The Effects of *N*-acetyltransferase 1 Gene Knockout on the Cytotoxicity of Pyrimidine Biosynthesis Inhibitors in Human Breast Cancer Cells

Afi H. Tagnedji, Kyung U. Hong and David W. Hein

Department of Pharmacology and Toxicology and James Brown Cancer Center, School of Medicine, University of Louisville

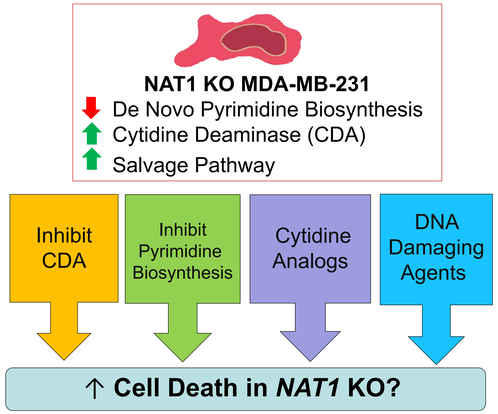
Introduction

Arylamine *N*-acetyltransferase 1 (NAT1) is a polymorphic drug metabolism enzyme that participates in detoxification and bioactivation of arylamines, arylhydrazines and similar compounds.

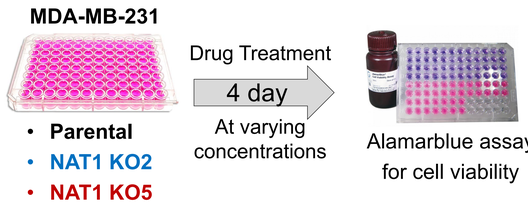
In recent years, it was discovered that **NAT1 is upregulated in breast cancer** [1]. However, how NAT1 contributes to breast cancer development and progression remains unclear. To develop novel hypotheses, NAT1 knockout (KO) cell lines (KO2 and KO5) were created from MDA-MB-231 (a triple-negative breast cancer cell line) using CRISPR/Cas9 technology.

According to our proteomics and RNAseq analyses, **NAT1 KO cells have increased cytidine deaminase (CDA)**, a player in the pyrimidine salvage pathway. Metabolomics data showed NAT1 KO cells had **defects in the *de novo* pyrimidine pathway**, which can explain the upregulation of the salvage pathway. Pyrimidine *de novo* synthesis and salvage pathways are essential for DNA and RNA synthesis and cell growth [2].

Hypothesis:

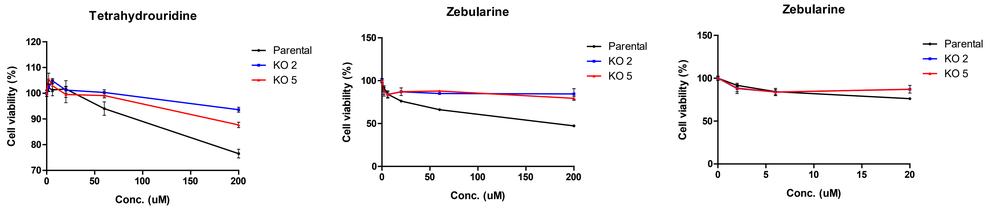


Method:



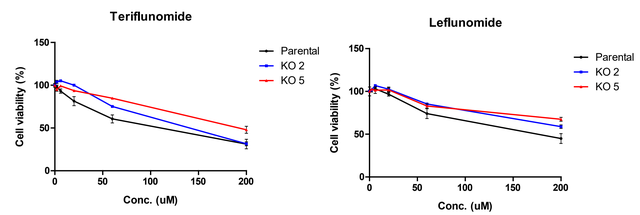
Results

CDA Inhibitors



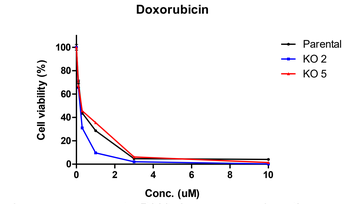
Rationale: CDA helps maintain the pyrimidine levels in the cells.[3] Pyrimidines such as cytosine, uracil (found an RNA) and thymine (found in DNA) are all essential for the growth and survival. Since NAT1 KO cells are more dependent on the pyrimidine biosynthetic salvage pathway, they should be more sensitive to cytidine deaminase inhibitors like tetrahydro uridine and zebularine.

Pyrimidine Biosynthesis Inhibitors



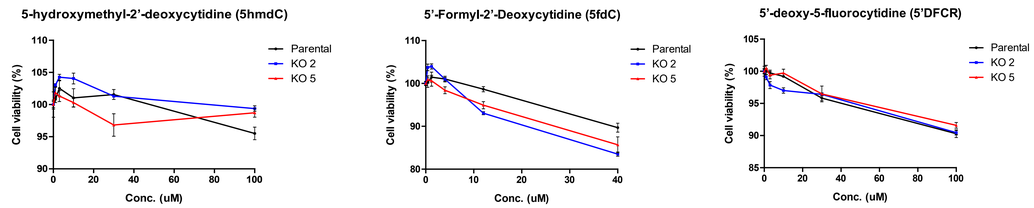
Reasoning: If there is defect in the pyrimidine biosynthetic pathway the NAT 1 breast cancer cells should have increased sensitivity pyrimidine biosynthesis inhibitors like leflunomide and teriflunomide

DNA Damaging Agent



Seeing as response to DNA damage requires the upregulation of the de novo synthesis pathway which we hypothesize to be down in knock out cells, we expect the cells to be more sensitive to DNA damage in agents such as doxorubicin[4]

Cytidine Analogs



Reasoning : Naturally occurring modified cytidines are poor substrates of the salvage pathway. In cells that express unusually high levels of CDA like NAT 1 KO's these modified cytidines are processed to uridine variants which are then incorporated into the DNA and may lead two DNA damage and cell death.[5]

Summary of Results

Drug Category	Compound	Expected Results (in NAT1 KOs)	Actual Results (in NAT1 KOs)	Statistical Summary
CDA Inhibitors	Tetrahydrouridine	↑Cell death	↓Cell death	No statistically significant variation between parental and NAT1 KOs **except** at 200 uM concentration . Statistically significant variation in cell types at p= 0.0002
	Zebularine	↑Cell death	↓Cell death	Statistically significant variation between parental and NAT1 KOs at concentrations above 60 uM Statistically significant variation n in cell types at p < 0.0001
Pyrimidine Biosynthesis Inhibitors	Teriflunomide	↑Cell death	↓Cell death	Statistically significant variation between parental and NAT1 KOs between 20 and 60 uM concentration Statistically significant variation in cell types at p < 0.0001
	Leflunomide	↑Cell death	↓Cell death	No statistically significant variation between parental and NAT1 KOs **except** at 200 uM concentration Statistically significant variation in cell types at p = 0.0006
DNA Damaging Agent	Doxorubincin	↑Cell death	Inconclusive	No statistically significant variation between parental and NAT1 KOs Statistically significant variation in cell types at p < 0.0001
Cytidine Analogs	5-Hydroxymethyl-2'-deoxycytidine (5hmdC)	↑Cell death	Inconclusive	No statistically significant variation between parental and NAT1 KOs Statistically significant variation in cell types at p = 0.0005
	5'- Formyl- 2'-Deoxycytidine (5'fdC)	↑Cell death	↑Cell death	Statistically significant variation between parental and NAT1 KOs above 12 uM Statistically significant variation in cell types at p = 0.0042
	5'-Deoxy-5-fluorocytidine (5'DFCR)	↑Cell death	Inconclusive	No statistically significant variation between parental and NAT1 KOs Statistically significant variation in cell types at p = 0.0021

Conclusions and Future Directions

Whereas the results with many of the drugs were inconsistent with our initial hypothesis, it is possible that the NAT1 KO cells are more resistant to CDA or pyrimidine biosynthesis inhibitors because of the upregulations of CDA.

The next step will be to first investigate inconsistencies in our hypothesis. What does Tetrahydrouridine, zebularine and teriflunomide have in common when in comes to their pharmacodynamics? This may help develop novel hypothesis and better direct future experiments.

Bibliography

[1] Carlisle, S. M., Trainor, P. J., Doll, M. A., Stepp, M. W., Klinge, C. M., & Hein, D. W. (2018). Knockout of human arylamine N-acetyltransferase 1 (NAT1) in MDA-MB-231 breast cancer cells leads to increased reserve capacity, maximum mitochondrial capacity, and glycolytic reserve capacity. *Molecular Carcinogenesis*, *57*(11), 1458–1466. https://doi-org.echo.louisville.edu/10.1002/mc.22869
[2] Samantha M Carlise, PhD and Jonathan Gardner, Unpublished Observations.
[3] Navaratnam, N., & Sarwar, R. (2006). An overview of cytidine deaminases. *International Journal of Hematology*, *83*(3), 195–200. https://doi-org.echo.louisville.edu/10.1532/IJH97.06032
[4] Adaptive Reprogramming of De Novo Pyrimidine Synthesis Is a Metabolic Vulnerability in Triple-Negative Breast Cancer. (2017). *Cancer Discovery*, *7*(7), 782. https://doi-org.echo.louisville.edu/10.1158/2159-8290.CD-17-0565
[5] Mark A. Doll , Unpublished observations.

Acknowledgements

This research was partially supported by the by USPHS grant R25-CA134283 from the National Cancer Institute .

Special thanks to the Hein lab of the Department of Pharmacology and Toxicology at the University of Louisville School of Medicine.