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Effect of Bak and Bax on Oxidative Stress in A549 Cells

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Monitoring the Effect of Bak and Bax on Oxidative Stress in A549 Cells in the Presence of Trichostatin A

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ABSTRACT

The BCL-2 pathway, known for its role in regulating apoptosis, has been extensively studied since the discovery of its function in 1988 [1]. Importantly, abnormalities in the function of the BCL-2 pathway have been linked to several diseases including cancer and neurodegenerative diseases [2]. Despite new insights into the role of BCL-2 proteins within and beyond the intrinsic apoptosis pathway, much remains unclear or debated about the function of specific BCL-2 proteins, such as Bak and Bax. Bak and Bax are two proteins within the BCL-2 family of proteins that play a role in mitochondrial outer membrane permeabilization (MOMP), leading to the release of cytochrome c from the mitochondria [2]. Cytochrome c then activates a cascade of caspases 3 and 9 that lead to the activation of proteases [2]. These proteases break down cellular structures leading to cell death [2]. One thing that is unknown about Bak and Bax is their contribution to oxidative stress within cancer cells treated with chemotherapy. One such agent is the anti-cancer drug Trichostatin A (TSA). TSA is a histone deacetylase inhibitor (HDACi). TSA arrests cancer cells in the G2/M phase in a mitochondrial derived reactive oxygen species (ROS) dependent matter leading to apoptosis [3]. Testing the effect of Bak and Bax on oxidative stress in the presence of TSA can shed new light on the functions of Bak and Bax. This can lead to a new understanding of how Bak and Bax interact with different parts the cell and could lead to a new mechanistic model for Bak and Bax, which can be tested in future projects, including therapeutic design. Thus, I decided to monitor how Bak and Bax modify oxidative stress within the BCL-2 pathway in A549 cell treated with Trichostatin A.

BCL-2

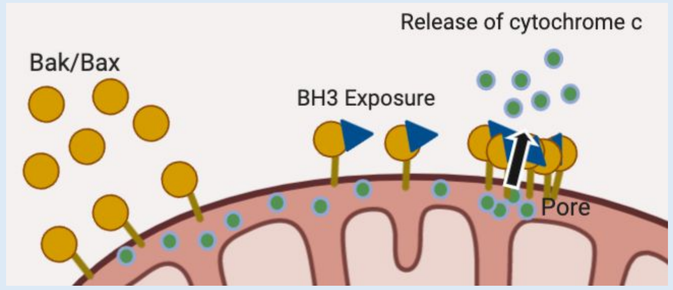

Figure 1. Bak and Bax in the BCL-2 pathway. The BCL-2 pathway is comprised of pro and anti-apoptotic proteins.[6] Bak and Bax are pro-apoptotic proteins (gold) that interact with the mitochondrial outer membrane (MOM). Bak and Bax attach to the MOM and are activated through the binding of BH3 (blue) so they can permeabilize the MOM.[7] This leads to the release of cytochrome c (green), initiating a caspase cascade that leads to cell death.[8,9]

METHODS

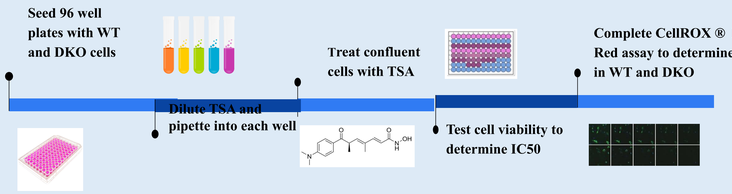

Figure 2. Treatment of Wild-Type and Double-Knockout A549 cells with Trichostatin A. A cell viability assay was performed using WT and DKO A549 cells treated with varying concentrations of TSA. An IC_{50} value was then obtained for each cell line. A CellRox® assay was performed to determine the overall oxidative stress in both the WT and DKO A549 cells. Images of WT and DKO A549 were then taken using fluorescent microscopy and used to calculate the fluorescent intensity per pixel.

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BCL-2 Pathway protein **Bak** may decrease **oxidative stress** in A549 cells when treated with the HDACi **TSA**.

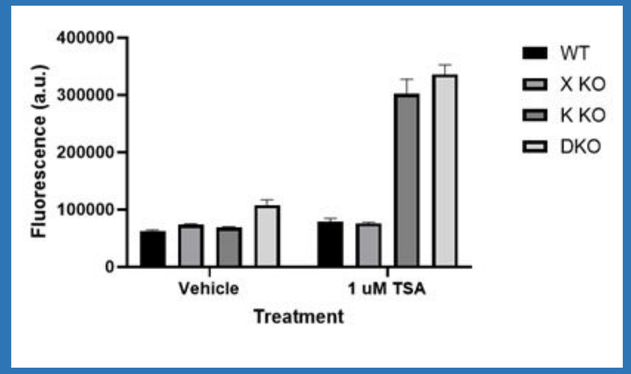

Figure 2: Normalized Fluorescence of Wild-type and Knockout Cell Lines in Presence of 1 uM TSA. The bak knockout and double knockout cell lines showed increased fluorescence over vehicle controls, while the wild-type and bax knockout showed no significant increase over the vehicle controls. This suggests that the absence of bak increases oxidative stress or bak decreases oxidative stress. The oxidative stress was monitored post 24 hours utilizing the CellROX® Deep Red reagent and fluorescent microscopy and normalized using an SRB assay.

IMPACT OF TSA ON A549 and DKO

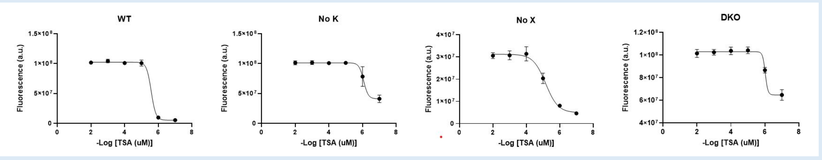

Figure 3. Fluorescence (a.u) of Wild-Type, Bak-Knockout, Bax-Knockout, and Double-Knockout A549 Cells in the Presence of Trichostatin A. The corresponding EC_{50} values were 38 nM, 116 nM, 14 nM, and 105 nM respectively.

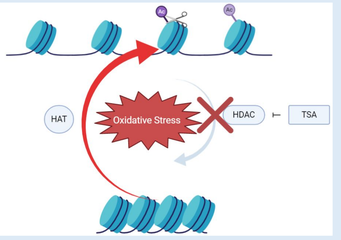

Figure 4. Trichostatin A inhibit HDACs. HDACs and HATs play opposing roles in the nucleus. HDACs cleave acetyl groups allowing DNA to condense, while HATs add acetyl groups to DNA, expanding the DNA and allowing for transcription.[10] TSA inhibits HDAC, causing an imbalance between HDACs and HATs leading to oxidative stress in the cell.

MONITORING OXIDATIVE STRESS

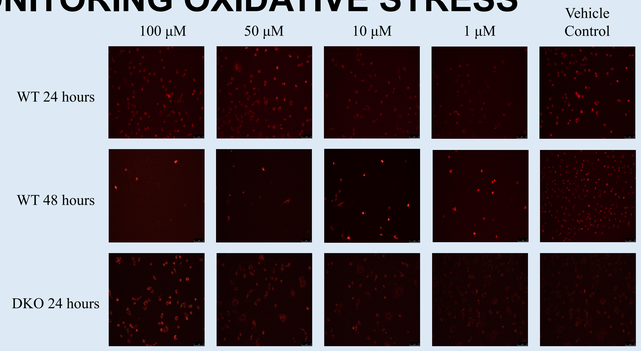

Figure 5. Monitoring the impact on oxidative stress upon treatment with a dose response of chemotherapy to wild-type and modified lung carcinoma cells. Wild-type and double-knockout (DKO) A549 cells were treated with a range of TSA, a chemotherapy agent, concentrations (1-100 µM) and a vehicle control. TSA and incubated for either 24 or 48 hours. The oxidative stress was monitored post 24 hours and 48 hours utilizing the CellROX® Deep Red reagent and fluorescent microscopy (20X objective, DMi8 Leica filter Ex. 644nm Em. 665nm, 100ms exposure). Further analysis will yield a change in oxidative stress as a result of the chemotherapy agent providing insight for future treatments and understanding of the BCL-2 pathway.

FUTURE INVESTIGATIONS

Monitor OS in DKO A549 cells incubated with TSA for 48 hours	Monitor OS in Bak knockout and Bax knockout A549 cells	Apply a co-chemotherapy treatment with TSA	Bak and Bax impact on OS in additional cancer cell lines

Figure 6. Further investigation of the impact of Bak and Bax on oxidative stress and chemotherapy treatment. Further work could include testing HDAC activity to see if Trichostatin A is acting as a true HDAC inhibitor. This will also allow for novel comparison investigations with other chemotherapy treatments. Additionally, it has been shown that Bak plays a role in peroxisome permeabilization [4]. It is possible that the release of catalase from the peroxisome. following permeabilization from Bak could decrease reactive oxygen species in the cytosol. This hypothesis is consistent with the results obtained. Further research could confirm this. It is also possible that reduced peroxisome biogenesis could increase oxidative stress by disrupting normal cellular metabolism. However, this hypothesis does not appear to be supported. In addition, we could test different cell lines to see if they respond in a similar fashion to treatment with Trichostatin A.