Role of Arylamine *N*-acetyltransferase 1 (NAT1) in Breast Cancer Growth and Metastasis

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Introduction

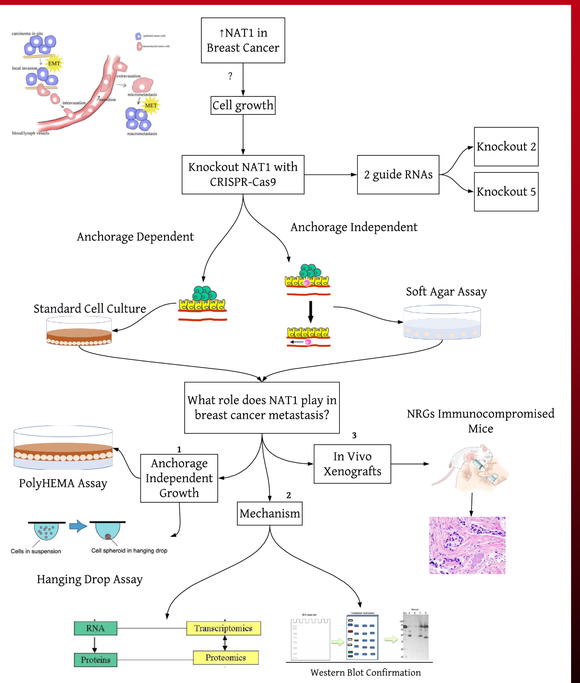
1. Metastasis from the primary solid tumor site is the leading cause of mortality in breast cancer patients.
2. In order for cancer cells to metastasize, they must undergo a complex process known as epithelial-to-mesenchymal transition (EMT).
3. Arylamine *N*-acetyltransferase 1 (NAT1) is key metabolic enzyme responsible for acetylation of arylamines for subsequent clearance in urine.

4. Recently, it was discovered that NAT1 is over-expressed in several forms of estrogen receptor positive breast cancer.
5. Knockout of NAT1 activity in several breast cancer cells lines causes cells to lose the ability to grow detached from a basement membrane (soft agar assay), which is a critical step in achieving metastasis and may implicate NAT1 having an effect on EMT.
6. For the above reasons, NAT1 is an exciting potential target for the treatment of metastatic breast cancer.

Hypothesis

Knockout of NAT1 was expected to decrease the anchorage independent growth, metastatic potential, and markers of epithelial-to-mesenchymal transition (EMT) in MDA-MB-231 (ER+/PR-/Her2-), MCF-7 (ER+/PR+/Her2-), and ZR-75 (ER+/PR+/Her2-) breast cancer cell lines.

Experimental Scheme



Results

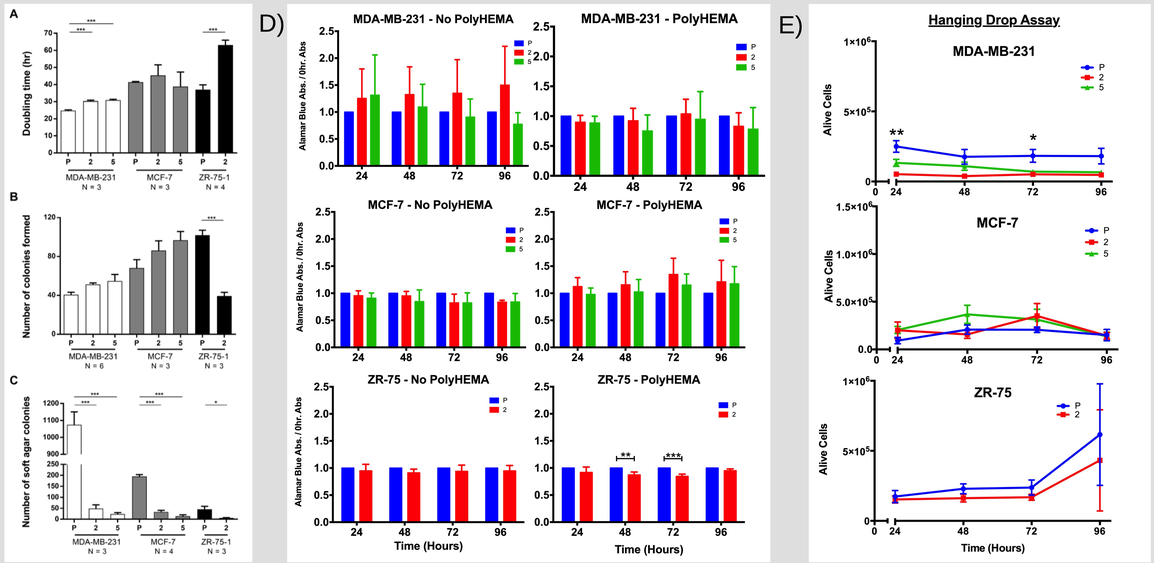


Figure 1 – Effects of NAT1 Knockout on Anchorage Independent Growth of MDA-MB-231, MCF-7, and ZR-75 Breast Cancer – A) Doubling time[1] **B)** Colony formation[1] **C)** Soft agar growth[1] **D)** PolyHEMA growth – P represents data from parental cells, 2 represents data from NAT1 knockout with gRNA #2 cells, and 5 represents data from NAT1 knockout with gRNA #5 cells **(same throughout poster) E)** Hanging drop. Three individual replicates were conducted (N=3) for all experiments and are represented as the mean ± SEM. Statistical significance was determined by one-way ANOVA followed by Bonferroni Post Hoc testing (3 or more comparisons) or student T-test for comparison of values. * (P<0.05), ** (P<0.005), *** (P<0.0005).

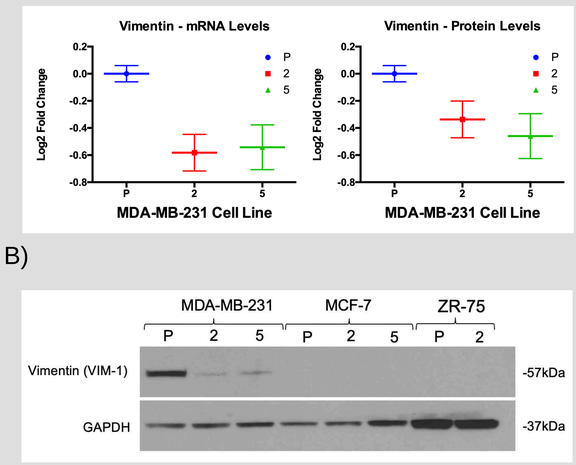


Figure 2 - Effect of NAT1 Knockout on Vimentin (EMT marker) Expression– A) Vimentin RNA and protein expression from transcriptomic and proteomic analysis of MDA-MB-231 breast cancer cells lysates. **B)** Western blot analysis of vimentin in breast cancer cells.

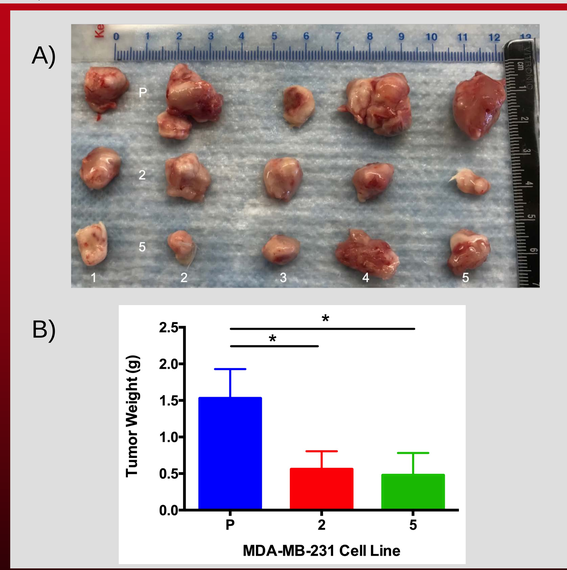


Figure 3 - Effect of NAT1 Knockout on MDA-MB-231 Xenografts – A) Harvested flank tumors from NRGs injections. (N=5) **B)** Mean flank tumor weight ± SEM. *(P<0.05) - one-way ANOVA and Bonferroni post hoc testing.

Assay	Cells Attached?	Cells Alone?	Cells Forced Together?
Doubling Time	+	-	-
Plastic/Colony Formation	+	+	+
Soft Agar	-	+	+
PolyHEMA	-	-	-
Hanging Drop	-	-	+

Table 1 – NAT1 Knockout Cell Growth Assay Characteristics

Conclusions

1. NAT1 knockout drastically decreases the ability of breast cancer cells to grow in an anchorage-independent manner (soft agar). However, does not effect growth in polyHEMA or hanging drop assays.
2. NAT1 knockout decreases expression of vimentin - a key marker of epithelial-to-mesenchymal transition.
3. NAT1 knockout results in impaired *in vivo* growth of MDA-MB-231 breast cancer in NRGs mice.

The results suggest that NAT1 contributes to breast cancer metastasis by potentially promoting anchorage-independent colony formation (soft agar). NAT1 may also drive metastasis of MDA-MB-231 breast cancer via increased expression of vimentin. The results suggest that NAT1 could serve as a potential treatment for metastatic breast cancer.

Clinical Significance

These studies suggest that arylamine *N*-acetyltransferase 1 (NAT1) may contribute to cancer metastasis and EMT and would potentially serve as an effective target for the treatment of metastatic breast cancer.

Future Work

Further study is necessary to understand the role of NAT1 on metastatic behavior of MCF-7 and ZR-75 cells injected into NRGs mice (experiments in progress).

References

[1] Stepp, M. W., Salazar-González, R. A., Hong, K. U., Doll, M. A. & Hein, D. W. N-Acetyltransferase 1 Knockout Elevates Acetyl Coenzyme A Levels and Reduces Anchorage-Independent Growth in Human Breast Cancer Cell Lines. *Journal of Oncology* **2019**, 1-11, doi:10.1155/2019/3860426 (2019).

Acknowledgements

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