

MASTER'S THESIS

Study of the anticarcinogenic mechanisms of astragalus membranaceus in colon cancer cells and tumor xenograft

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Date of Award:
2006

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**Study of the Anticarcinogenic Mechanisms of
Astragalus Membranaceus in Colon
Cancer Cells and Tumor Xenograft**

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

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December 2006

Abstract

Colorectal cancer is one of the leading causes of cancer-related deaths in developed countries. Conventional chemotherapy based on 5-fluorouracil and related compounds has been used for over four decades. However, these treatments do not have a high response rate and may lead to severe toxicities such as myelosuppression, stomatitis, nausea, vomiting and diarrhea in patients. More effective and less toxic chemotherapeutic regimens are urgently needed, which include many novel herbal derivatives. The dried root of *Astragalus membranaceus* (HuangQi) has been used in many Chinese medicinal formulations in treating immune deficiency conditions. In recent years, it has also been used clinically as an adjuvant agent in cancer treatments to alleviate the side effects of conventional chemotherapeutic drugs. In this study, we investigated the effects of different Astragali extracts in HT-29 human colorectal cancer cells and tumor xenograft. We have shown that total Astragali saponins (AST), but not its total polysaccharides (APT) and the pure saponin Astragaloside IV (AS IV) possess anticarcinogenic effects in HT-29 cells. Cytotoxicity of AST in HT-29 cells was demonstrated using the MTT assay, with EC₅₀ of 39.8 µg/ml and 31.6 µg/ml after 48 and 72h of treatment, respectively. AST was also found to cause profound proliferative inhibition in HT-29 cell proliferation as determined by the BrdU ELISA. Such anti-proliferative activity was associated with accumulation of cells in the S phase and G₂/M arrest as determined by flow cytometry. Subsequent Western analysis revealed that the arrest could be due to the overexpression of cyclin dependent kinase inhibitor p21, cyclin A, and decreased activity of the cyclin dependent kinase cdc-2. Apart from that, AST also possess pro-apoptotic effects in HT-29 cells, which were exhibited by chromatin condensation DNA fragmentation. The associated apoptotic signaling includes a

significant decrease in Bcl-xL protein expression, caspase-3 activation and Poly (ADP-ribose) polymerase (PARP) cleavage. Further mechanistic studies have demonstrated that the anti-carcinogenic effects of AST are associated with overexpression of a novel transcriptional factor nonsteroidal anti-inflammatory drug (NSAID)-activated gene (NAG-1). By using Western blotting and real time polymerase chain reaction (PCR), we have shown a time-dependent increase in protein and mRNA expression of NAG-1 in HT-29 cells. The upregulation of NAG-1 expression is suggested to be caused by activation of upstream transcription factors such as the early growth response gene-1 (Egr-1), with prior induction at both protein and mRNA levels. Results from the kinase inhibitor studies demonstrate that activation of Egr-1 and NAG-1 by AST could be phosphatidylinositol 3-kinase (PI3K)-dependent, but may not rely on its downstream regulator protein kinase B (AKT). This represents a novel pathway for differential regulation of NAG-1 by the PI3K/AKT pathway. The anti-tumorigenic effects of AST were further displayed in a xenograft nude mice model. Reduction of tumor volume was evident in HT-29 xenografted nude mice following AST treatments. The anti-tumor effect of AST was comparable to that included by 5-FU based chemotherapy while producing less toxic side effects. An immunomodulating effect of AST was in part due to its ability to counteract the leukopenic action of conventional chemotherapy. Taken together, our results indicate that the total Astragali saponins AST could be established as an effective chemotherapeutic agent in colon cancer treatment without great toxicity. It might also be used as an adjuvant in combination with conventional chemotherapy with reduced systemic side effects.

Total: 547 words

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