, and samples were harvested at the indicated time points (0, 0.5, 1, 2, 4, and 8 h) as previously described [17].

RNA extraction, reverse transcription and quantitative RT-PCR and PCR array
Total RNA and miRNA were extracted from cells using the E.Z.N.A.* Total RNA Kit I (Catalogue Number R6834–01, Omega Bio-Tek, USA). First-strand cDNA was prepared with the Transcriptor First Strand cDNA Synthesis Kit (Catalogue Number 04897030001, Roche, USA). Real-time PCR was performed using FastStart Universal SYBR Green Master (ROX) (Catalogue Number 04913914001, Roche) on a 7500 Fast Real-Time PCR system (ABI, USA). For quantification of gene expression, we used the 2^(-ΔΔCt) method. GAPDH expression was used for normalization. The primer sequences were synthesized by Shanghai Generary Biotech Co., Ltd. and are included in Additional file 1: Table S1.

Lentivirus production and infection
Recombinant lentiviruses expressing the lncRNA EGOT, shEGOT, shITPR1, shESR, EGOT-MS2, Flag-MCP2 and controls were constructed by Sangon Biotech Company (China). Concentrated viruses were used to infect 5 x 10^5 cells in a 6-well plate with 4–6 μg/ml polybrene (107,689, Sigma). The infected cells were then subjected to selection with 1 μg/ml puromycin (Catalogue Number 540411, Calbiochem, USA) for two weeks. Stable overexpression cell lines or knockdown cell lines were identified using qRT-PCR or western blotting. The shRNA sequences are provided in Additional file 1: Table S1.

Co-immunoprecipitation, western blot assay and antibodies
Co-immunoprecipitation assays were carried out by using the Pierce™ Crosslink Magnetic IP/Co-IP Kit (Catalogue Number 88805, Thermo Fisher, USA) according to the manufacturer's protocol. Western blotting was performed according to the previously described procedures [18]. Anti-LC3B (ab48394, 1:1000), anti-IP3R (ab5804, 1:1000) antibody, anti-SQSTM1/P62 (ab91526, 1:1000) antibody, anti-hnRNPH1 (ab10374, 1:1000) antibody, anti-HMGB1 (ab79823, 1:1000) antibody, anti-IP140 (ab42126,1:500) antibody, goat anti-rabbit IgG H&L (HRP) (ab6721, 1:10,000), and goat anti-mouse IgG H&L (HRP) (ab6789, 1:10,000) were obtained from Abcam. Anti-tubulin (sc-73,242, 1:1000) antibody was purchased from Santa Cruz Biotechnology. Anti-estrogen receptor (WL00940, 1:1000) antibody and anti-GAPDH (WL01114, 1:1000) antibody were obtained from Wanleibio (China). Anti-Bax (D2E11, 1:1000) (5023) antibody, anti-Bcl2 (2872, 1:1000) antibody and c-Jun (60A8) Rabbit mAb (#9165, 1:500) antibody were obtained from Cell Signaling Technologies (CST). Total cell lysates were prepared using a 1 x sodium dodecyl sulfate buffer. Identical quantities of proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose filter membranes. After incubation with specific antibodies, the blots were incubated with goat anti-rabbit IgG H&L (HRP) and goat anti-mouse IgG H&L (HRP) for 1 h at room temperature. The proteins were detected using a FluorChem HD2 (Protein Simple, USA).

Tandem mRFP-GFP fluorescence microscopy
Tandem monomeric RFP-GFP-tagged LC3 (tfLC3) (HBAP2100001, Hanbio, China) was used to monitor autophagic flux as previously reported. LC3-II relocalized to the autophagosomal membranes during autophagy. Thus, the accumulation of mRFP-GFP-LC3 puncta is an effective way to detect autophagosomes. When tfLC3 is located in autolysosomes, this form of LC3 displays only acidic condition in the lysosome lumen, whereas the RFP signal is more stable. To evaluate tandem fluorescent LC3 puncta, 48 h after tfLC3 transfection, cells were washed once with 1 x PBS, incubated with EBSS (E2888, Sigma) for the indicated durations and then directly sent out for confocal microscopy analysis. Images of samples were captured using a Zeiss LSM 710 confocal microscope system (Carl Zeiss, Germany) and processed with ZEN LE software (Carl Zeiss).
Subcellular fractionation
Nuclear/cytoplasmic isolation was carried out by using the NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Catalogue Number 78835, Thermo Fisher) according to the manufacturer’s protocol. Subcellular fractions were prepared as follows. Cytoplasmic and nuclear fractions were divided for RNA extraction. GAPDH and U1 were used as qRT-PCR markers of cytoplasmic and nuclear RNAs, respectively.

Transmission electron microscopy
Conventional electron microscopy was performed as previously described [19]. In brief, cells were fixed with 2.5% glutaraldehyde and then postfixed with 1% osmium tetroxide, dehydrated in a graded ethanol series and embedded in Embed 812 resin. Ultrathin sections were mounted on copper grids and then double-stained with uranyl acetate and lead citrate. The samples were examined and photographed with an FEI Tecnai transmission electron microscope.

RNA-FISH assay
To detect EGOT and pre-ITPR1 mRNAs, we used the QuantiGene® ViewRNAISH Cell Assay Kit (Catalogue Number QVC0001, Thermo Fisher) to perform the QuantiGene® ViewRNA FISH assay according to the manufacturer’s protocol. EGOT, ITPRI and ACTB (as control) hybridization was carried out using cy3, cy5, and 488-nm DNA-oligonucleotide probes in a moist chamber, respectively. After digestion with a working protease solution, slides were incubated with RNase III (AM2290, Life Technologies, USA) or RNase A (AM2272, Life Technologies) for 2 h if RNase enzymatic activity was to be determined. Standard immunofluorescence and imaging were performed by confocal microscopy. The details of the probe sets and corresponding gene sequences are provided in Additional file 1: Table S2.

TUNEL assay
To detect apoptosis in sections of tumor tissues, TUNEL assay was performed according to the manufacturer’s instructions (Catalogue Number 11684795910, Roche) as previously described [20]. The sections were analyzed by fluorescence microscopy (Olympus, Japan).

RNA pull-down assay
The Flag-MS2bp-MS2bs-based RNA pull-down assay was carried out by using the Anti-Flag M2 Affinity Gel (Catalogue Number A2220, Sigma) as previously described [21]. In short, lentivirus Flag-MS2bp and lentivirus EGOT MS2bs were cotransfected into breast cancer cells, and the cells were harvested after 48 h. Approximately 1 × 10^7 cells were lysed in soft lysis buffer (20 mM Tris-Cl, pH 8.0, 10 mM NaCl, 1 mM EDTA, and 0.5% NP-40) containing RNasin (80 units/ml). Fifty microliters of Anti-Flag M2 Magnetic beads was added to each binding reaction tube and incubated at 4 °C overnight. The beads were washed three times with lysis buffer and boiled in 1x loading buffer for 10 min. Finally, the retrieved proteins were analyzed by a NanoLC-ESI-MS/MS system (ProtTech, China).

Chromatin immunoprecipitation (ChIP) and ChIP-seq
Analysis of genome-wide NRIP1 and AP-1 occupancy was carried out using specific and internally validated antibodies. We purchased the EZ-ChIP™ Chromatin Immunoprecipitation Kit (Catalogue Number #17–371, Millipore, USA) to perform ChIP and ChIP-seq using MCF7 cells as previously described [22]. Briefly, MCF7 cells were subjected to hormone deprivation for up to 3 days and then treated with 1 mM E2 or ethanol (control) for 6 h. Approximately 2 × 10^7 cells were used for each ChIP or ChIP-Seq assay. Chromatin DNA precipitated by polyclonal antibodies against AP-1 or NRIP1 was purified with the Universal DNA purification kit (DP214, Tiangen, China) according to the manufacturer’s protocol. Rabbit anti-RIP140 (ab42126, Abcam) and rabbit anti-c-JUN (60A8) (Catalogue Number #9165, CST) antibodies and rabbit IgG (sc-2027, Santa Cruz, USA) were used. ChIP-PCR enrichment of target loci was normalized to input DNA and reported as % input ± s.e.m. ChIP libraries were prepared using ChIP DNA according to the BGISEQ-500ChIP-Seq library preparation protocol. In-depth whole-genome DNA sequencing was performed by BGI (Shenzhen, China). ChIP-seq data were deposited in the NCBI SRA: SRP149488 (https://www.ncbi.nlm.nih.gov/sra/SRP149488).

Transient transfection of cells
For downregulation of NRIP1 and hnRNPH1 expression, siRNAs targeting NRIP1 and hnRNPH1 were purchased from Sigma. Cancer cells were transfected with 100 pM siRNA using Lipofectamine 2000 (11668–019, Invitrogen, USA). The siRNA sequences are provided in Additional file 1: Table S1. After HeLa and T47D cells (5 × 10^5 cells/well) were cultured in 6-well plates for 24 h, siRNA or the corresponding controls were transfected into the cells by Lipofectamine 2000 reagent in accordance with the manufacturer’s protocol. Total RNA was extracted 48 h after transfection.

Animal experiment
Female athymic BALB/c nude mice (5 weeks old) were obtained from Beijing Vital River Laboratory Animal Technology Co, Ltd. (Beijing, China). All of the animal experiments were performed according to approved protocols and in accordance with the guidelines of the Guide for the Care and Use of Laboratory Animals.
Access and analysis of public data

GDS723, GDS4121, GDS1453 and GSE11324 were downloaded from GEO datasets (http://www.ncbi.nlm.nih.gov/geo/) and processed according to our previous study [23]. Genome-wide EGO T and ITPR1 expression profiles and clinical pathology information for human cancers were downloaded from The Cancer Genome Atlas (TCGA) (https://tcga-data.nci.nih.gov/), International Cancer Genome Consortium (ICGC) (http://icgc.org/) and Cancer Cell Line Encyclopedia (CCLE) (http://www.broadinstitute.org/ccle). All transcripts were normalized by log2 transformation. The expression of EGO T or ITPR1 was dichotomized using a study-specific median expression as the cut-off to define high values (at or above the median) versus low values (below the median). Guilt-by-association analysis was performed according to our previous study [23]. Correlations between genes were assessed by Pearson correlation coefficients. Unpaired Student’s t-tests were used to detect significant differences among tumors or between tumor and normal samples. The overall survival (OS) and relapse-free survival (RFS) were calculated as the time from surgery until the occurrence of death and relapse, respectively. The log-rank test was used to examine the survival difference between different patient groups. All statistical tests were two-sided, and P < 0.05 indicated statistical significance.

Statistical analysis

Data are presented as the mean ± s.d. of at least three independent experiments for each cellular experimental group and at least five independent experiments for each animal group. Student’s t-tests were used to determine statistically significant differences between groups. Correlations between EGO T expression and a pathological response were determined by the chi-square test. All statistical tests were two-sided, and P < 0.05 indicated statistical significance. Statistical analysis was performed using R.3.4 graphics software and GraphPad Prism software (GraphPad Software, USA).

Results

Ai-IncRNA EGO T enhances paclitaxel sensitivity in human cancer

Whole transcriptome sequencing of 33 breast specimens showed that Ai-IncRNA EGO T was expressed at low levels in cancer tissues compared with its levels in adjacent normal tissues (Fig. 1a and Additional file 2: Figure S1a). This result was subsequently validated in 258 pairs of breast cancer tissues and adjacent normal tissues in the Harbin Medical University Cancer Centre (HMUCC) cohort (Fig. 1b) and in data from other cancer contexts in TCGA (Additional file 2: Figure S1b).

Immunohistochemistry

Immunohistochemical (IHC) detection of ITPR1 and LC3B was performed on each slide. Each section was incubated with anti-LC3B (1:200) antibody or anti-IP3R (1:200) antibody solution. The proportion and intensity of ITPR1 and LC3 staining were evaluated in a series of 10 randomly selected high-power fields (magnification, 400x), which were considered to represent the average expression. The IHC staining intensity was graded as 0 (no staining), 1 (weak staining = light yellow), 2 (moderate staining = yellow brown) or 3 (strong staining = brown). The proportion of positively stained tumor cells in a field was scored as 0 (no positive tumor cells), 1 (fewer than 10% positive tumor cells), 2 (10–50% positive tumor cells) or 3 (more than 50% positive tumor cells). The staining index (SI) for each sample was obtained by multiplying the intensity and proportion values, with a score of less than 4 being classified as low expression.

Library preparation for IncRNA sequencing

A total of 3 μg of RNA per sample was used for downstream RNA sample preparation. Ribosomal RNA was removed using the Ribo-Zero™ Gold kit (Epicentre, Wisconsin, USA). Subsequently, sequencing libraries were generated according to the manufacturer’s recommendations, and the libraries were sequenced on an Illumina HiSeq 2500 platform to generate 100-bp paired-end reads. Raw sequencing and processed RNAseq data from this study have been deposited into the NCBI GEO database under accession number GSE71651 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=obcxosaur xoppwx & acc=GSE71651).
Fig. 1 (See legend on next page.)
To further investigate the biological functions of EOGT, we performed guilt-by-association analysis and found that EOGT was involved in multiple cellular microtubule-associated functions (Additional file 1: Table S3). Because paclitaxel is commonly used as a microtubule-disrupting agent, we suspected that EOGT may impact paclitaxel sensitivity in tumor cells. As expected, analysis of public data from CCLE showed that EOGT increases the sensitivity of tumor cells to paclitaxel (Additional file 2: Figure S1c). To validate the potential function of EOGT in mediating the paclitaxel response, we then generated stable overexpression and knockdown of EOGT in cancer cell lines (Additional file 2: Figure S1d-f). As shown in Fig. 1c and Additional file 2: Figure S1g, we determined that EOGT overexpression significantly increased the sensitivity of cancer cells to paclitaxel. Conversely, silencing EOGT protected cancer cells from paclitaxel (Fig. 1d).

Furthermore, we found that there was no distinct difference of xenografts in the presence of EOGT or control without treatment of paclitaxel in UACC-812 cell xenograft models (Fig. 1e-g). However, EOGT overexpression (Additional file 2: Figure S1h) in UACC-812 and HeLa cell xenografts grew more slowly than control ones when treated with paclitaxel (Fig. 1e and Additional file 2: Figure S1i). Moreover, the weight and volume of the tumors were decreased in the EOGT overexpression group compared with those in the control group with treatment of paclitaxel (Fig. 1f and g). Similar results were obtained in HeLa cell xenografts (Additional file 2: Figure S1j and k). More importantly, cell apoptosis was much higher in the EOGT overexpression group than in the control group when treated with paclitaxel (Fig. 1h). Collectively, these data demonstrate that EOGT enhances the sensitivity of human cancer cells to paclitaxel both in vitro and in vivo.

**EOGT expression is positively correlated with ITPRI expression**

We then sought to identify interacting partner(s) involved in EOGT-mediated paclitaxel sensitivity. Certain mammalian lncRNAs are embedded in the intronic-antisense regions of protein-coding genes and regulate the genes to exert their function [24]. Thus, we hypothesized the existence of a functional relationship between EOGT and ITPRI. To study this possibility, by analyzing whole-transcriptome sequencing data, we found that EOGT expression was positively correlated with ITPRI expression at the mRNA level (Additional file 2: Figure S2a). The result was validated using breast cancer specimens in the HMUCC cohort (Fig. 2a); moreover, EOGT expression was also positively correlated with ITPRI protein expression in the HMUCC cohort (Additional file 2: Figure S2b). To expand the generalizability of our results, human cancer data from 33 cancer contexts, including breast cancer, in TCGA were analyzed, and the results further validated as above (Fig. 2b and c; Additional file 1: Table S4). Furthermore, examination of CCLE data also revealed that EOGT expression was positively correlated with ITPRI mRNA expression in human breast cancer cell lines and other cancer cell lines (Fig. 2d and e; Additional file 2: Figure S2c-f). These results indicate that EOGT expression is positively correlated with ITPRI expression in cancer.

To further validate the relationship between EOGT and ITPRI expression, we detected the genes near the EOGT locus (less than 2 Mb). EOGT overexpression was shown to increase the expression of ITPRI mRNA (Fig. 2f and g), while EOGT knockdown decreased the expression of ITPRI mRNA (Additional file 2: Figure S2g). Consistent with these results, ITPRI protein levels were also increased following EOGT overexpression and were decreased following EOGT knockdown (Fig. 2h). However, EOGT expression did not significantly change following ITPRI knockdown (Additional file 2: Figure S2h). Collectively, these data suggest that EOGT may induce ITPRI expression in human cancer.

**EOGT induces autophagy to enhance paclitaxel sensitivity through ITPRI**

To illustrate that ITPRI plays a functional role in autophagy in human cancer cells, we investigated autophagic activity in cells after 48 h of growth under low-serum (0.1%) conditions or in Earl’s balanced salt solution (EBSS) to simulate stress-induced autophagy, as previously described [15]. Knockdown of ITPRI decreased LC3-II/
Fig. 2 EGOt expression is positively related to ITPR1 expression. a Correlation between EGOt and ITPR1 expression in 258 breast cancer tissues in the HMUCC cohort. EGOt and ITPR1 levels (normalized to GAPDH) were subjected to Pearson’s correlation analysis. b, c Correlation between EGOt and ITPR1 expression in 33 cancer types (n = 9664) (b) and in breast cancer (n = 1085) (c). Data were downloaded from the TCGA portal, and the expression levels of EGOt and ITPR1 are indicated by TPM values (log2). b Correlation between EGOt and ITPR1 expression in 1057 human cancer cell lines (d) and 57 breast cancer cell lines (e). Data were downloaded from the CCLE. EGOt and ITPR1 levels (normalized to GAPDH) were subjected to Pearson’s correlation analysis. f Schematic illustration of genes near the EGOt locus (less than 2 Mb). Location information was obtained from the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway). g Expression of nearby genes in MCF7 EGOt overexpression cells (left) and HeLa EGOt overexpression cells (right). Data are shown as the means ± s.d. Student’s t-test was applied for statistical analysis: * P < 0.05; ** P < 0.01; and *** P < 0.001. h Protein expression levels of ITPR1 in EGOt overexpression and knockdown cells. Data represent at least three independent experiments.
LC3-I levels and increased P62 expression in the presence of chloroquine (CQ) or EBSS (Additional file 2: Figure S3a and b). Moreover, nutrient starvation failed to induce autophagy when ITPR1 was knocked down in cancer cells, as shown by confocal microscopy examination 48 h after mRFp-GFP-LC3 adenovirus vector transfection (Additional file 2: Figure S3c and d). Additionally, electron microscopy examination indicated that ITPR1 knockdown reduced the number of autophagic vesicles (Additional file 2: Figure S3e).

We next explored whether EGOT was involved in autophagy via ITPR1 in the presence of CQ or EBSS. As expected, overexpression of EGOT increased LC3-II/LC3-I levels and reduced P62 expression in HeLa and breast cancer cells (Fig. 3a and b; Additional file 2: Figure S3f and g), while knockdown of EGOT reduced LC3-II/LC3-I levels and increased P62 expression (Fig. 3c and d). Meanwhile, overexpression of EGOT induced autophagy, as shown by the increases in mRFp-GFP-LC3 puncta accumulation and the number of autophagic vesicles (Fig. 3e and f; Additional file 2: Figure S3h). In contrast, knockdown of EGOT caused the opposite changes (Fig. 3f and h). Moreover, EGOT-induced autophagy could be reversed by ITPR1 knockdown in HeLa and MCF7 cells (Fig. 3k; Additional file 2: Figure S3i). These results revealed that EGOT can at least partly convey the autophagic signal via ITPR1.

Furthermore, by analyzing CCLE data, we found that ITPR1 may impact paclitaxel sensitivity in tumor cells (Additional file 2: Figure S3j). The result was validated by experimental evidence showing that ITPR1 knockdown conferred protection to cancer cells from paclitaxel (Additional file 2: Figure S3k). Moreover, paclitaxel sensitivity induced by EGOT overexpression could be repressed by ITPR1 knockdown in vitro (Additional file 2: Figure S3l). In addition, EGOT-overexpressing tumors exposed to paclitaxel treatment in vivo expressed high levels of ITPR1 and LC3B proteins (Fig. 3i; Additional file 2: Figure S3m and n). Consistently, breast cancer tissues with higher EGOT levels showed higher ITPR1 and LC3B levels in the HMUCC cohort, suggesting that the increased expression of EGOT may force a connection with the autophagic pathway via ITPR1 (Fig. 3j). These results indicate that EGOT enhances paclitaxel sensitivity through ITPR1 in human cancer.

**EGOT regulates ITPR1 expression both in cis and in trans in human cancer**

Emerging evidence shows that lncRNAs can regulate gene expression and protein functions in *trans* or *in cis* [9]. Some lncRNAs are known to be involved in regulating protein synthesis or protein stability in mammalian cells [25]. We found that neither ITPR1 protein synthesis nor protein stability appeared to have any significant effect on the overexpression of EGOT via assays using the protein synthesis inhibitor cycloheximide (CHX) and the proteasome inhibitor MG132 (Additional file 2: Figure S4a and b).

By fractionated nuclear and cytoplasmic RNA analysis and RNA fluorescence in situ hybridization (RNA-FISH) examination in four cancer cell lines, we found that EGOT is mainly expressed in the nucleus (Fig. 4a and b; Additional file 2: Figure S4c and d), suggesting that EGOT exerts its regulatory function for ITPR1 at the transcriptional level. Of note, ectopic expression of EGOT induced endogenous upregulation of pre-ITPR1 (Fig. 4c), while EGOT knockdown suppressed endogenous expression of pre-ITPR1 (Fig. 4d). Moreover, the stability of pre-ITPR1 mRNA was increased in EGOT-overexpressing cancer cells compared with that in control cells, as shown in the transcriptional inhibition experiments (Additional file 2: Figure S4e).

Given that EGOT is complementary to intronic sequences of the ITPR1 and is transcribed in the antisense direction, we hypothesized that a double-stranded (ds) RNA forms between EGOT and pre-ITPR1. As expected, dual-RNA-FISH assay indicated that EGOT and pre-ITPR1 hybridized in the same nuclear foci (Fig. 4e). These foci were completely depleted upon treatment with RNase III, which degrades only dsRNA, but not with RNase A, which degrades only single-stranded (ss) RNA (Fig. 4e), thereby indicating the formation of dsRNA from the physical association of EGOT and pre-ITPR1 (Additional file 2: Figure S4f). Furthermore, through domain mapping, two exons of EGOT were divided into four fragments (Fig. 4f). Interestingly, EGOT fragment 2 (324–645 nucleotides) in exon 1 increases ITPR1 protein levels as well as ITPR1 and pre-ITPR1 mRNA levels, whereas other fragments do not possess these capacities (Fig. 4g and h). Taken together, these data indicate the pivotal roles of EGOT fragment 2 (324–645 nucleotides) in exon 1, which binds to pre-ITPR1 to control ITPR1 expression in *cis* in human cancer.

Accumulating evidence suggests that lncRNA activity is centered on its ability to bind and regulate epigenetic complexes of methylation, acetylation and phosphorylation [26, 27]. Therefore, by using the Flag-MS2bp-MS2bs-based system (Additional file 2: Figure S4g) and, subsequently, mass spectrometry examination (Additional file 1: Table S5), hnRNPH1 was pulled down in this process, and the result was validated in four other cancer cell lines (Fig. 4i). RNA immunoprecipitation (RIP) further revealed that hnRNPH1 bound to both EGOT and pre-ITPR1 mRNA (Fig. 4j). Given the earlier evidence that hnRNPH1 interacts with RNA-binding proteins to improve alternative splicing [28], inhibition of hnRNPH1 expression may suppress alternative splicing of its target. As expected, knockdown of hnRNPH1 decreased ITPR1
Fig. 3 (See legend on next page.)
mRNA and protein levels (Fig. 4k and l). As EGOT fragment 2 (324–645 nucleotides) in exon 1 binds to pre-ITPR1 mRNA in cis (Fig. 4g), ectopic expression of EGOT fragment 2 (324–645) resulted in enrichment of hnRNPH1 by the RNA pull-down assays (Fig. 4m). hnRNPH1 has been reported to regulate alternative splicing by binding to specific motifs (GGGA) [29]. Moreover, mutation of the specific binding motifs (mut-A and mut-C) in EGOT fragment 2 led to the failure of hnRNPH1 enrichment (Fig. 4n). Altogether, these data demonstrated that hnRNPH1 is recruited by EGOT fragment 2 to enhance the alternative splicing of pre-ITPR1 in trans.

**EGOT expression is transcriptionally regulated by stress in human cancer**

Through guilt-by-association analysis, we found that EGOT is involved in stress-associated conditions (Additional file 1: Table S1). Stress, including hypoxia, estrogen depletion, radiotherapy, and chemotherapy, has been shown to affect autophagy induction [30–33]. Thus, we first aimed to determine whether EGOT is regulated by hypoxia. Hypoxia (1% oxygen) treatment induced a sustained upregulation of EGOT in a time-dependent manner in several cell types (Additional file 2: Figure S5a-e). Moreover, by analyzing previously published GEO datasets (GDS4121, GDS1453 and GDS723) involving different stress treatments, we found that EGOT was also upregulated in the absence of HGF stimulation or treatment with camptothecin or radiation (Additional file 2: Figure S5f-h). Furthermore, EGOT was upregulated in breast cancer patients who underwent neoadjuvant chemotherapy compared with its expression in patients who did not receive such therapy in the HMUCC cohort (Additional file 2: Figure S5i).

Estrogen (E2) is strongly related to breast cancer and has been shown to inhibit autophagy in a previous report [34]. Regarding the potential role of E2 in regulating EGOT expression, E2 indeed suppressed EGOT expression in a dose-dependent manner (Fig. 5a). Moreover, analysis from the GSE11324 dataset further certified the relationship of EGOT inhibition by E2, which was according with our results above (Additional file 2: Figure S5a).

Concordantly, the expression levels of several positive control genes that are negatively regulated by E2, such as IRX4, GLISB, BCAS4 and MUC1, were decreased after E2 stimulation (Fig. 5b). In contrast, EGOT expression was induced in response to treatment with the anti-estrogen agents tamoxifen and fulvestrant (ICI) (Fig. 5c and Additional file 2: Figure S6b). In addition, knockdown of the estrogen receptor (ER) in MCF7 cells was shown to induce the expression of EGOT by > 10-fold (Fig. 5d), while the expression of the positive control gene GREB1, which was reported to be positively regulated by E2, was decreased, and that of the IRX4, which was reported to be negatively regulated by E2, was increased (Additional file 2: Figure S6c). Furthermore, time course analysis demonstrated that E2 suppressed EGOT expression in a time-dependent manner (Fig. 5e). Moreover, dose-dependent inhibition of EGOT expression by E2 was also observed in other ER-positive T47D breast cancer cells (Additional file 2: Figure S6d). In all, these results demonstrate that EGOT is transcriptionally inhibited by E2 in ER-positive breast cancer cells.

Next, we determined the molecular mechanism underlying such transcriptional repression. Estrogen-inducible NRIP1 acts as a nuclear receptor co-repressor and interacts with AP-1 to directly repress target genes via ERE or AP-1 elements [35]. The expression of NRIP1 was induced, whereas EGOT expression was decreased after E2 treatment in MCF7 cells (Fig. 5f). As expected, transcriptional repression of EGOT by E2 was markedly reversed and the positive control gene GREB1 was upregulated in the presence of siNRIP1 (Fig. 5g and h; Additional file 2: Figure S6e), demonstrating that NRIP1 is necessary and involved in this process. Furthermore, co-immunoprecipitation assays revealed that NRIP1 bound to AP-1 (Fig. 5i). Moreover, NRIP1 and AP-1 chromatin immunoprecipitation sequencing experiments showed that AP-1 and NRIP1 binding sites were enriched near the transcription
Fig. 4 (See legend on next page.)
start sites (5 kb to 5 kb) of target genes (Additional file 2: Figure S6f). Interestingly, significant enrichment peaks for AP-1 and NRIP1 were detected at a genomic locus (chr 3: 4757428–4,757,590) upstream of the transcription start site of EGOT (Fig. 5j). Next, the binding of AP-1 and NRIP1 to the promoter region of EGOT was validated by ChIP-quantitative PCR (ChIP-PCR) (Fig. 5k). Taken together, our results indicated that NRIP1 physically interacts with AP-1 and transcriptionally represses the expression of EGOT in presence of E2 (Fig. 5l).

**EGOT/ITPR1 expression is associated with a favorable prognosis and enhances paclitaxel sensitivity in human cancer**

The prognostic role of EGOT and ITPR1 was finally examined in the HMUCC and public cohort data. High expression of EGOT was associated with favorable OS and RFS in breast cancer patients, while low expression of ITPR1 indicated worse RFS in the HMUCC cohort (Fig. 6a and b; Additional file 2: Figure S7a). These results were then validated in 14,586 cancer patients in TCGA and ICGC human cancer cohorts, and patients with high expression levels of EGOT showed favorable OS and RFS in both of 33 human cancer types and breast cancer cohorts (Fig. 6c and d; Additional file 2: Figure S7b and c), while patients with high ITPR1 expression showed favorable OS in the human cancer cohort (Additional file 2: Figure S7d and e).

Regarding paclitaxel sensitivity, breast cancer patients who were treated with paclitaxel and exhibited high expression levels of EGOT demonstrated better OS and RFS than patients with low expression levels of EGOT in the HMUCC cohort (Fig. 6e and f). Moreover, the results were further validated in TCGA breast cancer patients treated with paclitaxel, which showed that high expression of EGOT was also associated with favorable OS, in accordance with our study (Fig. 6g). Similarly, loss of ITPR1 was associated with poor RFS in breast cancer patients treated with paclitaxel in the HMUCC cohort (Additional file 2: Figure S7f). More importantly, breast cancer patients with high expression of EGOT achieved a higher complete pathological response ratio than patients with low expression of EGOT when treated with paclitaxel-containing adjuvant chemotherapy regimens in the HMUCC cohort (Fig. 6h). Therefore, EGOT/ITPR1 expression is associated with a favorable prognosis and enhances paclitaxel sensitivity in human cancer.

**Discussion**

Regarding the function of Ai-lncRNA EGOT, we first reported that low levels of EGOT expression were significantly correlated with increased tumor size, lymph node metastasis, and Ki-67 expression in human breast cancer [14]. In this study, we identified an uncharacterized lncRNA, EGOT, and its roles in enhancing paclitaxel sensitivity by triggering autophagosome accumulation. Mechanistically, EGOT regulates ITPR1 expression in cis and in trans, leading to the activation of autophagy signaling. We also found that EGOT is transcriptionally regulated by various stress conditions that are associated with paclitaxel resistance. Overall, we provide compelling evidence that in response to stress, EGOT activates autophagy via ITPR1, which sensitizes paclitaxel cytotoxicity in human cancer (Additional file 2: Figure S8).

Several studies have investigated the role of macrophages in decreasing the cytotoxic effects of chemotherapy and demonstrated a therapeutic window for autophagy inhibition in cancer therapy and prevention [36, 37]. Paradoxically, a previous study showed that autophagy enhances paclitaxel-induced cell death and that reduced autophagy may contribute to clinical chemotherapeutic resistance in primary breast tumors [6]. Of note, paclitaxel blocks the activation of PI3K and Vps34 and inhibits the movement and maturation of autophagosomes to induce cell death [6]. In vitro, autophagosome accumulation sensitizes cells to...
Fig. 5 (See legend on next page.)
paltaxel toxicity [6]. Using in vivo and in vitro experiments in this study, we found that EGO7 overexpression activated autophagy signaling, which sensitzes cells to paclitaxel, whereas EGO7 knockdown has the opposite effect, in accordance with previous study. Mechanistically, EGO7 upregulates the expression of ITPR1, which promotes autophagosome accumulation and sensitzes cells to paclitaxel toxicity, thereby highlighting a novel function for these molecules.

Loss or low expression of autophagy-related genes, indicating a decrease of autophagic flux, was associated with poor prognosis [38, 39]. High expression of EGO7 is significantly associated with favorable OS and RFS in patients in the HMUCC cohort and TCGA and ICGC public datasets. Furthermore, recent study showed that autophagy-related genes/proteins could be possible predictive markers for paclitaxel efficacy in the clinic [6]. High expression of EGO7 significantly associated with enhanced autophagosome sensitivity in patients in the HMUCC cohort and TCGA and ICGC public datasets, while the lack of EGO7 expression indicates resistance to paclitaxel therapy. In addition, high expression of EGO7 indicated an increased complete pathological response ratio following treatment with paclitaxel-containing adjuvant chemotherapy regimens, in contrast to patients with low EGO7 expression in the HMUCC cohort. However, paclitaxel-containing adjuvant chemotherapy sensitivity has only been evaluated in a small study cohort; thus, a multicenter clinical trial with a larger patient number will be performed in a future study to validate these results. Hence, our study provides a proof of concept showing that EGO7 may be a predictive marker of the clinical efficacy of paclitaxel treatment.

lncRNAs can function in cis to regulate the expression of neighboring genes or in trans to carry out many roles by various modes [9]. Compared to other category of long non-coding RNAs, the function of Ai-lncRNAs including EGO7 is still largely unknown. In this study, we clearly demonstrated that EGO7 regulates ITPR1 expression both in cis and in trans. On one hand, EGO7 regulates ITPR1 levels via a unique regulatory mechanism involving the formation of a pre-ITPR1/EGO7 dsRNA that induces pre-ITPR1 accumulation to increase ITPR1 protein expression in cis. However, we should mention that although it was initially proposed that lncRNA mainly functions to regulate neighboring gene transcription, other studies have shown that many lncRNAs do not exert such function [40, 41]. Thus, whether EGO7 regulates any other neighboring gene transcription in cis await further investigation. Furthermore, EGO7 recruits hnRNPH1 to promote ITPR1 expression in trans. hnRNPH1, a splicing factor, has been shown to regulate alternative splicing and polyadenylation [42, 43]. EGO7 fragment 2 (324–645 nucleotides) in exon 1 was found to bind pre-ITPR1 mRNA and the hnRNPH1 protein, thereby mediating alternative splicing of pre-ITPR1 mRNA. hnRNPH1 has been shown to bind G-rich sequences interspersed with adenosines around exons [28, 42]. ITPR1 contains 59 exons and multiple GGGA/C/G motifs distributed in these exons. Thus, we propose that hnRNPH1 mediates pre-ITPR1 splicing in human cancer by binding these GGGA/C/G motifs. Taken together, these findings broaden the understanding of the autophagy-associated lncRNA landscape and provide novel insights into the exploration of the mechanism of autophagy regulation in human cancer.

Our data also show that EGO7 is positively and negatively transcriptionally regulated by hypoxia and estrogen-related stress, respectively, in human cancer. Studies have shown that hypoxia can promote tumor invasion, metastasis, autophagy, and angiogenesis, can regulate tumor metabolism and is closely related to the poor prognosis of cancers [44, 45]. Hypoxia is associated with the expression of numerous noncoding RNAs, including miRNAs and lncRNAs [46]. In this study, EGO7 was induced in vitro by subjecting different cancer cell lines to hypoxia. Apart from its positive regulation by hypoxia, EGO7 can be transcriptionally repressed by estrogen. Estrogen, a paracrine mediator throughout life, is an important factor in tumorigenesis in humans. Estrogen binds to estrogen receptors to control vast gene networks that are involved in glycolysis, glutaminolysis, oxidative

**Fig. 5** EGO7 expression is directly repressed by the NRIP1/AP-1 complex. a EGO7 expression determined by qRT-PCR (normalized to GAPDH) in MCF7 cells treated with E2. b Expression of the estrogen-repressed genes, IRMA, GUSB, BCA54, MUC1 and EGO7, determined by qRT-PCR in MCF7 cells treated with 1 nM E2 or ethanol (Ctrl) for 6 h. c EGO7 expression determined by qRT-PCR in MCF7 cells treated with different doses of tamoxifen. d EGO7 expression determined by qRT-PCR in MCF7 cells following ESR knockdown. e EGO7 and GREB1 expression in MCF7 cells treated with 1 nM E2 stimulation at different time points. f NRIP1 mRNA and EGO7 expression in MCF7 cells treated with 1 nM E2 stimulation at different time points. g, h Control siRNA (siCtrl) or siRNA against NRIP1 (siNRIP1) was transfected into MCF7 cells for 48 h, followed by 1 nM E2 stimulation. NRIP1 mRNA, GREB1, and EGO7 levels were assessed after estrogen stimulation in the presence of siCtrl or siNRIP1. i Co-IP assays were conducted using anti-NRIP1 or anti-Ap-1 antibodies for endogenous proteins in MCF7 cells treated with or without E2 stimulation. j Genome browser view of AP-1 and NRIP1 binding at the promoter of the EGO7 locus. AP-1 and NRIP1 ChIP-seq was performed in MCF7 cells stimulated with ethanol (Ctrl) or E2. k ChIP-PCR showed the binding of AP-1 and NRIP1 to the EGO7 promoter. The BCA54 gene was utilized as a positive control. l Schematic illustration of NRIP1/AP-1 complex-mediated transcription suppression of EGO7 expression. MCF7 cells were hormonoe starved for 3 days and further treated. In A, C, E, and F, GREB1 expression determined by qRT-PCR in MCF7 cells following ESR knockdown.
phosphorylation, nutrient sensing and biosynthesis pathways in cancer [47]. Several lncRNAs have been shown to be regulated by estrogen in breast cancer [22, 48]. In this study, EGOТ was found to be a direct target of AP-1/NRIP1-mediated transcriptional repression complex in the presence of estrogen. Previous studies have shown that NRIP1 is an obligate co-factor of the estrogen receptor, and germline single-nucleotide polymorphisms (SNPs) near NRIP1 have been associated with ER-positive breast cancer [49]. Estrogen mediates NRIP1 induction,

![Fig. 6](image_url)

**Fig. 6** EGOТ/ITPR1 expression is associated with a favorable prognosis and enhances paclitaxel sensitivity in human cancer. a, b Kaplan-Meier analyses of the relationships between EGOТ expression and OS (a) or RFS (b) in breast cancer patients in the HMUCC cohort. c, d Kaplan-Meier analyses of the relationships between EGOТ expression and OS among 33 cancer types in TCGA (c) and ICGC cohorts (d). e, f Kaplan-Meier analyses of the relationships between EGOТ expression and OS (e) or RFS (f) in breast cancer patients treated with paclitaxel in the HMUCC cohort. g Kaplan-Meier analyses of the relationship between EGOТ expression and OS in breast cancer patients treated with paclitaxel in the TCGA cohort. h The correlations between EGOТ expression and ITPR1 mRNA expression were measured in 15 breast cancer patients treated with paclitaxel-containing adjuvant chemotherapy regimens (left). EGOТ and ITPR1 levels (normalized to GAPDH) were subjected to Pearson's correlation analysis. Correlations between EGOТ expression and a pathological response were determined by the chi-square test (right). A complete pathological response is denoted by CR, while a partial response is denoted by PR. The median expression level was used as the cut-off value. Patients with EGOТ expression values below the 50th percentile were classified as EGOТ-Low. Patients with EGOТ expression values above the 50th percentile were classified as EGOТ-High.
which subsequently interacts with estrogen receptor AP-1 complexes to directly repress adjacent target genes, including BCAS4, IRX4, GLISB and MUC1 [35]. In this study, EGOT was inhibited by estrogen at physiological concentrations (0–10^{-10} M), which led to a gradual decrease in EGOT expression; meanwhile NRIP1 expression was gradually increased with time. Knockdown of NRIP1 reversed the estrogen-mediated transcriptional repression of EGOT expression. Interestingly, a previous study reported that the expression of ITPR1 protein decreased in an estrogen-receptor-dependent manner and that the growth of MCF7 cells induced by estrogen was sensitive to pharmacological inhibitors of ITPR1 [16]. Our study provides a proof of concept indicating that EGOT is transcriptionally repressed by the AP-1/NRIP1 complex in the presence of estrogen and that this axis may send regulatory feedback signals to repress the excessive activation of autophagy.

In conclusion, these findings broaden comprehensive understanding of the biology function of Ai-lncRNAs. Moreover, our findings demonstrate that EGOT my act as a clinical biomarker of paclitaxel resistance, and that proper regulation of EGOT may be a novel synergistic strategy for enhancing paclitaxel sensitivity, thereby enhancing its clinical benefits for cancer patients.

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Availability of data and materials
Raw sequencing and processed RNASeq data from this study have been deposited into the NCBI GEO database under accession number GSE71651 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=obxosaurusxoppxw & acc = GSE71651). ChiP-seq data were deposited in the NCBI SRA: SRP149488 (https://www.ncbi.nlm.nih.gov/sra/SRP149488).

Authors’ contributions
D. P. and S.P.X. designed the study and performed the bioinformatics analyses, P.Y.W., J. Z., S.Y.S., J.E.Z. and H.W. performed the experiments, Q.W., W.W.Y., K.Q. and H.B.X. performed in vivo experiments. D. P. and S.P.X. supervised the study and wrote the manuscript. All authors have read and approved the manuscript.

Ethics approval and consent to participate
All patients consented to an institutional review board-approved protocol that allows comprehensive analysis of tumor samples (Ethics committee of Harbin Medical University). This study conforms to the Declaration of Helsinki.

Consent for publication
Written informed consent for publication was obtained from the patients. All authors have agreed to publish this manuscript.

Competing interests
The authors declare that they have no competing interests.

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Author details
1Department of Breast Surgery, Harbin Medical University Cancer Hospital, 150 Haping Road, Harbin 150081, China. 2Department of Pathology, Harbin Medical University, Harbin, China. 3Heilongjiang Academy of Medical Sciences, 157 Baojian Road, Harbin 150086, China.

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References
1. Yu Y, Gaillard S, Phillips JM, Huang TC, Pinto SM, Testarollo NG, Zhang Z, Pandey A, Wirtz D, Ayhan A, et al. Inhibition of spleen tyrosine kinase potentiates paclitaxel-induced cytotoxicity in ovarian Cancer cells by stabilizing microtubules. Cancer Cell. 2015;28:62–02.
2. Luo Y, Li D, Ran J, Yan B, Chen J, Dong X, Liu Z, Liu R, Zhou J, Liu M, End-binding protein 1 stimulates paclitaxel sensitivity in breast cancer by promoting its actions toward microtubule assembly and stability. Protein Cell. 2014;5:469–79.
3. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. Cell. 2011;147:728–41.
4. Jeon YJ, Khelifa S, Ratnikov B, Scott DA, Feng Y, Parisi F, Ruller C, Lau E, Kim H, Brill LM, et al. Regulation of glutamine carrier proteins by RNFS determines breast cancer response to ER stress-inducing chemotherapies. Cancer Cell. 2015;27:354–69.

5. Domenech E, Maestre C, Esteban-Martinez L, Partida D, Pascual R, Fernandez-Miranda G, Seco E, Campos-Olivas R, Perez M, Megias D, et al. AMPK and PFKFB3 mediate glycolysis and survival in response to mitophagy during mitotic arrest. Nat Cell Biol. 2015;17:1304–16.

6. Veldhoen RA, Banman SL, Hemmerling DR, Odsen R, Simmen T, Simmonds AU, Underhill DA, Goping IS. The chemotherapeutic agent paclitaxel inhibits autophagy through two distinct mechanisms that regulate apoptosis. Oncogene. 2013;32:7336–46.

7. Cech TR, Stitz JA. The noncoding RNA revolution-trashing old rules to forge new ones. Cell. 2014;157:77–94.

8. Battista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell. 2013;152:1298–307.

9. Joung J, Engreit JM, Kornemann S, Abudayyeh OD, Verdin K, Aguet F, Gootenberg JS, Sanjana NE, Wright J, Fulco CP, et al. Genome-scale activation screen identifies a lincRNA locus regulating a gene neighbourhood. Nature. 2017;548:343–6.

10. Zhang J, Wang P, Pan L, Xu S, Pan D. The emergence of noncoding RNAs as Heracles in autophagy. Autophagy. 2017;13:1004–24.

11. Lin A, Hu Q, Li C, Xu Z, Ma G, Wang C, Li J, Ye Y, Yao J, Liang K, et al. The LINK-A lncRNA interacts with PtdIns(3,4,5)P3 to hyperactivate AKT and confer resistance to AKT inhibitors. Nat Cell Biol. 2017;19:238–51.

12. Echevarria W, Leite MF, Rezaei J, Guerra MT, Zipfel WR, Nathanson MH. Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum. Nat Cell Biol. 2003;5:440–6.

13. Adkins CE, Morris SA, De Smedt H, Partida D, Pascual R, Fernández-Miranda G, Seco E, Campos-Olivas R, Perez M, Megias D, et al. AMPK and PFKFB3 mediate glycolysis and survival in response to mitophagy during mitotic arrest. Nat Cell Biol. 2015;17:1304–16.

14. Zhang J, Wang P, Pan L, Xu S, Pan D. The emergence of noncoding RNAs as Heracles in autophagy. Autophagy. 2017;13:1004–24.

15. Lin A, Hu Q, Li C, Xu Z, Ma G, Wang C, Li J, Ye Y, Yao J, Liang K, et al. The LINK-A lncRNA interacts with PtdIns(3,4,5)P3 to hyperactivate AKT and confer resistance to AKT inhibitors. Nat Cell Biol. 2017;19:238–51.

16. Echevarria W, Leite MF, Guerra MT, Zipfel WR, Nathanson MH. Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum. Nat Cell Biol. 2003;5:440–6.

17. Adkins CE, Morris SA, De Smedt H, Sinaeart I, Torok K, Taylor CW. Ca2+ trisphosphate receptors. Biochem J. 2000;345(Pt 2):357–67.

18. Xu SP, Zheng JF, Sun SY, Bai NX, Gao S, Zhang GW, Shi QY, You ZL, Zhan C, Pang D. Downregulation of the long noncoding RNA EGOT correlates with malignant status and poor prognosis in breast cancer. Tumour Biol. 2015;36:9807–12.

19. Hall DP, Cost NG, Hegde S, Kellner E, Mikhaylova O, Stratton M, Underhill DA, Goping IS. The chemotherapeutic agent paclitaxel inhibits autophagy through two distinct mechanisms that regulate apoptosis. Oncogene. 2013;32:736–49.

20. Zhang J, Wang P, Pan L, Xu S, Pan D. The emergence of noncoding RNAs as Heracles in autophagy. Autophagy. 2017;13:1004–24.

21. Lin A, Hu Q, Li C, Xu Z, Ma G, Wang C, Li J, Ye Y, Yao J, Liang K, et al. The LINK-A lncRNA interacts with PtdIns(3,4,5)P3 to hyperactivate AKT and confer resistance to AKT inhibitors. Nat Cell Biol. 2017;19:238–51.

22. Echevarria W, Leite MF, Guerra MT, Zipfel WR, Nathanson MH. Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum. Nat Cell Biol. 2003;5:440–6.

23. Adkins CE, Morris SA, De Smedt H, Sinaeart I, Torok K, Taylor CW. Ca2+ trisphosphate receptors. Biochem J. 2000;345(Pt 2):357–67.

24. Salameh A, Lee AK, Cardo-Vila M, Nunes DN, Efstathiou E, Staquicini FI, et al. Molecular Cancer Ther. 2019;18:89.