Abstract

Our goal is to investigate the effect of Panobinostat on the level of FACT complexes in malignant neuroblastoma cells. Neuroblastoma is an extracranial childhood malignancy which is developed from progenitors of nerve cells in children. Current chemotherapy of neuroblastoma is known to cause long term side effects such as a decrease in cognitive skills, and predisposition to secondary cancer. Panobinostat is an Histone deacetylase (HDAC) inhibitor, which is currently approved for the treatment of multiple myeloma. CBL0137 is novel anti-cancer agent, an inhibitor of histone chaperone FACT, which is highly overexpressed in neuroblastoma. Previous research studies show that combined treatment of mouse with neuroblastoma with Panobinostat and CBL0137 increased survival rate, induced rapid tumor regression, and increased apoptosis in tumor cells to a greater degree than Panobinostat and CBL0137 alone. Due to the increased efficacy of CBL0137 treatment in combination with Panobinostat we asked if Panobinostat may also act on the FACT complex to promote apoptosis of aggressive tumor cells. In order to test the effect of Panobinostat on the FACT complex, I treated 2 aggressive neuroblastoma cell lines, Be2C and Kelly, with a different doses of Panobinostat and measured protein level of each subunit of the FACT complex (SSRP1 and SPT16).

Results

- **Tissue Culture**
  - Cultured Be2C and Kelly human neuroblastoma cells in which Myc oncogene is overexpressed
  - Each cell line was plated in two 6-well plates, one for protein extraction and one for cell viability
  - Cells were treated with the following concentrations of Panobinostat for 24 hours: 0nM, 5nM, 10nM, 20nM, 40nM, 50nM

- **Western Blot**
  - I collected cell lysates from each sample using 200 µL of 1X CCLR lysis buffer
  - Standard western blot procedure was followed to identify and compare the protein level of SSRP1, SPT16, and Caspase 3 proteins from each cell lysates.
  - Antibody to beta-actin was used for the normalization of protein loading
  - Secondary antibodies conjugated with HRP was used to detect protein specific primary antibodies

- **Resourfin Assay**
  - Resourfin is a stain used for quantitative measurement for cell viability
  - Resazurin is added to samples, and in the presence of viable cells which can with active oxidative phosphorylation, Resazurin is irreversibly reduced into Resourfin which emits fluorescence.

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Conclusion and future plans

- SPT16 and SSRP1 levels decreased with increase in dose of Panobinostat.
- Total Caspase 3 (35kDa) protein levels decreased with higher dose of treatment, suggesting increased caspase 3 cleavage and apoptosis with higher doses of treatment
- Panobinostat treatment is more toxic to Kelly cells than Be2C cells.
- After observing decrease protein levels of SSRP1 and SPT16 upon Panobinostat treatment, I will be preforming RT-qPCR to determine if the decrease in protein levels of SSRP1 and SPT 16 is due to Panobinostat acting on the level of transcription of the FACT complex or from other possible variables such as Panobinostat causing degradation of FACT protein subunits

References

- Wikipedia, Histone Acetylation and Deacetylation.
- Children’s Cancer Institute, “Combination of CBL0137 and panobinostat is highly potent in vitro and in vivo for Diffuse Intrinsic Pontine Glioma” National Cancer Institute, “FACT Complex-targeting curasin CBL0137.”

Acknowledgement

- Special thanks to Dr. Gurova and lab members for dedication and mentorship
- University at Buffalo Honors College for research opportunity

Background

Histone chaperone FACT

There are multiple alterations in chromatin in cancer cells. FACT (Facilitates Chromatin Transcription Complex) is histone chaperone. It plays a critical role in interaction between the histones and DNA, by destabilizing the nucleosome structure. It functions by removing a H2A/H2B histone dimer from the nucleosome, promoting transcriptional elongation. Research studies show that FACT is overexpressed in aggressive tumor cells, but not expressed in normal cells, making it a promising target for development of anticancer drugs.

Panobinostat (HDAC Inhibitor)

Panobinostat is a HDAC inhibitor. HDAC is histone deacetylase, enzyme which removes acetyl group from histones. Acetyl groups make the nucleosome structure more open by making the histone more negative. DNA is a highly negative due to a phosphate group on the backbone. Adding a negatively charged acetyl group to histones through post-translational regulation loosens the interaction between histones and DNA, thus transforming chromatin to a more open state. Studies suggest blocking the removal of these acetyl groups with HDAC inhibitors results in greater opening of chromatin and is more toxic for tumor than normal cells.

Acetylation in histone alters nucleosome structure

Figure 1b: FACT complex removes H2A/H2B histone dimers

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Results

- **Western Blot results both Be2C and Kelly cells on levels of FACT subunit SPT16**

- **Western Blot results both Be2C and Kelly cells on levels of FACT subunit SSRP1**

- **Western Blot results both Be2C and Kelly cells on levels of Total Caspase3**

- **Western Blot results both Be2C and Kelly cells on levels of Actin**

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