ABCC4 as a Possible Target for Neuroblastoma Treatment

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Introduction

Neuroblastoma is a tumor that forms from the immature cells of the autonomic nervous system, and commonly occurs in very young children, with the median age of diagnosis being 17 months. [1] More than 50% of cases present as high risk disease, where long-term survival rates are below 50%. [2] The amplification of the MYCN oncogene is one of the prominent indicators of aggressive disease and is found in 20% of neuroblastoma cases. What is particularly problematic is the growing evidence that aggressive neuroblastomas are resistant to standard therapies such as chemotherapy or radiation. ATP-binding transporters such as ABCC4 are direct transcriptional targets of MYCN and are associated with therapeutic resistance.[3] Here, we investigate the IC50 concentration of Ceefourin 1 for the neuroblastoma cell lines.

Materials/Methods

Cell Culture of Neuroblastoma cells

The neuroblastoma cell lines CHLA15 and NB1643, where NB1643 contains the MYCN amplification were obtained and cultured in IMDM medium, supplement with 10% FBS, 1% ITS, and 100 U/ ml Penicillin-Streptomycin.

Drug testing in Cell Lines

The cell lines were incubated with IMDM media with Ceefourin 1 (ab145144; abcam), which interferes with the function of ABCC4 ATP-binding transporters, at concentrations of 1, 5, 7.5, 10, 20, 50, 100, 200, and 250 uM for 72 hours, and cell viability was assessed through Cell-Titer Glo viability assays (Promega). The cells were plated in triplicate in 96-well plates at 10,000 cells/well and treated with the drug at the indicated concentrations. Percent cell growth was normalized to 0.5% DMSO vehicle treated cells (set to 100%) as well as wells with the medium alone without viable cells. IC50 values were determined busing GraphPad Prism.

Result

The IC50 Values of CHLA15 and NB1643 were calculated to be 113.1 uM and NB1643 uM respectively. Cell viability remained high for lower concentrations of Ceefourin1, which was consistent with what was found in existing literature.

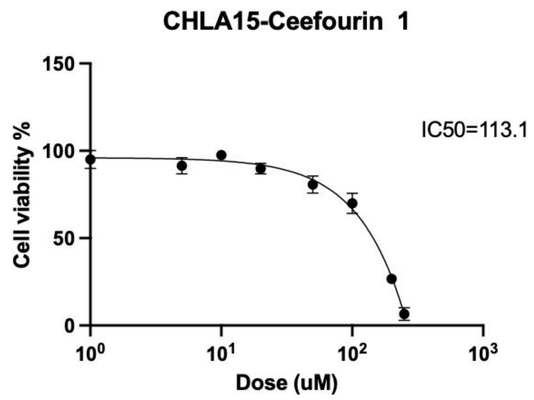

Fig 1. The IC50 curve for Neuroblastoma cell line CHLA15

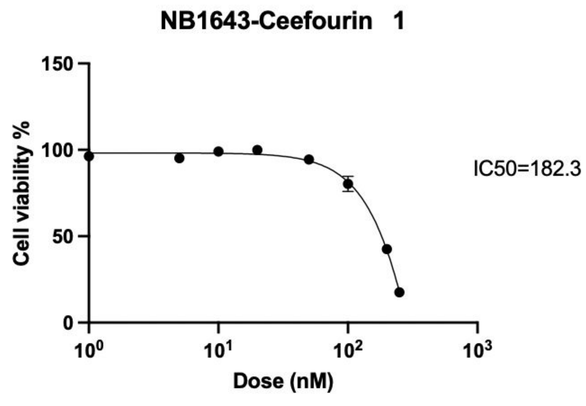

Fig 2: The IC50 curve for Neuroblastoma cell line NB1643.

Conclusion / Future Direction

The Ceefourin 1 IC50 concentration for NB1643, which contains the MYCN amplification, was greater, comparing with CHLA15 cell line. This indicates that NB1643 may be more resistant to the ABCC4 inhibitor treatment. This study shows that the inhibition of ABCC4 transporters does lead to cell death and decreased cell viability.

For future work, a drug combination experiment with the chemotherapy drugs, such as doxorubicin, can be performed in order to assess whether this targeted treatment can be used in conjunction with an existing chemotherapy treatment particularly against aggressive neuroblastomas.

References

[1] Colon NC, Chung DH. Neuroblastoma. Adv Pediatr. 2011;58(1):297-311.

[2]Matthay KK, Maris JM, Schleiermacher G, Nakagawara A, Mackall CL, Diller L, Weiss WA. Neuroblastoma. Nat Rev Dis Primers. 2016 Nov 10;2:16078.

[3] Huynh T, Norris MD, Haber M, Henderson MJ. ABCC4/MRP4: a MYCN-regulated transporter and potential therapeutic target in neuroblastoma.

Acknowledgments

This project, under the direction of Kai Tan and Kristina Cole, from the department of pediatrics at the Perelman School of Medicine and the Children's Hospital of Pennsylvania, along with Chia-Hui Chen is funded by the Penn Undergraduate Research mentorship Program.