

DOCTORAL THESIS

Application of differential proteomic strategies to investigate the anti-cancer effects of *Gynostemma pentaphyllum* saponins in rat 6 fibroblast cell system

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**Application of Differential Proteomic Strategies to
Investigate the Anti-cancer Effects of *Gynostemma pentaphyllum*
Saponins in Rat 6 Fibroblast Cell System**

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A thesis submitted in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

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ABSTRACT

Gynostemma pentaphyllum (Gp), also known as Jiaogulan, is a popular Chinese medicinal herb and health supplement. Gp is rich in triterpenoid saponins (over 100 gypenosides were identified) and regarded as a complex drug system. Their anti-inflammatory and anti-cancer activities were documented in various reports. Studies in our laboratory showed that total saponins from Gp effectively inhibit the growth of tumor cells in the presence of co-cultivated normal cells. The co-culture model mimics the development of tumor *in vivo*. Animal studies in our laboratory revealed the chemotherapeutic and chemopreventive potential of Gp saponins. The Gp saponins were able to substantially reduce the number of polyps in the Min/+ mice that are predisposed to colon cancer.

In the previous investigation of our laboratory, Raf-1 protein kinase, a component of the Ras-Raf-Erk MAPK signal transduction pathway, was discovered as a potential molecular target of Gp saponins. The present study further demonstrated that Gp saponins induce the degradation of Raf-1 protein via the proteasome machinery. In addition, evidence shown that Gp saponins may exert inhibitory effect on Heat shock protein 90 leading to the dissociation of Raf-1 protein from the chaperone complex, and proteosomal degradation of the unprotected Raf-1 protein.

Today, there are sweet and bitter taste variants of Gp in the market. However, information on the chemical and biological properties between the sweet and the bitter taste variants is limited. The present study investigated the chemical constituents and anti-cancer activities of eleven Gp saponins obtained from different regions of growth in China. They were subdivided according to the taste (sweet/bitter/unspecified) of herbs. The saponin fingerprints were performed by HPLC-UV and LC-ESI-MS methods. Their anti-cancer effects were accessed by co-culture assay. Data showed that by HPLC chromatography in conjunction with ESI/MS analysis, the sweet and bitter taste variants of Gp are clearly differentiated and display strikingly different in their chemical constituents. The two taste groups display distinct and non-overlapping gypenosides profiles between sweet and bitter taste Gp herbs. The differences are reflected both in the complexity and constituents of the total saponins. Results showed that all of the samples from the sweet taste Gp and two of the bitter taste Gp exhibited the growth inhibitory effect within a non-toxic dosages assessed by the co-culture assay. The sweet taste Gp showed broader dosage margin between the desired anti-cancer effect and the toxic effect. The distinct and unique saponin profile of

different Gp variants may direct the biological activity. The combination of the chemical and biological analysis in the present work provides useful information for the quality assessment of Gp products.

The anti-cancer mechanism of Gp saponins is remained elusive. In order gain further insight of the underlying mechanism of the drug, especially the possible signaling cascade involved, the effect of Gp saponins on the phosphoproteome in R6 cells was investigated. Two strategies were employed in the present study to profile the differential phosphoproteome in the early event of Gp saponins treatment on R6 cells: two dimensional gel electrophoresis (2-DE) with immunodetection using phosphotyrosine- and phosphoserine/threonine-specific antibodies; a combined strategy comprising phosphoprotein enrichment, SILAC, and time course analysis. In total, we have identified 72 altered phosphoproteins responding to Gp treatment. Integrating the data obtained from these experiments, bioinformatics-assisted analysis revealed that a number of interaction networks with distinct function were involved. Gp saponins may modulate the glycolysis and TCA cycle via phosphorylation-dependent functional regulation of enzymes involved in the pathways. Gp saponins may also down-regulate the MAPK/Erk signaling pathway through hypophosphorylation of PLC β 3, MEK-1, PP1A and ROCK2. In addition, the protein translation and synthesis machinery may be regulated by Gp saponins through activation of eEF2. Finally, we observed the changes of phosphorylation of FBP aldolase, G3PDH, PEA-15, PLC β 3, citrate synthase and hnRNP A1. These protein molecules are known to be regulated by the CaMK and PKA family. This observation poses the potential effect of Gp saponins on CaMK and PKA.

In summary, this study provides new information on the chemical constituents and biological activities of Gp variants. It will benefit quality control and drug safety of a daily consumed health supplement. This study also provides new insight into mechanisms responsible for part of the Gp saponins activities. Application of the state-of-the-art proteomics technology was demonstrated to be an advantageous approach for system biology research of Chinese medicine.

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