Modulation of Apoptosis in Breast Cancer Cells MDA-MB-157, 93A and 93B by Aqueous Extract of Chinese Medical Herb *Scutellaria barbata*.

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Abstract

Scutellaria barbata (SB), a Chinese medical herb, has been known to contain anti-cancer properties. In this study, the effectiveness of SB in apoptotic modulation of APC-mutant breast cancer cell lines MDA-MB-157, 93A, and 93B was investigated. Assessments were performed using the green/red/blue fluorescent Apoptosis/Necrosis Detection Kit and the Human Apoptosis Antibody Array - Membrane (43 Targets) test by the Abcam cooperation. Our data demonstrated that 1-hour and 3-hour incubation treatments with aqueous extract of SB induced apoptosis in all cell lines. Additionally, modulation of pro-apoptotic markers (Bad, Bax,BID, BIM, C3, C8, p53, p27) and anti-apoptotic markers (BcL-2, Bcl-w, p21) was observed.

Introduction

Breast cancer cell lines MDA-MB-157 and its APC-mutants (93A and 93B) are more resistant to cancer treatment due to inhibition of APC tumor suppressor gene. SB, a Chinese herb used in traditional Chinese medicine has been known to contain biologically active phytochemicals that are known to induce apoptosis (guided multi-step pathway leading to programmed cell death). This study investigates the modulation effect of SB on these breast cancer cell lines.

Methods

- Cell cultures of breast cancer cell lines MDA-MB-157, 93A, and 93B were grown and maintained in RPIM.
- Abcam Apoptosis/Necrosis detection kit
  - Each cell line was divided in three groups: negative control (distilled water), positive control (1µlM Staurosporine) and treatment group (2 mg SB). Incubation with treatment and apoptotic markers (Apopxin green, red 7-ADD and CytoCalcein violet 450) was performed for 1 and 3 hours.
  - Fluorescent microscopy pictures were used to count the number of apoptotic (green), necrotic (red) and live cells (blue) (Figure 1). Obtained data was analyzed using paired-t-test ($p<0.05$).
- Abcam Human Apoptosis Antibody Array – (43) Membrane
  - Each cell line was divided into a negative control group (distilled water), positive control group (Staurosporine) and treatment group (SB).
  - Blocking and incubation was performed. Chemiluminescence detection was used to obtain results through X-ray films.
  - Densitometry software ImageJ was used to obtain spot signal densities from scanned images to perform a semi-quantitative comparison (Figure 2)
  - Results were analyzed by one-way ANOVA and Tukey post hoc test.

Results

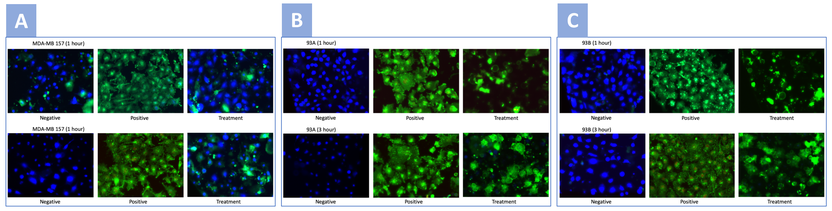

Figure 1. Fluorescent microscopy pictures demonstrating 1-hour and 3-hour incubation results after treatment with 2mg of aqueous SB extract for cell lines (A) MDA-MB 157, (B) 93A and (C) 93B. Blue fluorescence indicates cytoplasm staining (normal live-cells), green fluorescence indicates inner phosphatidylserine exposure (apoptosis) and red fluorescence of the nucleus indicates loss of cell membrane (necrosis).

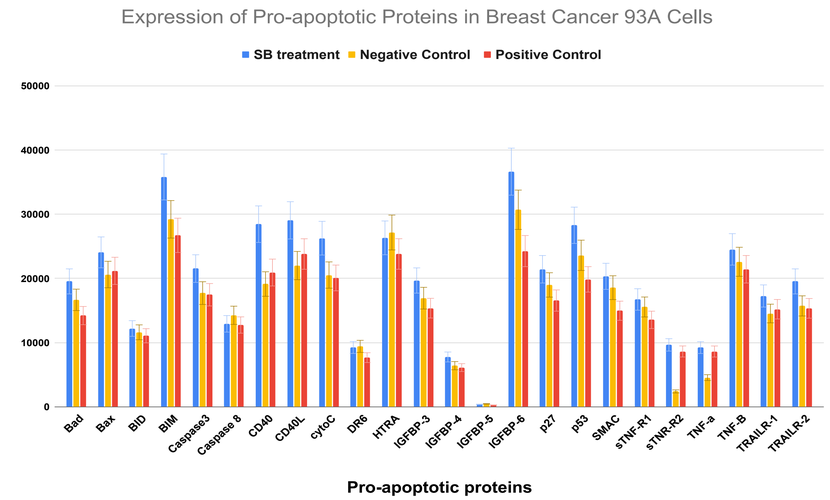

Figure 3. Expression of Pro-apoptotic Proteins in APC-Mutant Breast Cancer Cell line 93A from spot signal densities obtained through ImageJ software (NIH) data from Figure 2B.

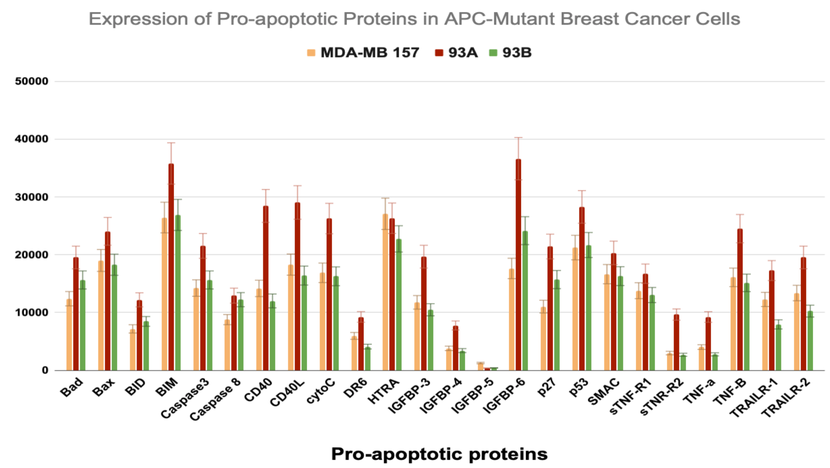

Figure 4. Comparison of Pro-apoptotic Protein expression between Breast Cancer Cell lines MDA-MB 157 and APC-Mutants (93A and 93B) after 2mg SB treatment.

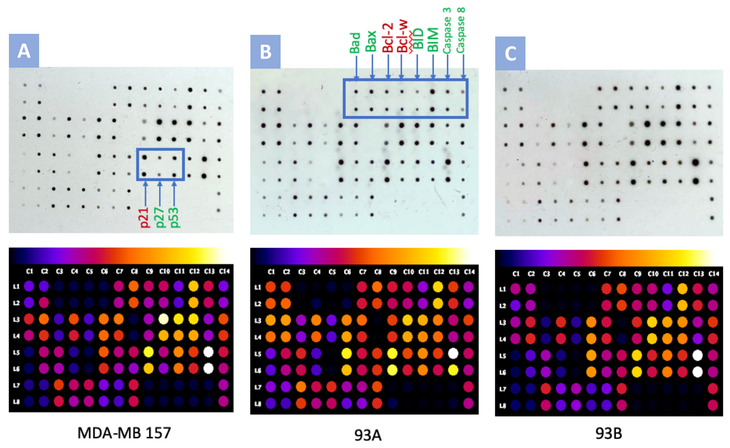

Figure 2. Human Apoptosis Antibody Array X-ray film with respective ImageJ software protein profiling analysis using color intensities to reveal spot signal densities of various apoptotic protein concentration for Breast Cancer cell lines (A) MDA-MB 157, (B) 93A and (C) 93B after treatment with 2mg SB. Some significant apoptotic proteins observed were: Bad, Bax, Bid, Bim, C3, C8, cytoC, DR6, HTRA, IGFBP-5, p27, p53, TNF-B, TRAILR-1, and TRAILR-2.

Conclusion

- Fluorescent apoptosis results reveal that aqueous extract of SB induced a statistically significant percentage of apoptosis in MDA-MB- 157 ($46.5 \pm 7.5\% > 16\% \pm 1.0\%$, $p < 0.05$); 93A ($54.5 \pm 2.5\% > 0\%$, $p < 0.05$); and 93 B ($65.5 \pm 13.5\% > 0\%$, $p < 0.05$). Similar results were obtained with 3-hour incubation.
- Antibody Array Membrane results demonstrate that there was a statistically significant difference between groups in the expression of the following pro-apoptotic proteins:
  - MDA-MB-157: BID ($p=0.019$) and BIM ($p=0.007$).
  - 93A: Bad ($p=0.02$), BIM ($p=0.001$), C3($p=0.015$) and p53 ($p=0.004$).
  - 93B: Bad ($p=0.002$), Bax ($p=0.020$), BID ($p=0.001$), p27 (0.004), p53 (0.022).
- Cell line 93A expressed the highest protein signal intensity for pro-apoptotic proteins with SB treatment in comparison to MDA-MB 157 and 93B.

References

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