Electronic Supplementary Material (ESI) for

Liposomal Curcumol Nanocomposite for Magnetic Resonance Imaging and Endoplasmic Reticulum Stress Mediated Chemotherapy of Human Primary Ovarian Cancer

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Additional Materials and Methods

Cell viability assay

$SKOV_3$ cells and hPOCCs were seeded into 96-well plates in sextuplicate and incubated with LC at different concentrations (0, 15, 30, 45, 60, 75 and 90μg/mL) for 24h, respectively. According to the IC_{50} of LC, $SKOV_3$ cells were treated with 30μg/mL LC for 12h, 24h, or 48h, while hPOCCs were treated with 60μg/mL LC. Curcumol powder was directly added into culture medium at the same concentrations for comparison. Cell viability was measured by standard MTT method. To assess general toxicity of this liposomal composite, hNOECs were treated with LC (0, 30, 60μg/mL).

Cell migration assay

To evaluate the migration of tumor cells, scratch assay was performed. When the cells were in the logarithmic growth, a 200μL tip was used to scratch the wells. Then they were treated with LC 30μg/mL for $SKOV_3$, and 60μg/mL for hPOCCs. The cell scratch length was measured at different times, and each experiment was repeated three times.

Cell invasion assay

To evaluate the invasion of tumor cells, transwell assay was performed. The cells were pretreated with LC in the indicated concentrations for indicated time. Then they were reclaimed for following study. Upper chambers were paved by Matrigel 5-6h before. Then the cells were seeded respectively into the upper chambers (8-μm pore) contained 200μL culturemedium without FBS at a density of $3x10^5$ cells/mL, and the lower well contained 500μL culture medium containing FBS (10% for $SKOV_3$ and 15% for hPOCCs). After 33-36h for $SKOV_3$ cells, 24-28h for hPOCCs, the chambers were stained with crystal violet. Each experiment was repeated three times.

Apoptosis assay

Annexin-V FITC and PI staining. $SKOV_3$ cells and hPOCCs were treated with LC at the indicated concentration for indicated time. Apoptosis of cells were stained by Annexin-V FITC and PI (Sigma, USA). $SKOV_3$ groups and hPOCCs groups were further analyzed by flow cytometry.

Western blotting assay

Cells were divided into different groups according to the LC concentration and treatment time. Proteins from the different groups were separated by SDS-PAGE and transferred to PVDF membrane. These membranes were incubated with antibodies (Table S1, 1:1,000), and then incubated with secondary antibody (1:8,000). GADPH was used as control. Images were captured by Gel DocTM gel documentation system (Bio-Rad, USA) and intensities were quantified by Quantity-One software version 4.62 (Bio-Rad).

qRT-PCR

qRT-PCR was used to analyze the mRNA levels of BIP, PERK, eIF2α, ATF4, CHOP, and Caspase3. Primers were synthesized by Shanghai Sangon Biological Engineering Technology. These primer sequences were listed in Table S2. The qRT-PCR Kits are from Takara. The procedure was executed by Cobasz 480.

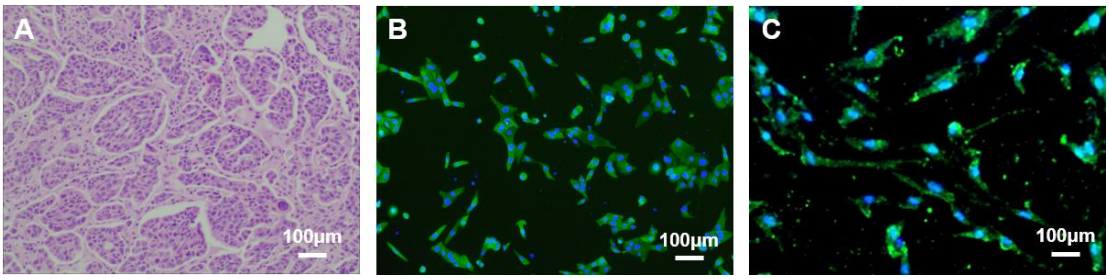

Figure S1. (a) Histology of human ovarian cancer tissue (H&E staining); (b) hPOCCs identified by cytokeratin 7-FITC; (c) hNOECs identified by cytokeratin 19-FITC.

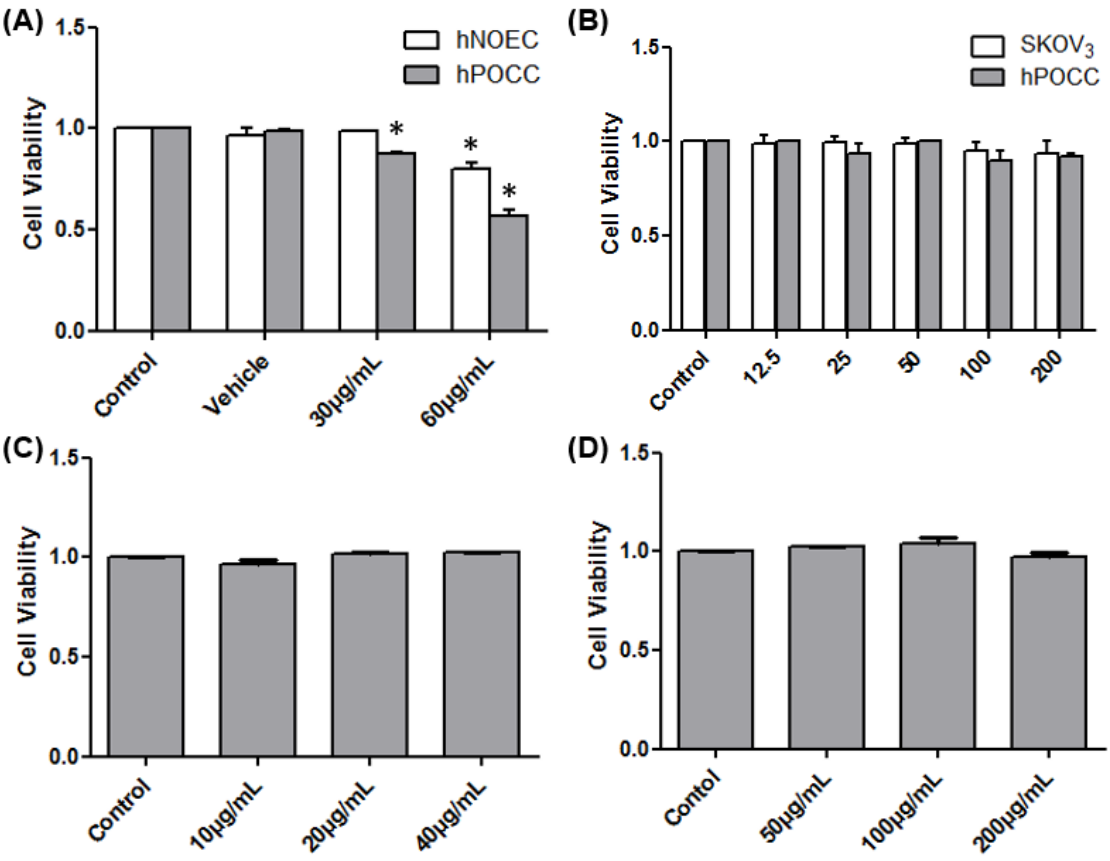

Figure S2. (A) Toxicity assay: hNOECS or hPOCCs were treated with LC (0, 30, 60μg/mL) for 24h. (B) Cells were exposed to liposome (0, 12.5, 25, 50, 100, 200μg/mL) for 24h. $SKOV_3$ Cells were exposed to (C) Gd-DTPA and (D) Gd-liposome for 24h.

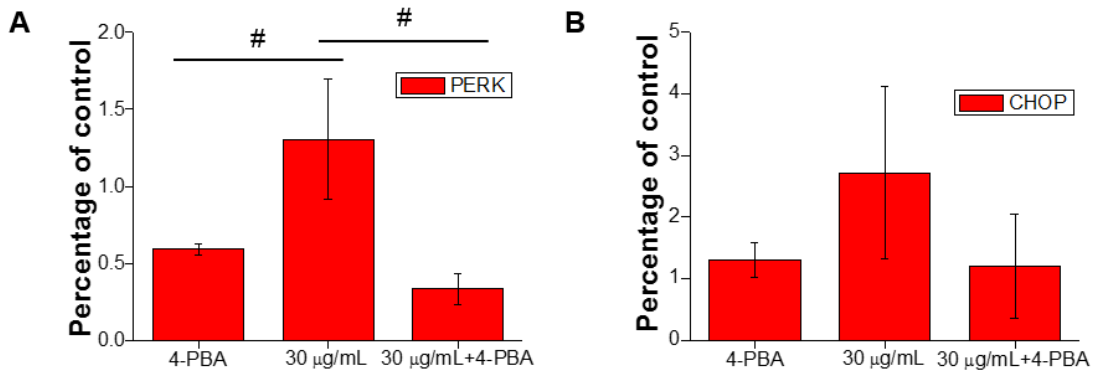

Figure S3. $SKOV_3$ cells were pre-treated with the ERS inhibitor 4-PBA (15 μM) for 2h and incubated with/without LC (30μg/mL) for 24h. The protein levels of (A) PERK and (B) CHOP were measured by WB assay in $SKOV_3$ cells. Data represented means±S.D. of three separate experiments. *#P≤0.01* compared with control group. As shown in (B), the results of CHOP were not significant between LC group and LC+4-PBA group (P=0.055), which indicating that the ERS was important but not the only pathway inducing ovarian cancer cells to apoptosis in this study.

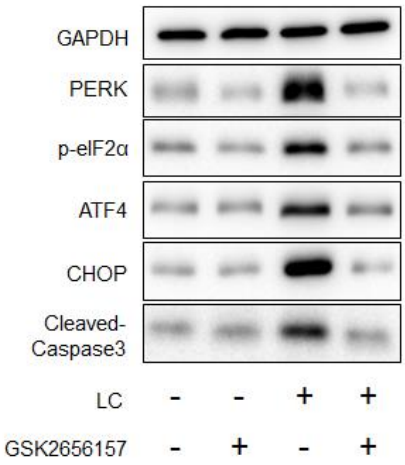

Figure S4. GSK2656157, a PERK inhibitor, reduced LC induced apoptosis mediated by PERK-CHOP branch of ERS. Cells were pretreated with GSK2656157(10μM) for 0.5h and incubated with/without LC (30μg/mL) for 24h. The protein levels of PERK, p-eIF2α, ATF4, CHOP, and cleaved-Caspase3 were measured by WB assay in $SKOV_3$ cells only.

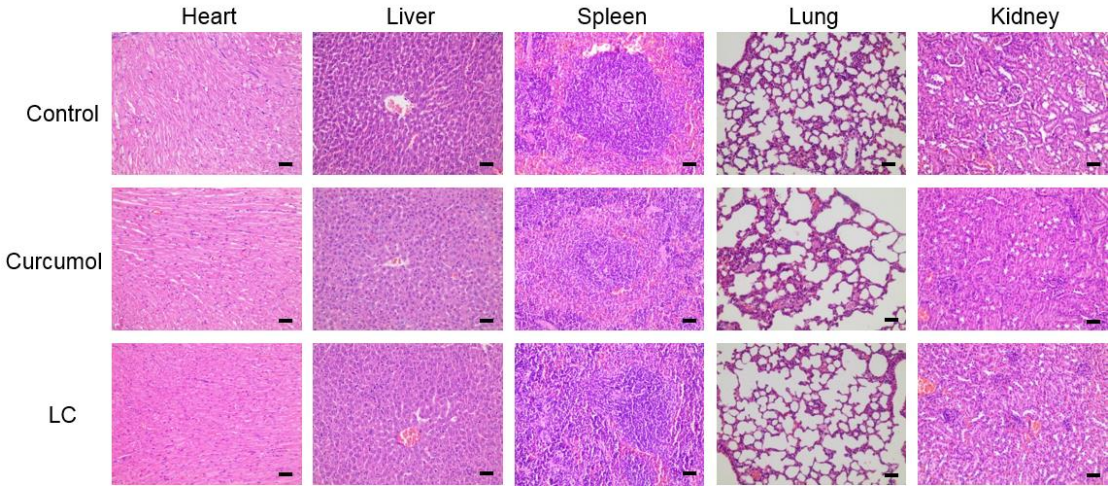

Figure S5. H&E staining of major organs in different groups. Scale bars, 50μm.

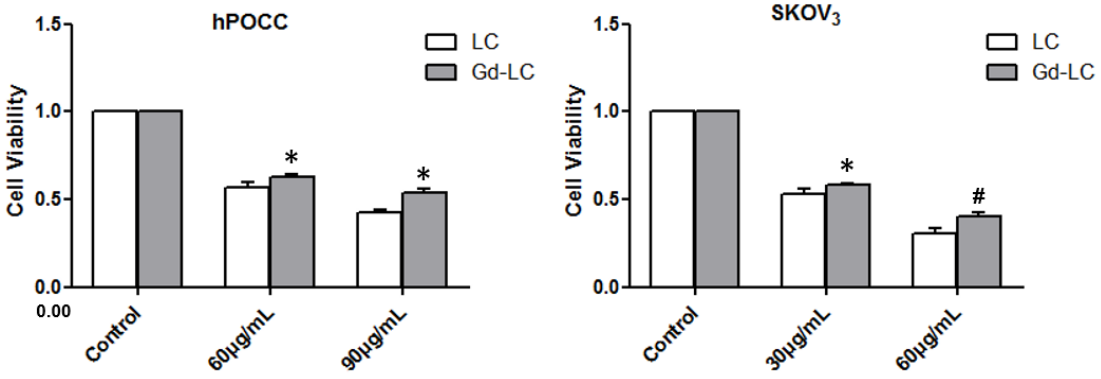

Figure S6. Cells were exposed to LC or Gd-LC (0, 60, 90 μg/mL for hPOCCs , and 0, 30, 60 μg/mL for $SKOV_3$) for 24h.

Table S1. Information of antibodies.

Antibody	Company	Source
GAPDH	Proteintech	mouse
BIP	Proteintech	rabbit
PERK	Proteintech	rabbit
p-eIf2α	CST	rabbit
ATF4	Proteintech	rabbit
CHOP	Santa Cruz	rabbit
cleaved-Caspase3	CST	rabbit

Table S2. Primer of target genes.

Primer	Forward	Reverse
BIP	5'-GCA CAG ACG GGT CAT TCC AC-3'	5'-TCC TAT GTC GCC TTC ACT CC-3'
PERK	5'-GCC CAC TTT CAC CTT CAG AG-3'	5'-CTG GTT CTT TGG TTG CTT GG-3'
eIf2α	5'-AGG ACT GCC TGG GTC TTT G-3'	5'-CTT CCC GTT CAT CTT CAT TCA-3'
ATF4	5'-GTC CTG TCC ACT CCA GA-3'	5'-GGG TGT CTT CCT TTA TGC-3'
CHOP	5'-TCT TCC TCT TCC TG-3'	5'-CAC TCT TGA CCC TGC TTC TC-3'
Caspase3	5'-TTGAGACAGACAGTGGTG-3	5'-AGAGTTCTTTTGTGAGCA-3‘
GAPDH	5'-CCA CTC CAC CTT TG-3'	5'-CAC CAC CCT GTT GCT GT-3'