



Uvaria afzelii (UA) Induce Tumor Suppressor Proteins and Induction of ROS in SK-N-SH Human Neuroblastoma Cells

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Introduction

- Neuroblastoma is a rare cancer caused by a genetic mutation which leads to uncontrolled division of immature nerve cells. In the United States, there are around 800 incidences of neuroblastoma per year, which accounts for 50 percent of all cancer in infants younger than 1 year old.
- In the literature, it is reported that *Uvaria afzelii* (UA) is one of many species belonging to the Annonaceae family that exhibits a degree of antineoplastic activity.
- UA is a small tree or spreading shrub found in West tropical Africa known for many healing properties including antitussive, pyrexia, dyspnea, respiratory tract infections, antimicrobial, inflammation/infection of the liver, kidney and bladder.
- This investigation studies the effect of UA on SK-N-SH human neuroblastoma cell lines to analyze their dose-effect on the tumor cellular growth, death, and other cell signaling pathways.

Objectives of the Study

- To investigate which cell signaling cascade that were effected by UA i.e causing programmed cell death "apoptosis", cell cycling arrest or other pathways in neuroblastoma SKNSH cell line.
- To determine if there is a therapeutic potential for the use of UA in inhibiting neuroblastoma growth.

Materials

- SK-N-SH cell lines were purchased from the American Type Culture Collection (ATCC, USA). Fetal bovine serum (FBS), penicillin/streptomycin (P/S), DMEM cell mediums were purchased from VWR.
- UA was obtained from Dr. Omonike Ogbale lab. Primary and secondary antibodies from Cell Signaling Technology (Denver, MA). Acrylamide/Bis solution 29:1, PVD membrane, Bradford Reagent, EC solution from BioRad (Hercules, CA).

Methods

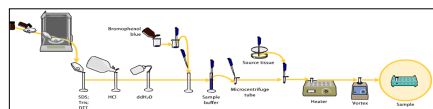
Cell Culture: Cells were grown in DMEM with L-glutamine supplemented with 10% FBS and 0.5% P/S and maintained in a monolayer at 37° C with 5% CO₂

Protein Extraction: Cells were extracted using RIPA buffer. Lysate was then prepared using a thawing and freezing method. The top layer containing protein was collected in a microfuge tube after being centrifuged at 12,000 RPM at 4° C for 30 minutes.

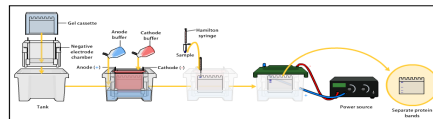
Determination of protein concentration: Using Bradford Protein Assay Reagent, the protein was measured using SpectraMax M3 plate reader, absorbency was read at 595 nM.

Western Blot: Equivalent amounts of protein from each sample were loaded onto 10% polyacrylamide gel. Electrophoresis of the gel was preformed using PowerPac200 system. The proteins were then transferred to nitrocellulose membranes. The membranes were then blotted using 5% milk and treated with primary and secondary antibodies.

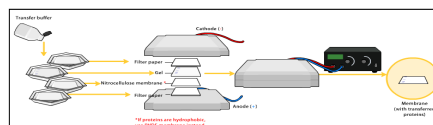
1. Tissue preparation



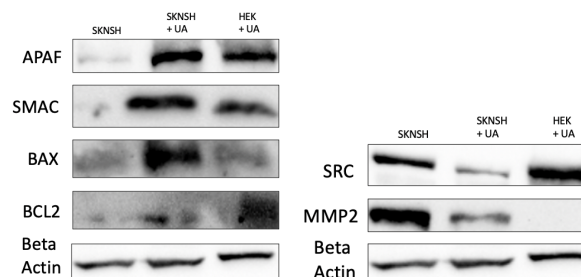
2. Gel Electrophoresis



3. Transferring protein from gel to membrane



Results



Discussion

- Apaf-1 is a protein involved in apoptosis. It was upregulated in SK-N-SH cells after treatment with UA indicating UA's promising effect in cancer by causing cell death.
- Smac/DIABLO is a protein that induces apoptosis and enhances chemotherapeutic sensitivity to antineoplastics. This protein expression increased in SK-N-SH cells after UA treatment.
- Generation of intracellular ROS (reactive oxygen species) plays a crucial role in apoptosis. ROS is also important for the modulation of pro-apoptotic Bax and anti-apoptotic Bcl2 proteins. UA treatment caused up-regulation of Bax and down-regulation of Bcl2 which plays an important role in ROS mitochondrial apoptotic pathway activation.
- Src is a proto-oncogenes that plays a critical role in the signal transduction pathways that control cancer cell development and growth. Src protein expression was less expressed in SK-N-SH cells after UA treatment.
- The polygenetic variants of MMPs play a critical roles in the progression and development of cancer through their effect of causing angiogenesis. MMP2 expression was down regulated after treatment of UA in SK-N-SH cells.

Bibliography

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