Selective induction of apoptosis by aqueous extract of Chinese medicinal herb *Scutellaria barbata* in HCT-116 colon cancer cells and CCD-841 colon epithelial cells.

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Abstract

Scutellaria barbata (SB) has been used in traditional Chinese medicine for treating liver, lung and rectal tumors. We previously found that aqueous extracts of SB inhibited mutagenesis, DNA binding, and metabolism of aflatoxin B1 and benzo(a)pyrene. They were also shown to inhibit foci formation in the colon of AOM-induced mice. Other researchers demonstrated that ethanol extracts of inhibit colorectal cancer growth, inhibit proliferation and induce apoptosis. In this study, the effects of aqueous extracts of SB on the induction and modulation of apoptosis in HCT-116 colon cancer cells and CCD-841 normal colon epithelial cells were assessed using TUNEL assay, Apoptosis/Necrosis Detection Kit, and Human Apoptosis Antibody Array. Our findings suggest that SB possess a wide range of antitumor activity and may be a useful drug for treating colon cancer.

Introduction

Colon cancer remains one of the leading cancers worldwide [1]. Individuals in developed countries have the highest risk for colon cancer which is the second most prevalent cause of cancer mortality in the United States [1-2]. Multiple abnormal intracellular signaling pathways such STAT3, Erk, and p38 have been associated with the development of colon cancer [3]. Polyps which are non-cancerous proliferations of mucosal epithelial cells can gradually develop over time into invasive forms of cancer [2]. Later stages are commonly treated with standard forms of chemotherapy which have a high level of toxicity to normal cells. This issue highlights the importance of developing novel chemotherapeutic drugs for the treatment of colon cancer.

Scutellaria barbata (SB) is a natural medicinal herb widely used in China and other regions of northeast Asia. Scutellarin, baicalin, luteolin, and apigenin, flavonoids found in *S. barbata,* have demonstrated anti-inflammatory, anti-viral, anti-angiogenic and anti-tumor effects. Previous research has reported that SB is able to induce colon cancer cell apoptosis, and tumor angiogenesis via modulation of several pathways.

Scutellaria barbata
(Ban Zhi Lian)

Results

Figure 1 Induction of apoptosis in HCT-116 colon cancer cells (3-6 hours)

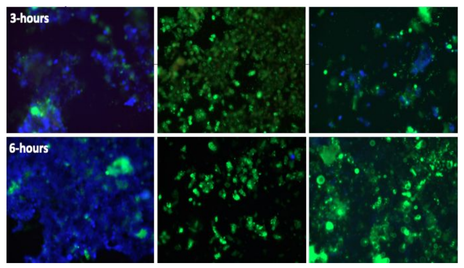


Figure 2 Induction of apoptosis in CCD-841 colon epithelial cells (3-6 hours)

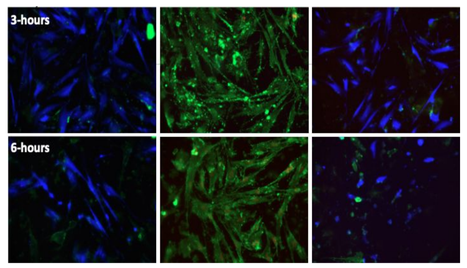


Figure 3 Model of HCT-116 cells treated with *S. barbata*

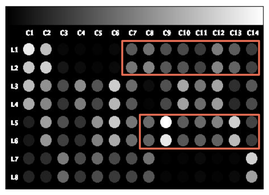


Figure 4 Model of CCD-841 cells treated with *S. barbata*

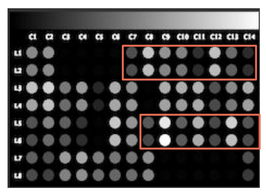


Figure 5 Expression of Pro-apoptotic Proteins in CCD-841 Cells

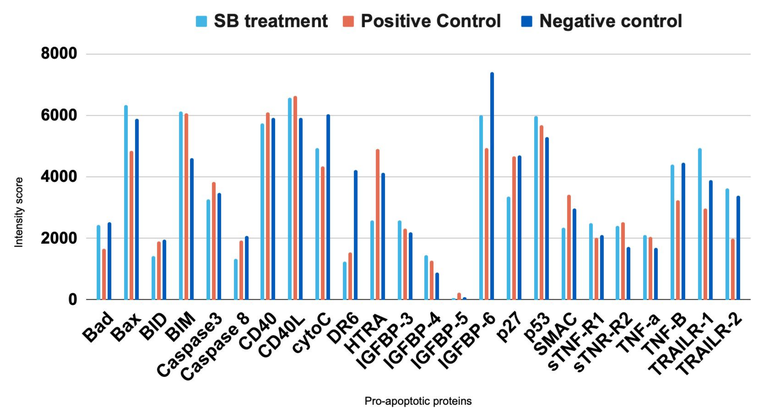
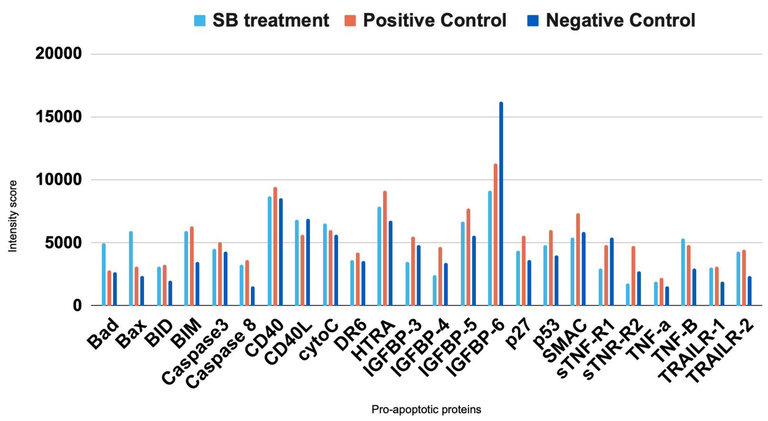


Figure 6 Expression of Pro-apoptotic Proteins in HCT-116 Cells



Methods

REAGENTS

Crude *S.barbata* powder were purchased from Nuherbs (Oakland, CA, USA). Stock solutions were diluted to a working concentration of 2.5mg/mL. Staurosporine was used as the control treatment.

CELL CULTURE

CCD-841 and HCT-116 cell lines were obtained from ATCC (Manassas, Virginia, MA), and cultured in Mccoy's 5a Medium. The CCD-841 cells were maintained in ATCC-formulated Eagle's Minimum Essential Medium. Cells were incubated at 37°C in 5% CO_2 environment.

APOPTOSIS/NECROSIS ASSAY KIT

Fluorescent analysis was conducted to determine healthy (blue), necrotic (red) and apoptotic (green) cells after treatment with 1.5mg/mL *S.barbata* using the Apoptosis/Necrosis Assay Kit (ab176749), Abcam, UK).

APOPTOSIS ANTIBODY ARRAY

Analysis of protein expression was performed using the Human Apoptosis Antibody Array kit (ab134001, Abcam, Cambridge, UK) which contains antibodies against 43 protein targets. Membranes were incubated with 200µL of protein.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software (Version 27; Chicago, IL). Student's *t* tests were used to analyze and determine differences between two treatment groups. Comparisons between three or more groups were analyzed using ANOVA. Triplicates were used in all experiments. A standard $p < 0.05$ was considered statistically significant.

Conclusion

Our apoptosis/necrosis data demonstrated that both the 3-hours and 6 hours of 1.5 mg/mL SB treatments induced apoptosis in HCT-116 significantly, $p < 0.05$; while there was no notable induction of apoptosis in normal CCD 841 cells. Preliminary data from the human apoptosis antibody array shows up-regulation of pro-apoptotic marker proteins Bax, BID, Bad, p27, p53, Caspases 8, and 3 in HCT-116 cells with the treatment of SB. These results suggest that SB contains phytochemicals that selectively induce apoptosis in HCT-116 cancer cells by modulating these markers while not significantly affecting the normal CCD 841 CoN colon epithelial cells. Further study of their specific modulation effects and phytochemicals on apoptosis is warranted to reveal their potential chemopreventive properties.

Selected References

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Aknowledgements

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