MASTER'S THESIS

Study of the modulating effects of Astragalus saponins on tumor angiogenesis and invasiveness in colon cancer cells

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Study of the Modulating Effects of Astragalus Saponins on Tumor Angiogenesis and Invasiveness in Colon Cancer Cells

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ABSTRACT

Colorectal cancer is prone to develop into invasive cancer, while metastatic development is the major cause of cancer-related deaths. Astragalus saponins (AST) exhibit anti-carcinogenic effects in various cancer cell lines in our previous studies. Our latest findings indicate that AST could suppress tumor growth in nude mice xenograft of HT-29 colon cancer cells. The results were comparable to the conventional chemotherapeutic drug 5-FU with fewer adverse effects. Here, we attempted to further explore the *in vitro* and *in vivo* effects of AST on colon tumor progression.

AST (80 μg/ml) significantly inhibited the growth of HCT 116 cells by 75% after 72 h of treatment. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are the key angiogenic growth factors for blood vessel formation and contribute to tumor angiogenesis. Matrix metallopeptidase, MMP-2 and MMP-9, which have been found to be elevated in colon cancer, are the collagen IV gelatinase responsible for the extracellular matrix (ECM) degradation that facilitate cell invasion. Our results revealed that AST could significantly downregulate the protein expression of these angiogenic factors and invasive molecules. Some signaling molecules involved in PI3K/AKT/mTOR pathway were also examined. AST increased PTEN expression, decreased the phosphorylation of Akt and its downstream target, mTOR. Rapamycin alone could reduce the protein expression of VEGF, bFGF, MMP-2 and MMP-9, while co-treatment of rapamycin and AST further decreased the expression. HIF-1α is the key transcriptional activator of the VEGF gene and will be elevated under hypoxic conditions. In cobalt chloride (II) ($CoCl_2$)-mimicked hypoxia, the induced HIF-1α and VEGF in HCT 116 cells could be suppressed by AST and the effect was intensified with rapamycin co-treatment. In HCT 116 xengrafted nude mice model, tumor volume and

tumor weight were found to be reduced by 42.7 % and 33.9 %, respectively, by AST treatment when compared to the control group. AST was found to markedly reduce the serum level of VEGF when compared to the control group. Immunohistochemical staining of the tumor sections also showed decreased VEGF expression in AST-treated group. The chemotherapeutic drug vinblastine (VBL) has been widely used for treating different human cancers and exhibited potential anti-carcinogenic effects in colon cancer cells in our pilot study. The therapeutic effects of the combined use of AST and VBL on colon cancer development were also investigated in the present study. The anti-invasion effects of AST were demonstrated using LoVo metastatic colon cancer cells. The number of invaded cells was decreased by AST treatment by using the cell invasion assay. AST also increased the localization of cadherin-catenin complex at the cell membrane, indicating that AST might hinder cell invasion by increasing cell-cell interaction. A splenic liver metastasis animal model was used to study the anti-metastatic effects of AST. A smaller extent of metastases was observed in the liver in AST-treated mice when compared to that in control animals. AST could also downregulate the expression of various metastasis-associated proteins and genes in the tumor-bearing liver biopsy.

In conclusion, our results suggest that AST could attenuate tumor progression of colon cancer through inhibition of angiogenesis, probably via downregulation of the AKT/mTOR signaling and inhibition of invasion by modulating the invasive factors.

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