

DOCTORAL THESIS

The impact of herbal saponins on gut microflora in animal models

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ABSTRACT

Human gut harbors 100 trillion microbial organisms that is intrinsically linked to individual's health and diseases, including cancer. Food fiber and phytochemicals such as polyphenols are considered as prebiotic-like dietary modifiers. They can influence the gut microbial communities, and in turn to modulate disease outcome and drug responses of the host. Saponins belong to a family of phytochemicals commonly found in many medicinal and edible plants. Herbal saponins have raised keen interest among scientists for their health-promoting effects, but have not been investigated for their potential as prebiotics. *Gynostemma pentaphyllum* (Gp) is riched in triterpenoid saponins and has been consumed in China and other part of the world as an herbal tea and as a folk medicine. In our lab, we have demonstrated that Gp possesses strong anticancer and anti-inflammatory effects. Whether Gp possesses prebiotic property and whether gut microbiota plays any part of the anticancer effect of Gp are the questions addressed in the present study. Thus, we hypothesized that Gp saponins (GpS) might modulate the gut microbiota, which in turn enhance its anticancer activities. In the study, the gut microbiome analysis were carried out using two main techniques, namely the enterobacterial repetitive intergenic consensus (ERIC-PCR) and 16S pyrosequencing approaches. Both xenograft nude mice and *Apc*^{min/+} mice were employed as the animal models to investigate the interaction between the herbal saponins and the gut microbiota in the host.

Athymic nude mice have been employed for tumorigenic research for decades, however, the relationships between the gut microbiome and host's response to the grafted tumors and drug treatments are unexplored. For the first part of the thesis, we investigated the relationship between the gut microbiota and grafted tumor in the nude mice under the treatment of Gp saponins. Partial least squared discriminant analysis (PLS-DA) of ERIC-PCR data showed that the microbiota profile of xenograft nude mice departed from that of the nonxenograft mice. However, prolonged treatment of GpS seems to realign the fecal microbiota with the pretreatment control. Pyrosequencing data reiterated the differences in fecal microbiome between the nonxenograft and xenograft animals. GpS treatment had a much stronger impact on the phylotypes of the xenograft than the nonxenograft mice. In addition, GpS treatment markedly induced the relative abundance of *Clostridium cocleatum* and *Bacteroides acidifaciens*, for which the beneficial effects on the host have been well documented.

Apc^{Min/+} colorectal cancer mouse model was further employed for the investigation of the association of the gut microbiota and cancer occurred inside the gut, which was a more direct site to interact with the gut microbiota. In the *Apc*^{Min/+} mouse model, we found distinct difference of fecal microbiome between the *Apc*^{Min/+} and the wild-type littermates. GpS treatment significantly reduced the number of intestinal polyps. GpS also increased the ratio of Bacteroidetes/Firmicutes and reduced the sulfate- and sulfur-reducing bacteria lineage and potential opportunistic pathogens, which might cause certain deleterious effects to the host. The impact of GpS on the gut mucosal environment was also examined. We found GpS treatment improved the gut barrier function by increasing the numbers of Paneth cells, goblet cells, up-regulating the expression of E-cadherin and down-regulating the expression of N-cadherin in the intestine. In addition, GpS treatment down-regulated the protein expression of beta-catenin and p-STAT3. Furthermore, higher levels of anti-inflammatory and tissue repair-related cytokines as well as Arginase I, but lower level of iNOS expression were found in GpS-treated *Apc*^{Min/+} mice, indicating increased anti-inflammatory macrophage phenotype M2 (associated with tissue repair) and reduced proinflammatory phenotype M1. Furthermore, in addition to GpS, other herbal saponins also showed prebiotic-like effects in C57BL/6 mice.

In summary, this study provides first hand evidence for the impact of herbal saponins on the gut microbial ecosystem and new insight into mechanisms responsible, at least in part, for the activities of GpS. We demonstrate that tumor growth induce intestinal dysbiosis. GpS treatment can inhibit tumor progression and concurrently alter the microbiome by increasing symbionts and/or decreasing pathobionts, which may contribute to its chemopreventive effect against tumorigenesis. Herbal saponins showing prebiotic-like effects may be used for improving the health of the host by manipulation of the gut microbiota.

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