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Original Article

# Physakengose G induces apoptosis via EGFR/mTOR signaling and inhibits autophagic flux in human osteosarcoma cells

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ABSTRACT

*Background:* Physakengose G (PG) is a new compound first isolated from *Physalis alkekengi* var. *franchetii*, an anticarcinogenic traditional Chinese medicine. PG has shown promising anti-tumor effects, but its underlying mechanisms remain unknown.

*Purpose:* To investigate the anti-cancer effects of PG on human osteosarcoma cells and the underlying mechanisms.

*Methods:* Cell viability was measured by MTT assay. Apoptosis rates, mitochondrial membrane potential (MMP), reactive oxygen species (ROS) generation, and acidic vesicular organelles (AVOs) formation were determined by flow cytometry. Protein levels were analyzed by immunofluorescence and western blotting.

*Results:* PG inhibited cell proliferation and induced apoptosis in human osteosarcoma cells. PG treatment blocked EGFR phosphorylation and suppressed epidermal growth factor (EGF)-induced activation of downstream signaling molecules, such as AKT and mTOR. PG treatment resulted in lysosome dysfunction by altering lysosome acidification and LAMP1 levels, which led to autophagosome accumulation and autophagic flux inhibition.

*Conclusion:* PG inhibits cell proliferation and EGFR/mTOR signaling in human osteosarcoma cells. Moreover, PG induces apoptosis through the mitochondrial pathway and impedes autophagic flux via lysosome dysfunction. Our findings indicate that PG has the potential to play a significant role in the treatment of osteosarcoma.

## Introduction

Osteosarcoma is the most common primary malignant tumor in the presentation age range of 10–25 years, with an annual incidence of approximately 3/1 million (Botter et al., 2014). Traditional treatment for osteosarcoma involves the integration of surgery and chemotherapy, however, the patient survival rate has remained stagnant. Surgery is a conservative treatment (limb salvage) in a majority of patients, and the current new adjuvant chemotherapy regimen has only a moderate effect on osteosarcoma. Due to early metastasis and chemotherapy resistance, prognosis of this disease is poor (Xian et al., 2017). Therefore, more effective therapies for osteosarcoma must be further developed.

Mitochondria have long been thought to be the key to cell survival. Evidence shows that mitochondrial metabolic pathways are closely related to tumor development (Kruspig et al., 2014). The proteins of Bcl-2 family induce membrane permeabilization in damaged mitochondria and regulate the release of apoptogenic factors such as cytochrome *c*, which is vital for subsequent caspase activation and the endogenous apoptosis pathway (Desagher and Martinou, 2000). Hence, modulating mitochondrial function is important for the discovery of new therapeutic strategies to treat cancer.

Autophagy is the process of recycling misfolded proteins and damaged organelles by lysosomal degradation to maintain cellular homeostasis (Kimmelman, 2011). Autophagy is regarded as a novel regulator of cancer processes, and the lysosome is a crucial organelle that regulates autophagy (Janku et al., 2011). Recent evidence shows that autophagy plays a cytoprotective role in tumors by allowing cells to adapt to environmental pressures during growth and metastasis (Shi et al., 2015). Therefore, autophagy inhibitors may theoretically be used as adjuvant therapy drugs for tumors.

*Abbreviations:* MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; EdU, 5-ethynyl-2′-deoxyuridine; MMP, mitochondrial membrane potential; ROS, reactive oxygen species; AVOs, acidic vesicular organelles; PI, propidium iodide; AO, acridine orange; MDC, monodansylcadaverine; 3-MA, 3-methyladenine; CQ, chloroquine; EGF, epidermal growth factor

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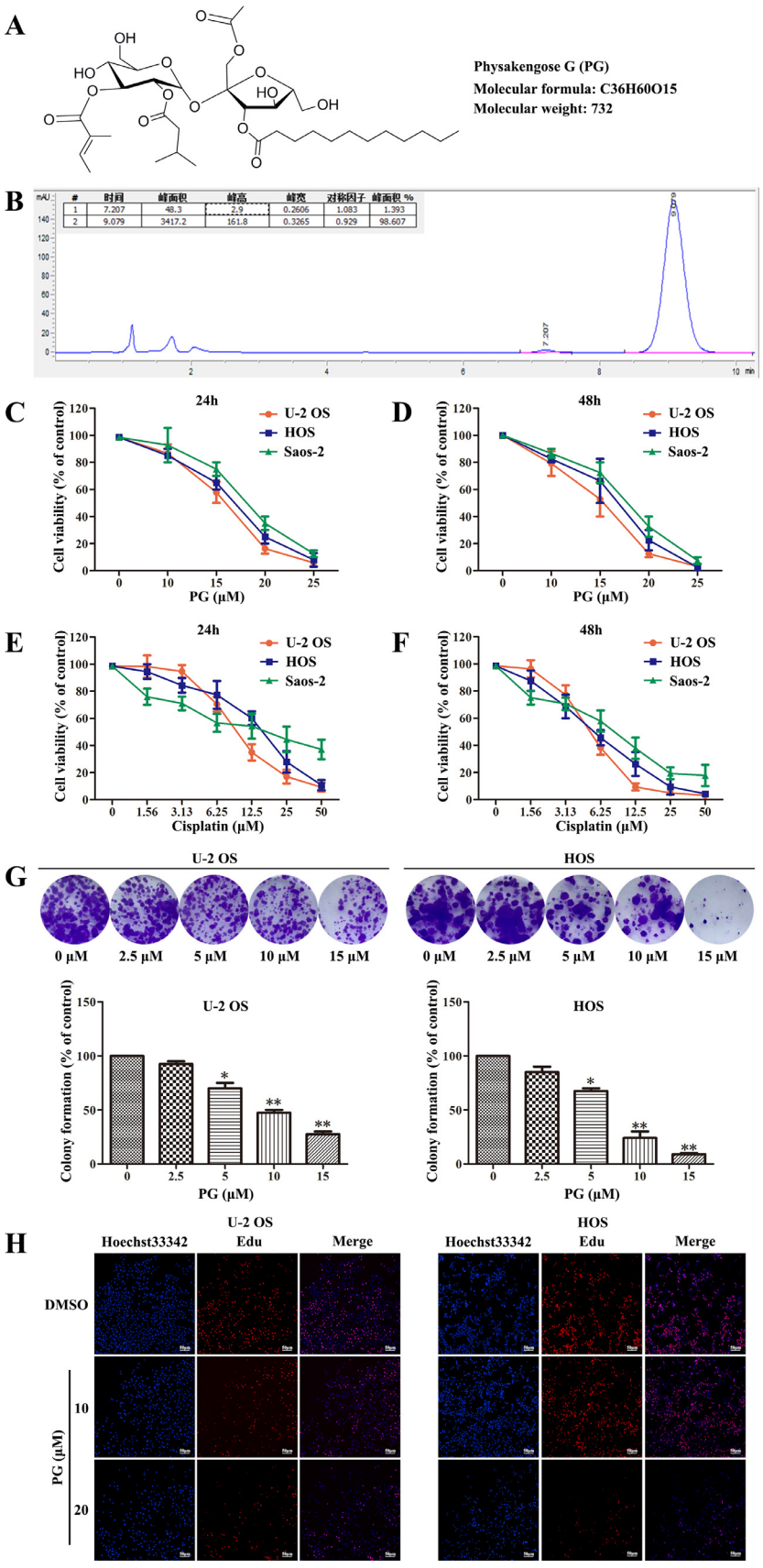


**Fig. 1.** PG inhibits the proliferation of human osteosarcoma cells. (A) and (B) Chemical structure, molecular weight and HPLC analysis of PG. (C)–(F) U-2 OS, HOS and Saos-2 cells were treated with PG (0–25 μM) or cisplatin (0–50 μM) for 24 h or 48 h. MTT assay was used to test cell viability. (G) U-2 OS and HOS cells were treated with PG (0–15 μM) for 24 h to investigate colony formation. (H) EdU staining assay was examined after cells were pretreated with PG (10, 20 μM). Data are presented as mean ± SEM from three independent experiments. *$P < 0.05$; **$P < 0.01$, compared to control.