Graduate Students
Population, Health & Wellness

Sanguinarine mediated anti-tumor activity via targeting JAK/STAT3 pathway in thyroid cancer

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ABSTRACT:

Sanguinarine (SNG), a natural compound with an array of pharmacological activities, has promising therapeutic potential against a number of pathological conditions, including malignancies. This research aimed to investigate the antiproliferative and anti-cancer potential of SNG against two well characterized papillary thyroid cancer (PTC) cell lines, BCPAP and TPC-1 .In both cell lines , SNG was able to inhibit cell proliferation in time and dose dependent manner. Western blot analysis revealed increased expression of apoptosis and autophagy markers , caspase-3,cleaved caspase-3 , P62, and LC3. SNG modulate its anticancer effect through ROS production, because NAC was able to reverse SNG effect. Interestingly, co-treatment of PTC with SNG and cisplatin amplified anticancer activity. Finally, SNG treatment of PTC spheroid suppressed its growth with downregulation of stemness markers including ALDH2 and SOX2 markers. In conclusion, SNG enhanced the anti cancer activity against PTC cells and the effect is amplified when cisplatin is added.

INTRODUCTION:

Thyroid cancers are the most malignant cancer of the endocrine system with papillary thyroid carcinoma (PTC) is one of the most important malignant thyroid cancer type (Ahn, 2014). There are three types of thyroid follicular epithelial-derived cancers include papillary (PTC), follicular (FTC), and anaplastic cancer. Both PTC and FTC are well differentiated type of cancers whereas anaplastic thyroid cancer is the rarest type and is poorly differentiated (Atashrazm, 2015). Papillary carcinoma is the predominant histological type followed by follicular carcinoma in both genders. Among female, Qatar has the highest incidence with an age standardized incidence rate of 13.5 per 100,000 during period 1998-2002 (Al-Zahrani & Ravichandran, 2007). The aim of this study is to investigate the antiproliferative and anti-cancer potential of SNG against two well-characterized PTC cell lines.

MATERIAS & METHODS:

Cell culture: BCPAP, TPC1 cells were cultured in RPMI 1640 medium supplemented with 10% (vol/vol) fetal bovine serum (FBS), 100 U/ml Penicillin and 100 U/ml Streptomycin at 37^{0}C in humidified atmosphere containing 5% CO2. **Cell viability**: Cell viability experiments were performed following treatment with curcumin for 24 hours using MTT assay. Apoptosis: **Apoptosis** was measured using fluorescent annexinV/PI staining and analyzed by flow cytometry. **Western blot**: Following treatment with curcumin and other compounds, cells were lysed and proteins were isolated. Equal amounts of proteins were separated by SDS-PAGE, transferred to PVDF membranes and probed with specific antibodies**. Statistical Analysis:** Results were analyzed by using one-way ANOVA followed by a paired Student's t-test using the software GraphPad Prism Values of * p < 0.05 were considered statistically significant.

RESULTS

1. SNG inhibits proliferation/cell viability of PTC cells dose dependently..
2. RTCA findings confirms that SNG mediated growth inhibition of PTC cells is time and dose dependent.
3. SNG treatment downregulates constitutive expression of JAK/STAT3 in PTC cells.
4. SNG mediated cell death occurs through activation of caspases and induction of double strand breaks.
5. z-VAD-FMK, a pan-inhibitor, partially prevents SNG mediated cell death suggesting involvement of caspases-cascades in SNG mediated apoptosis.
6. SNG induced molecular changes leading to apoptosis or growth inhibition is due to ROS involvement.
7. Autophagy induction in PTC cells when treated with SNG.
8. SNG attenuated stemness potential of PTC cells.

Results

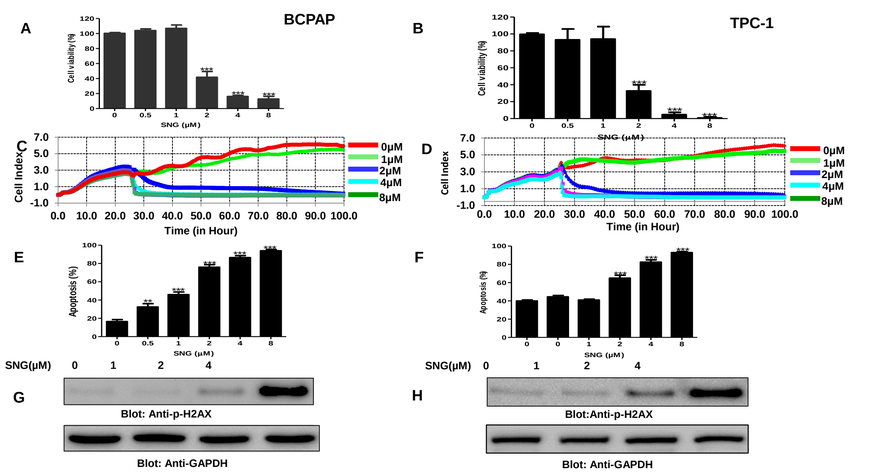

Figure-1: SNG-mediated inhibition of cytotoxic effects in PTC cells

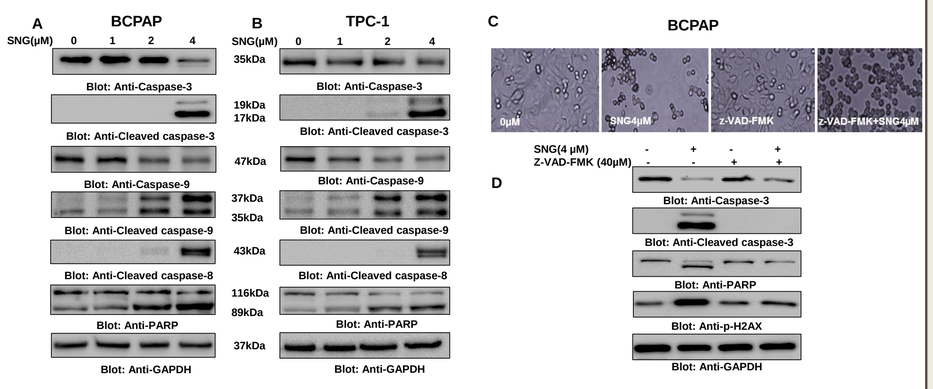

Figure-2: SNG mediated activation of caspase cascade in PTC cells.

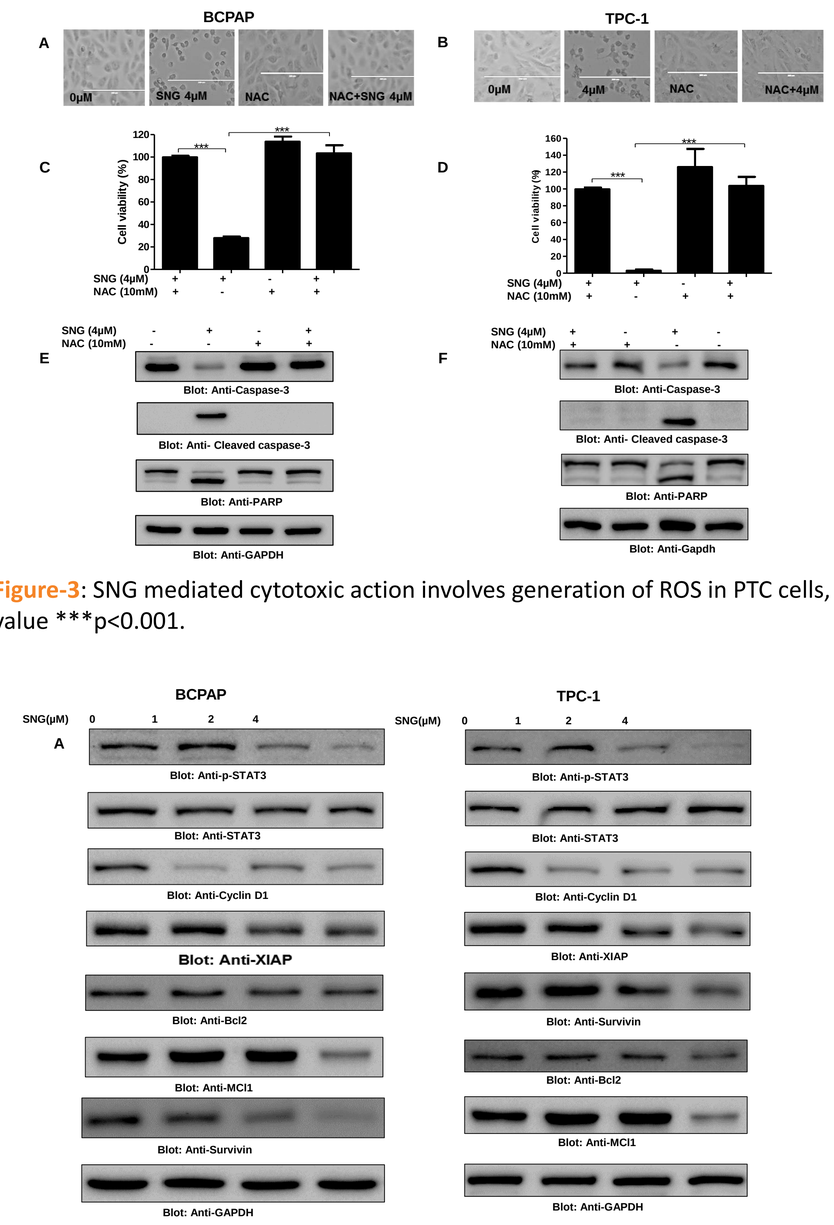

Figure-3: SNG mediated cytotoxic action involves generation of ROS in PTC cells, p value ***p<0.001.

Figure-4: SNG suppresses constitutively activated Stat3 signaling pathway.

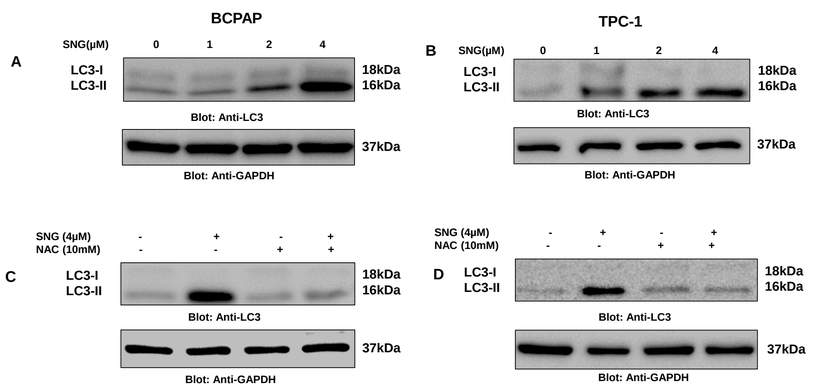

Figure-5: Induction of autophagy in PTC cells treated with SNG.

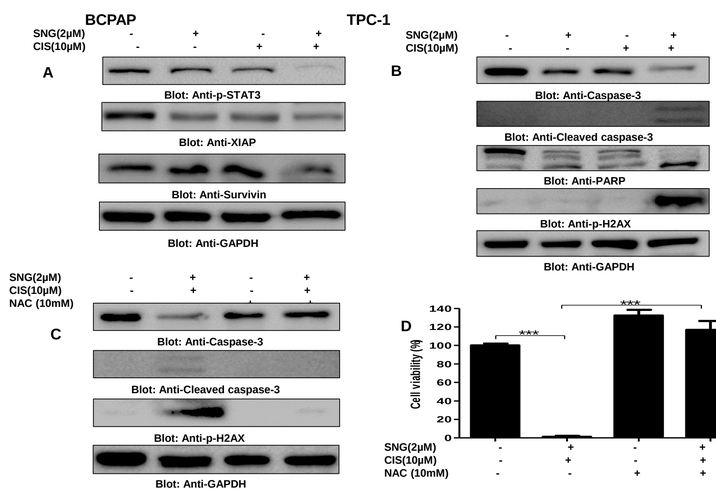

Figure-6: Sanguinarine sensitized PTC cells to anticancer drug cisplatin and enhanced apoptosis via inactivation of STAT3 and its associated proteins, p value ***p<0.001.

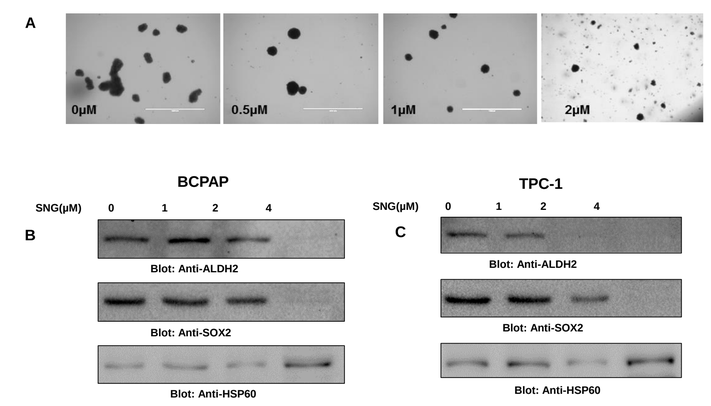

Figure-7: SNG-treatment inhibited thyrosphere formation of PTC cells.

CONCLUSION

In conclusion, overall findings of the present study showed the anticancer potential of SNG against PTC cells. SNG inhibits PTC cell proliferation and growth through ROS mediated apoptosis and autophagy most likely via downregulation of STAT3. SNG also inhibited the thyroid cancer stemness potential and sensitizes PTC cells with chemotherapeutic drug cisplatin.

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