Characterization of the Effect of Aspirin on the PI3K-Akt Pathway and the Unfolded Protein Response during Endoplasmic Reticulum Stress

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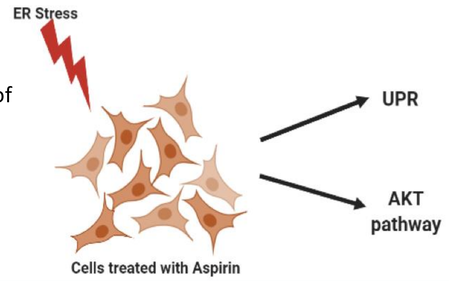
Abstract/Introduction:

The endoplasmic reticulum (ER) is a eukaryotic organelle that regulates various cellular processes such as protein synthesis, folding, and trafficking. Perturbations to ER function results in the accumulation of misfolded proteins within the ER lumen and subsequent ER stress. Upon induction of ER stress, cells activate both the Unfolded Protein Response (UPR) and the Phosphatidylinositol 3-Kinase (PI3K)-Akt pathway to attempt to restore cellular homeostasis. However, if the stress becomes chronic and homeostasis is not achieved within a reasonable timeframe, cells commit to apoptosis. Cancer cells are known to withstand and overcome ER stress better than regular cells. Sodium salicylate (NaSal), a metabolic derivative of Aspirin, is a non-steroidal anti-inflammatory drug that has been long purported to have chemo-preventive effects. However, the mechanism by which aspirin does so remains elusive. We hypothesize that the chemo-preventive effects of sodium salicylate may be mediated by modulation of UPR and PI3K-Akt signaling. In this experiment, we treated HeLa (cervical cancer) and HEK293 (human embryonic kidney) cells with NaSal and tunicamycin, an ER stress inducer, and analyzed the cellular lysates for markers of UPR and PI3k-Akt activation.

Hypothesis:

The chemo-preventive effects of aspirin is regulated by the UPR and PI3K-Akt Pathway.



Materials and Methods:

Cell Culture: Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), and Penicillin, Streptomycin, and Glutamine. Cells were maintained in a humidified atmosphere containing 5% CO_2 at 37°C.

Drug Treatments: Cells were treated with Sodium Salicylate and ER stress was induced by treatment with Tunicamycin (10 µg/mL), which inhibits N-linked glycosylation, 6h before cell lysis.

SDS-PAGE and Western Blotting: Samples were electrophoretically separated by molecular weight on a 10% SDS-PAGE gel, transferred to a PVDF membrane, blocked in 5% dry milk, and probed with primary and fluorescent secondary antibodies. All western blots were detected using a Li-COR Odyssey Fc and processed using Image Studio Lite.

The PI3K-Akt Pathway:

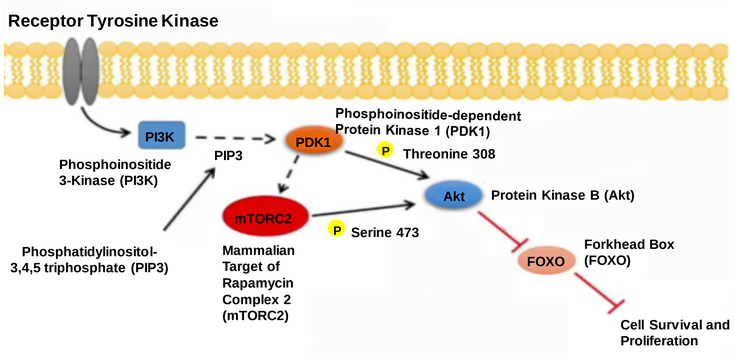


Figure 1. Schematic of the PI3K-Akt pathway.

The Unfolded Protein Response:

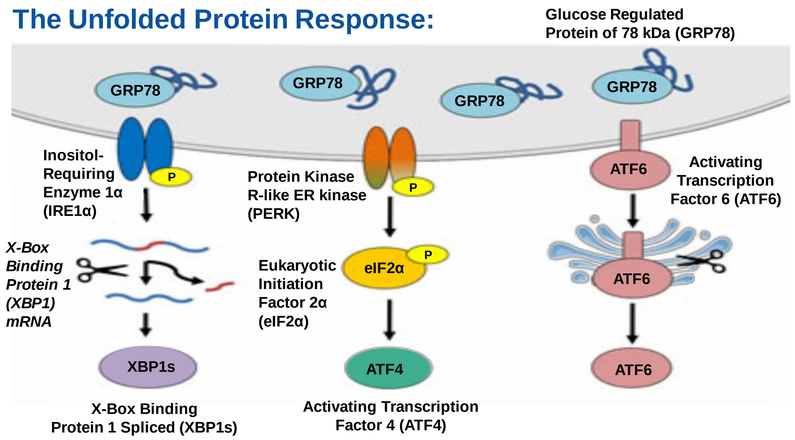


Figure 2. Schematic of the Unfolded Protein Response.

Results:

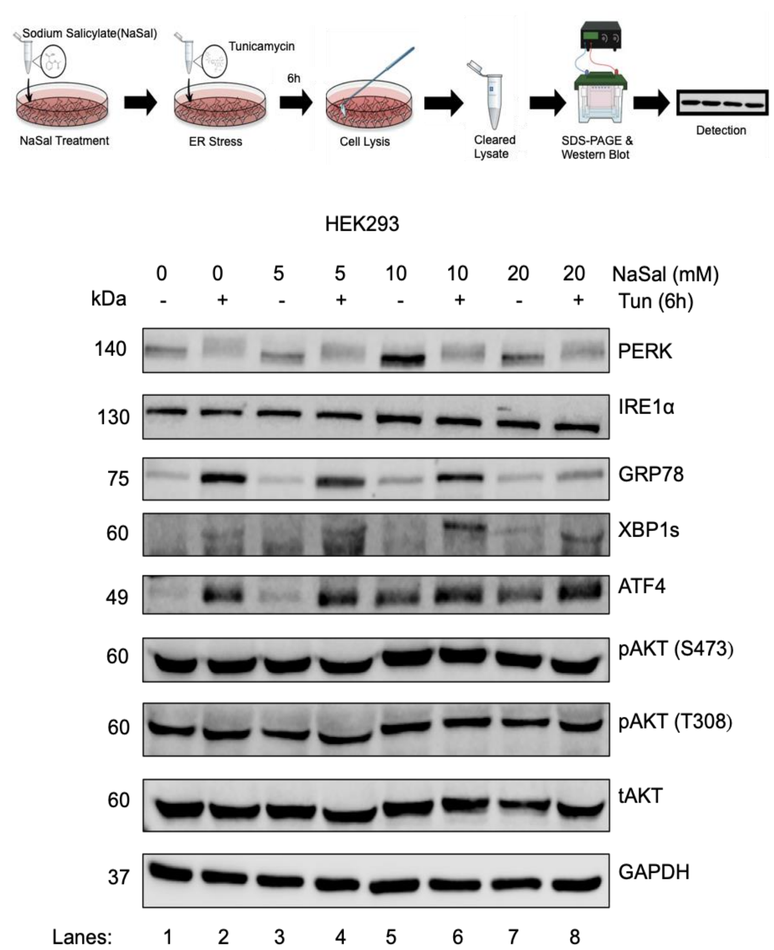


Figure 3. Lysates of HEK293 cells treated overnight (16hrs) with Sodium Salicylate and stressed with Tunicamycin were analyzed by western blotting. GAPDH serves as the loading control.

Results (continued):

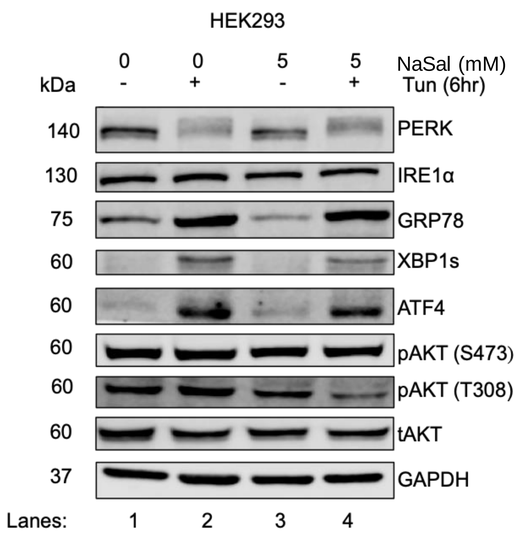


Figure 4. Lysates of HEK293 cells treated for 14 days with Sodium Salicylate and stressed with Tunicamycin were analyzed by western blotting. GAPDH serves as the loading control.

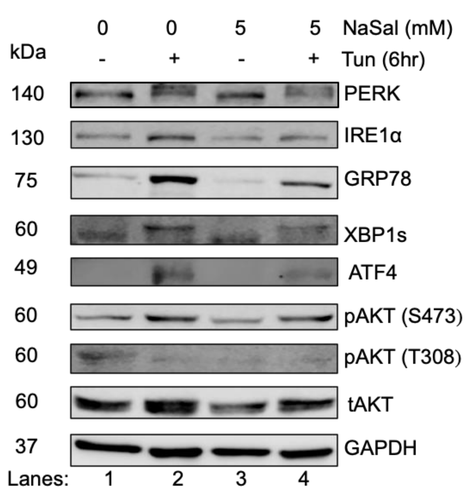


Figure 5. Lysates of HeLa cells treated for 24 days with Sodium Salicylate and stressed with Tunicamycin were analyzed by western blotting. GAPDH serves as the loading control.

Conclusions:

- NaSal (20mM) decreases expression of GRP78 and increases expression of ATF4, in HEK293 cells.
- Long term NaSal (5mM) treatment decreases expression of GRP78 in HeLa cells, otherwise not observed in HEK293 cells.

Future Directions:

- Replicate experiments and quantify data for statistical analysis.
- Conduct experiments on colorectal cancer cell lines.

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