DOCTORAL THESIS

The impact of herbal saponins on gut microflora in animal models

Chen, Lei

Date of Award:
2014

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, neamly 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.

ACKNOWLEDGEMENTS

It was really a nice journey, also a great adventure pursuing my Doctor of Philosophy study. I extremely enjoyed exploring the unknown world with my strong curiosity. In addition to gaining invaluable experiences, knowledge and joyfulness, I learned how to deal with tough situations, fallen expectations and setbacks, thus making me more mature. During this journey, I gradually felt that science is art. As *Richard Dawkins* says, "You need a combination of that and genuine inspiration, which is akin to poetry; it's akin to great art; it's the inventive, creative leap which great scientists have." This journey would not be possible without the continued support from my supervisor, colleagues, friends and family.

I would like to express my sincere gratitude to my principal supervisor Prof. Wendy Hsiao for providing me the opportunity to join her team and start my PhD study. I appreciate her great foresight for choosing such an interesting topic, which becomes more and more important and popular. I am grateful for her continuous guidance throughout my whole study and the preparation of this thesis. Thanks for her full support for allowing me to test all hypotheses for which we were interested in. I am also thankful to her encouragement when I encountered the difficulties. Spark of inspiration always came after pleasant discussions, and her insightful advices empowered me the power to discover more beautiful scenery. Thanks for continuously developing my potential and research interests. Thanks for giving me so many chances to share my research in overseas conferences, which had broadened my horizon, refreshed my knowledge and strengthened my communication skill. Altogether, I feel very fortunate to meet Prof. Hsiao and be her student in this wonderful journey.

I am very grateful to Dr. William Tai for his generous support and his good laboratory management. Thanks for his kind help on reagent ordering and animal supplying. I would like to thank all the present and past members of Prof. Hsiao's lab for creating such an excellent environment for learning and working. Special thanks to Mr. Huang Chen-hu for his kind assistance in animal experiments. I am also appreciated the technical support from our enthusiastic technicians, Mr. Michael Wong, Ms. Hilda Cheung, Ms. Nickie Chan, Mr. Alan Ho, Ms. Irene Koo, and Ms. Sally Lee, and the help from Ms. Lisa Song in literature retrieval. I would like to express my sincere thanks to Ms. Patty Lam and Mr. Lo Kam Fai for their help in my thesis submission

and oral examination. I am extremely thankful to our collaborators Prof. Frederick CC Leung in The University of Hong Kong and his student Manreet for their help in pyrosequencing. In addition, I particularly want to thank all the colleagues who give me a hand during my study. I thank Mr. Chan Chi Leung, Ms. Ma Xiao-qing, and Ms. Liang Xu for their help in metabolomics data analysis. I thank Dr. Qin Hong-yan for her kind help in discussion of histological techniques. I thank Dr. Alexander Leung for his helpful suggestions in microbiological experiments. Also, I would like to thank Dr. Tang Jing and Dr. Tu Xing in Model Animal Research Center of Nanjing University for their help in data collection of the comprehensive laboratory animal monitoring system.

Furthermore, I would like to give heartfelt thanks to my friends, our PhD ladies club, Dr. Qin Hong-yan, Dr. Zhu Lin, Dr. Wu Meng-hua, Dr. Chen Xiao-yu, Ms. Pook Supawadee Parhira, Ms. Liang Xu, Ms. Xiao Ting-ting, Ms. Cao Hui-hui, Ms. Cheng Zhen, Ms. Zhang Zui, and Ms. Chen Lei-lei. As the old saying goes, "Fixed barrack, floating soldiers." Although some of them have already left BU, the wonderful memories are timeless. Thanks for the numerous moments of joy, excitement and disappointment we shared together. I would particularly like to thank Ms. Xiao Ting-ting and Ms. Cao Hui-hui for their daily companionship and being my eternal sunshine. Long live our friendship. Finally, I would like to express my countless and sincere thanks to my family and my boyfriend for their unconditioned love and enormous support. Thanks for their understanding, toleration, and encouragement all the time. Thanks for giving me strength to overcome the moments of stress and frustrations. Thanks for always being by my side.

TABLE OF CONTENTS