NUDT5 Is a Critical Growth Regulator of Triple-Negative Breast Cancer

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Background

Triple negative breast cancer (TNBC) lacks the expression of all three receptors, the estrogen receptor (ER), the progesterone receptor (PR) and the epidermal growth receptor 2 (HER2). This types of cancer is about 15% of all breast cancers and have poor clinical outcome. Previous Brown lab's publications have identified several phosphatases that are overexpressed in TNBC compared to the ER-positive breast cancers. NUDT5, one of these phosphatases, is part of NUDIX family.[1] This family eliminates toxic nucleotide metabolites along with regulating the concentrations of nucleotide substrates and signaling molecules. NUDT5 has a key role in ADP-ribose and 8-oxo-guanine metabolism.[2,3]

Hypothesis

Inhibition of NUDT5 will suppress the growth of triple-negative breast cancer cells.

Method

1. NUDIX Family Overall Survival Analysis and mRNA Expression Level Comparison: Using publicly available datasets (Curtis, Esseman and Kao), mRNA expression level of NUDIX family was compared across overall breast cancer and based on their ER status. Using the same datasets, a survival analysis of this family has been conducted which log-rank p values were used to determine the difference of NUDIX family as prognosis factor for the overall survival of breast cancer patients.
2. NUDT5 Protein Level Across ER-positive vs. TNBC: 2 normal cells (MCF10A and MCF12A), 4 ER-positive breast cancer cells (MCF7,ZCF7,MDA361 and T47D) and 7 TNBC cells (MDA231, MDA436, BT20, HCC1143, HCC1937 and HCC70) were used in this study.
3. Test Knockout Efficiency of iCas9-sgNUDT5 MDA231 Pool: Inducible NUDT5-Knockout TNBC cell lines, iCas9-sgNUDT5 MDA231, have been generated to test the growth suppression effect of NUDT5 on TNBC. These cells have been induced with Dox to knockout NUDT5. Then their growth has been measured by counting cell numbers over seven days. Lastly, the knockout efficiency is demonstrated by western blot.
4. Test Small Molecule Inhibitor TH5427 in Breast Cancer Cell Lines: MDA231 cells were used in this study to investigate the effect of small molecule inhibitor, TH5427. Their growth has been measured by counting cell numbers over seven days.

NUDT5 is a Member of the NUDIX Family

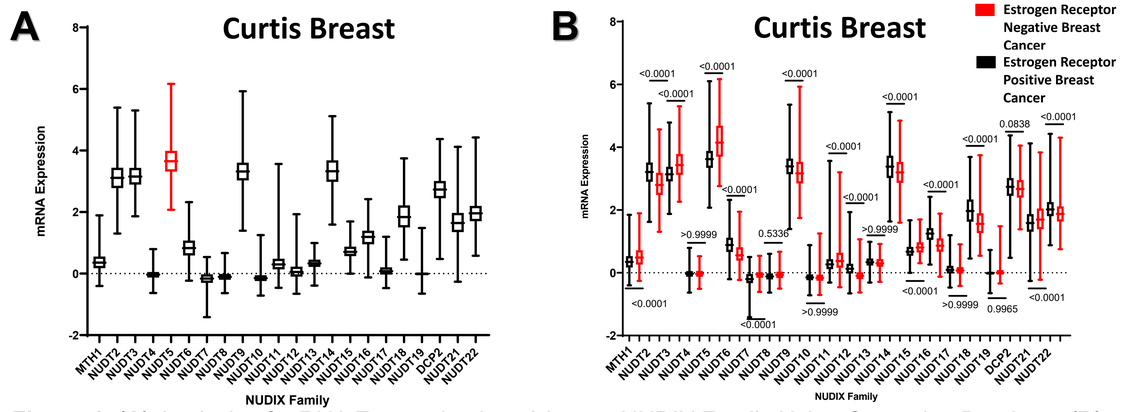


Figure 1. (A) Analysis of mRNA Expression Level Across NUDIX Family Using Oncomine Database **(B)** Analysis of mRNA Expression Level Based on ER Status Across NUDIX Family Using Oncomine

Survival Analysis of NUDIX Family

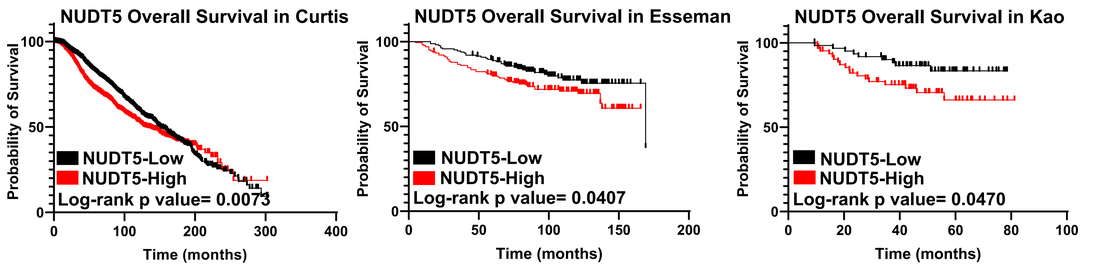


Figure 2. Kaplan-Meier Curves of Overall Survival in Breast Cancer Patients with High-NUDT5 (Red) and Low-NUDT5 Expression (Black) in Esserman, Curtis and Kao breast cancer datasets

NUDT5 Protein Level in Breast Cancer

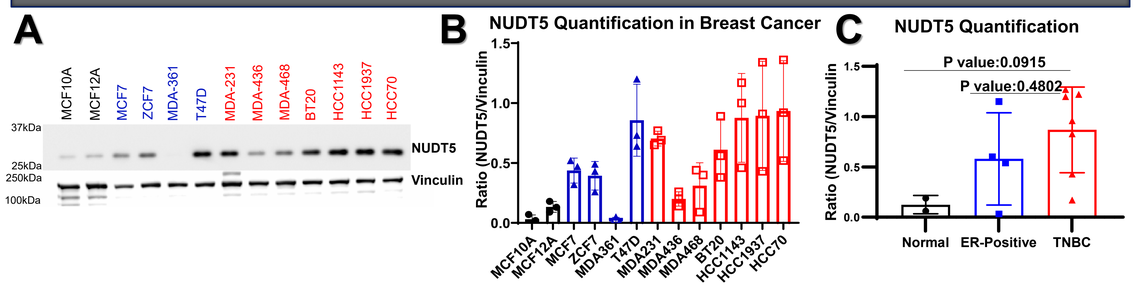
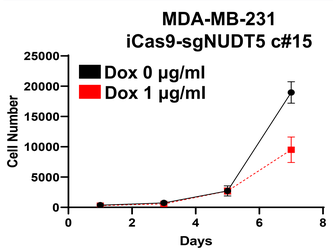


Figure 3. (A) NUDT5 Panel in Breast Cancer Cells by Western Blot **(B)** NUDT5 Quantification in Breast Cancer Cells **(C)** NUDT5 Quantification Based on the Type of the Breast Cancer

NUDT5 Knockout



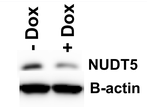
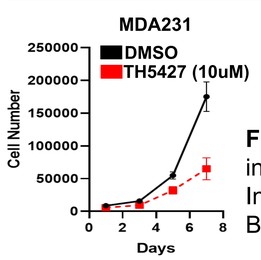


Figure 4. Testing the Knockout Efficiency of iCas9 sgNUDT5 MDA231 Pool

NUDT5 Inhibition



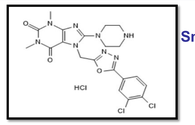


Figure 5. Testing NUDT5 inhibition by Small Molecule Inhibitor, TH5427 in MDA231 Breast Cancer Cells

Conclusion

1. NUDT5 has the highest mRNA expression level among other NUDIX family members in breast cancer. High NUDT5 expression level is correlated with poor overall survival in breast cancer.
2. NUDT5 has a trend of higher expression level in TNBC compared to the ER-positive breast cancer.
3. NUDT5 knockout and inhibition by a small molecule inhibitor reduces TNBC cell growth.

Future Direction

1. Determine the effect of NUDT5-knockout on TNBC growth via inducible NUDT5-knockout TNBC cell lines.
2. Investigate the biological mechanism of NUDT5 inhibition in TNBC to understand the suppressive effect of NUDT5 on TNBC growth.
3. Test the potential of NUDT5 inhibitors in preclinical animal models of triple-negative breast cancers.

Reference

[1]Qian J, et al . Abstract 1439: NUDT5 is a critical regulator of triple-negative breast cancer growth. In: Immunology. Vol. 80. American Association for Cancer Research; 2020. p. 1439–1439.
[2]Carreras-Puigvert J, et al. A comprehensive structural, biochemical and biological profiling of the human NUDIX hydrolase family. Nature communications. 2017; 8(1):1–17.
[3]Page BDG, Valerie NCK, Wright RHG, Wallner O, Isaksson R, Carter M, Rudd SG, Loseva O, Jemth A-S, Almlöf I, et al. Targeted NUDT5 inhibitors block hormone signaling in breast cancer cells. Nature communications. 2018; 9(1):250.
5. Yin L, Duan J-J, Bian X-W, Yu S-C. Triple-negative breast cancer molecular subtyping and treatment progress. Breast cancer research: BCR. 2020;22(1):61.

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