

Pulsatilla Decoction and its bioactive component β -peltatin induce G2/M cell cycle arrest and a poptosis in pancreatic cancer

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Abstract:

Introduction: Pancreatic cancer (PAC), a malignancy that is fatal and commonly diagnosed at a late stage. Despite considerable advancements in cancer treatment, the survival rate of PAC remains largely consistent for the past 60 years. The traditional Chinese medicine formula Pulsatilla Decoction (PD) has been clinically used to treat inflammatory diseases for millennia and recently as a supplementary anti-cancer treatment in China. However, the bioactive ingredients and mechanisms underlying its anti-cancer effect remains unclear.

Materials and Methods: The composition and quality control of PD were verified through analysis by high performance liquid chromatography. Cell viability was determined using Cell Counting Kit-8 assay. The cell cycle distribution was analyzed through PI staining and flow cytometry analysis, while apoptotic cells were measured by double staining with Annexin V-FITC and PI. We used immunoblotting to examine protein expressions. The *in vivo* effects of β -peltatin and podophyllotoxin were evaluated on a subcutaneously-xenografted BxPC-3 cell nude mice model.

Results: The current study demonstrated that PD markedly inhibited PAC cell proliferation and triggered their apoptosis. Four herbal PD formula was then disassembled into 15 combinations of herbal ingredients and a cytotoxicity assay showed that the Pulsatillae chinensis exerted the predominant anti-PAC effect. Further investigation indicated that β -peltatin was potently cytotoxic with IC₅₀ of ~2 nM. β -peltatin initially arrested PAC cells at G2/M phase, followed by apoptosis induction. Animal study confirmed that β -peltatin significantly suppressed the growth

of subcutaneous-implanted BxPC-3 cell xenografts. Importantly, compared to podophyllotoxin that is the parental isomer of β -peltatin but clinically obsoleted due to its severe toxicity, β -peltatin exhibited stronger anti-PAC effect and lower toxicity in mice.

Conclusions: Our results demonstrate that *Pulsatillae chinensis* and particularly its bioactive ingredient β -peltatin suppress PAC by triggering cell cycle arrest at G2/M phase and apoptosis.