Pulchinenoside E4 sensitizes chemotherapy against breast cancer through blocking autophagic flux

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Autophagy is an intracellular protective process involving degradation of misfolded proteins, damaged organelles, and nucleic acids in lysosomes to maintain homeostasis under stressful conditions. Present evidence shows that autophagy inhibitors can improve the pathologic tumor and serum biomarkers responding to chemotherapeutic drugs. Pulchinenoside E4 (PSE4) is a triterpenoid isolated from *Pulsatilla chinensis* (Bunge) Regel, a traditional Chinese medicinal herb used for the treatment of inflammation and infection. It was reported that some triterpenoids exhibited anti-cancer and autophagy inhibitory effect. The present study focuses on the autophagy inhibition caused by PSE4 and its underlying mechanisms. Results revealed that PSE4 induced autophagosome formation but interrupted the autophagosome-lysosome fusion process in breast cancer cells. As a result, autophagic flux was inhibited. Alexa Fluor 594-conjugated CTxB staining showed that lysosomal lipid rafts were disrupted by PSE4. However, when adding cholesterol, which is protective to lipid rafts, the decreased fluorescence of CTxB caused by PSE4 was partially recovered, further demonstrating the destructive effect of PSE4 on lipid rafts. The variation of protein LC3I, LC3II and p62 suggested this lipid raft disruption was directly related to the autophagic flux inhibition. Lysosomal acidification and activation of cathepsins in lysosomes were also interfered. PSE4 significantly elevated lysosomal pH, which is comparable to chloroquine, a known lysosomal alkalizing agent that strongly inhibited autophagy. Inhibition of cathepsin activity in lysosomes has been proved to be associated with increased lysosomal pH and lipid rafts destruction on lysosome membrane. Furthermore, inhibition of autophagy by PSE4 (1.5, 3, 6 μM) could enhance the anti-breast cancer activity of 5-fluorouracil in vitro and in vivo. Taken together, PSE4 inhibits autophagic flux and thereby sensitizes chemotherapy against breast cancer through disrupting lysosomal structure and function.

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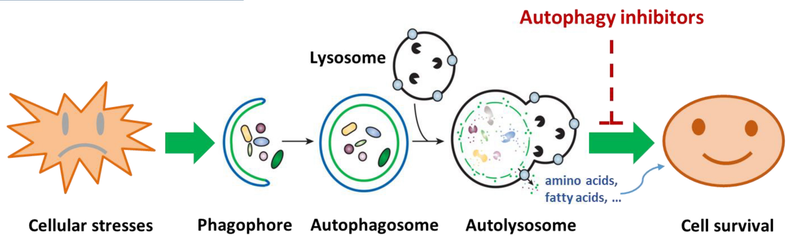
Introduction



Autophagy is an intracellular protective process involving degradation of misfolded proteins, damaged organelles and nucleic acids in lysosomes to maintain homeostasis under stressful conditions. Present evidence shows that autophagy inhibitors can improve the pathologic tumor and serum biomarkers responding to chemotherapeutic drugs. Pulchinenoside E4 (PSE4) is a triterpenoid isolated from Pulsatilla chinensis (Bunge) Regel, a traditional Chinese medicinal herb used for the treatment of inflammation and infection. It was reported that some triterpenoids exhibited anti-cancer and autophagy inhibitory effects.

Results

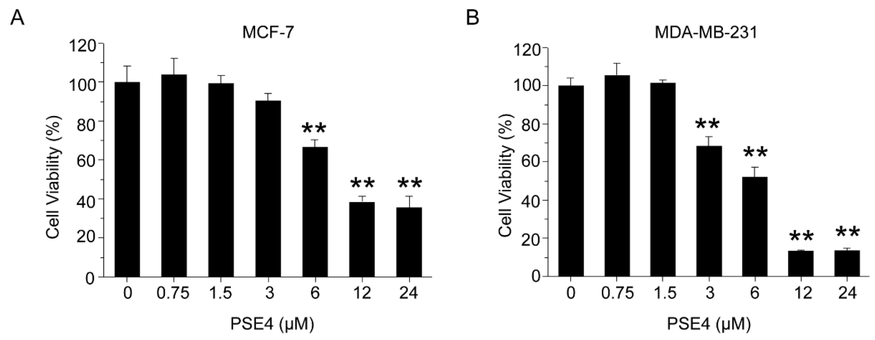


Fig. 1. Figure 1. PSE4 dose-dependently reduced cell viability of human breast cancer cells. MCF-7 (A) and MDA-MB-231 (B) cells were treated with increasing concentrations (0.75-12 μM) of PSE4 for 48 h. Values represent means ± SD (n = 3). **p < 0.01, compared with the control group (0 μM PSE4).

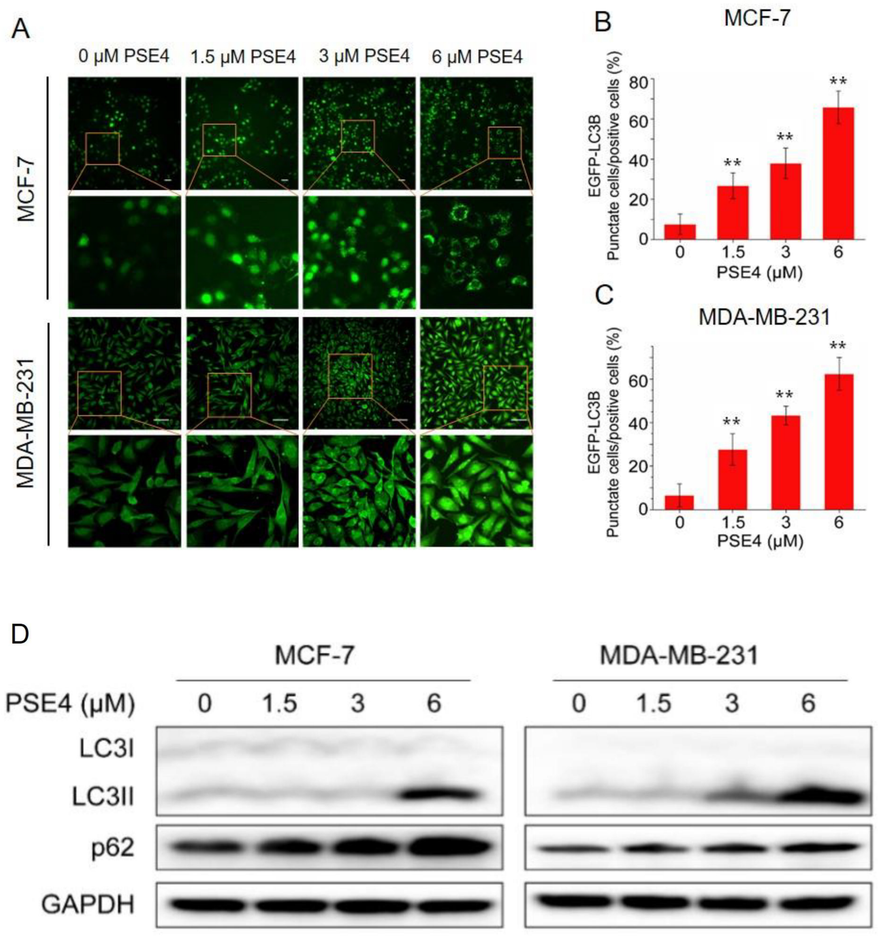


Fig. 2. PSE4 induced autophagosome formation but inhibited autophagic flux of MCF-7 and MDA-MB-231 cells. (A) was EGFP-LC3 punctate dots in MCF-7 and MDA-MB-231 cells were treated with increasing concentrations of PSE4 for 24 h. LC3B punctate dots were observed and measured using INCell Analyzer 2000 system. Scale bar: 40 μm. (B) and (C) were quantified results of panel (A). Protein levels of LC3-I, LC3-II and p62 in cancer cells were determined by Western blotting analysis (D).

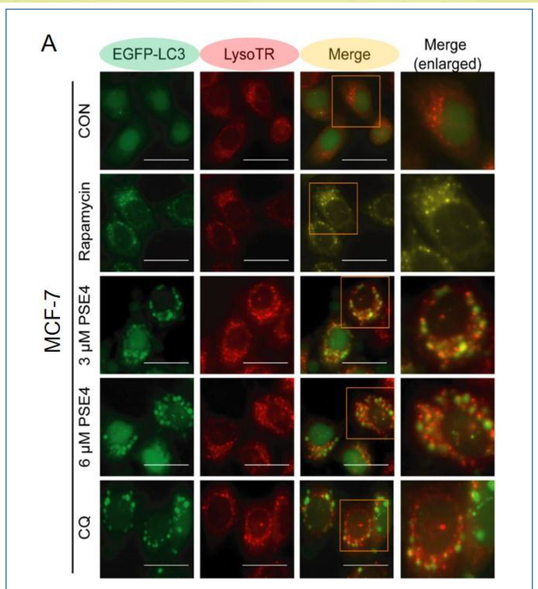


Fig. 3. PSE4 interrupted the autophagosome-lysosome fusion process in human breast cancer cells. Cells stably expressing EGFP-LC3B were treated with rapamycin (10 μM), PSE4 (3μM and 6μM) and CQ (20 μM) for 48 h in MCF-7 cells (A) and MDA-MB-231 cells (B).

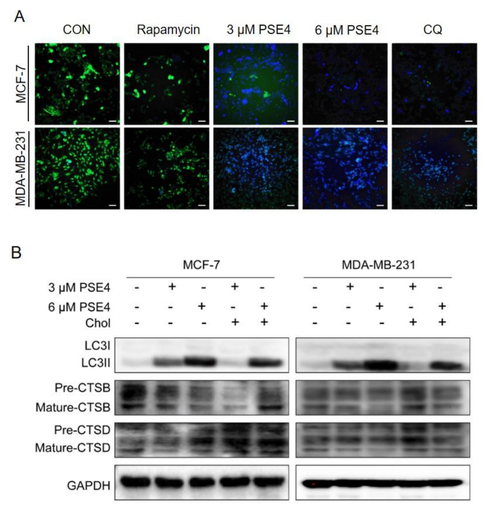


Fig. 5. PSE4 blocked the acidification of lysosomes and suppressed the activation of lysosomal cathepsins. Cells were treated with PSE4 (3 μM and 6 μM), rapamycin (10 μM) and CQ (20 μM) for 48 h. The intralysosomal pH values were detected by INCell Analyzer 2000 system using LysoSensor Yellow/Blue DND-160 probes (A). Scale bars: 40 μm. Western blotting was used to analyze protein levels of LC3II/LC3I, pre-CTSB, mature-CTSB, pre-CTSD and mature-CSTD in cells treated with PSE4 (3 μM and 6 μM) or Chol (10 μM) alone or their combination for 24 h (B).

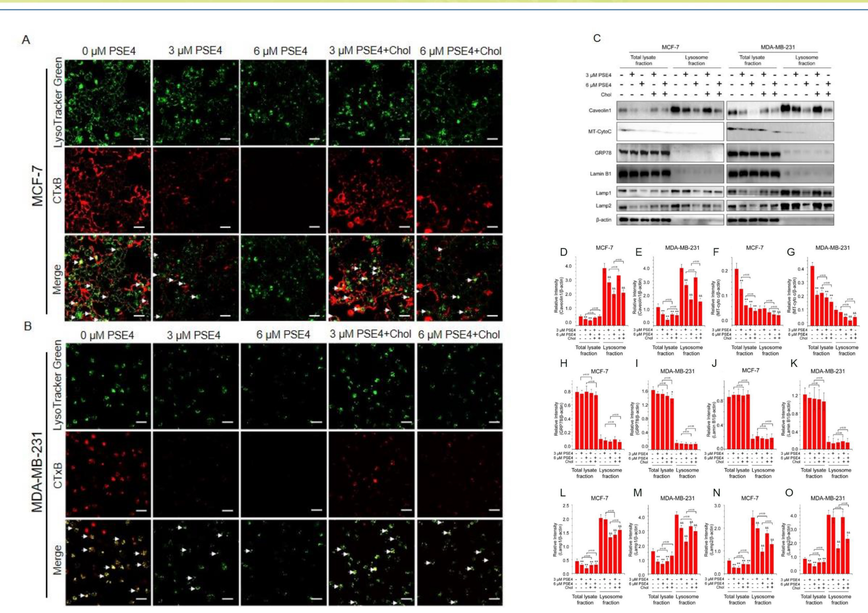


Fig. 4. PSE4 disrupted lysosomal lipid rafts in breast cancer cells. Cells were treated with PSE4 (3 μM and 6 μM) alone or in combination with Chol (10 μM) for 48 h. MCF-7 (A) and MDA-MB-231 (B) cells were stained with LysoTracker Green and lipid raft marker Alexa Fluor™ 594 Conjugated CTxB for 30 min, then the distribution and intensity of fluorescence were observed by confocal microscopy. Scale bar: 40 μm. (C) Western blotting was used to analyze the protein levels of caveolin1, MT-Cyto C, GRP78, lamin B1, lamp1 and lamp2 in the total lysate and lysosome fractions of MCF-7 and MDA-MB-231 cells. MT-Cyto C was used as a mitochondria marker. GRP78 was used as an endoplasmic reticulum marker.

Figure 8. Co-treatment of PSE4 and 5FU showed synergistic anti-breast cancer activity in vivo. Female athymic nude mice bearing MCF-7 and MDA-MB-231 tumor xenografts were randomly divided into four groups. Mice were then i.p. injected with saline, PSE4 (5 mg/kg), 5FU (15 mg/kg) and PSD + CPT (5 mg/kg PSD and 15 mg/kg 5FU) every other day for a total of 27 days. Tumor weight was measured at the end of the experiment (A and B). Tumor volume was measured every other day (C and D). Images in (E) are xenograft tumors from each group on day 27. (F and G) were quantified results of panel (E). Paraffin-embedded tumor tissue sections were stained with hematoxylin and eosin for histological analysis (H). Scale bars: 40 μm. Protein levels of LC3I, LC3II and p62 in MCF-7 and MDA-MB-231 tumor xenografts were analyzed by Western blotting (K).

Summary

PSE4 induced autophagosome formation but interrupted the autophagosome-lysosome fusion process in breast cancer cells (MCF-7 and MDA-MB-231 cell lines). Lysosomal lipid rafts were disrupted by PSE4. The variation of protein LC3I, LC3II and p62 suggested this lipid raft disruption was directly related to the autophagic flux inhibition. Lysosomal acidification and activation of cathepsins in lysosomes were also interfered. Inhibition of cathepsin activity in lysosomes has been proved to be associated with increased lysosomal pH and lipid rafts destruction on lysosome membrane. Furthermore, inhibition of autophagy by PSE4 (1.5, 3, 6 μM) could enhance the anti-breast cancer activity of 5-fluorouracil in vitro and in vivo. Taken together, PSE4 inhibits autophagic flux and thereby sensitizes chemotherapy against breast cancer through disrupting lysosomal structure and function.

Acknowledgement

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