

A Novel NSC Small Molecule Inhibitor, Inhibits Proliferation of Triple-Negative Breast Cancer Cells Through Upregulation of NR4A Family Genes



School of Medicine



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INTRODUCTION

Triple negative breast cancer (TNBC) is very aggressive, accounting for approximately 20% of clinical breast cancer (BC) subtypes found in patients. It is associated with early and advanced stages of the disease. TNBC patients harbor tumors that lack estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor type 2 (HER2) expression; therefore, these patients cannot undergo receptor targeted therapy. The lack of therapies for TNBC patients has intensified the need for more treatment options especially using chemotherapy agents such as small molecule compounds.

NSC007 was recently discovered after a screening of over 1000 small molecule drug compounds from the National Cancer Institute (NCI). Preliminary data show that NSC007 has anti-tumor effect on TNBC cell line, MDA-MB-231, and can activate the tumor suppressor gene family NR4A, especially NR4A2.

Here, we used bioinformatic analysis to analyze the gene expression levels of NR4A2 with the clinical data set from METABRIC (The Molecular Taxonomy of Breast Cancer International Consortium).

METHODS

Next generation sequencing (NGS)

NGS was used to measure the global gene expression levels in MDA-MB-231 cell line post 24hr treatment using NSC007. Data analysis show that NR4A gene family have the largest fold change amongst other genes.

NGS Validation

Preliminary *in vitro* research data show that: - NSC007 treated MDA-MB-231 cell line showed a dose-dependent increase in NR4A2 gene expression in a 24hr time point.

- NR4A2 gene expression was suppressed in BC cell lines and upregulated in normal cell line

Database Mining and Analysis

Raw data was mined and acquired for 2,000 clinically annotated BC patients from METABRIC. Next, bioinformatic analysis was done to analyze the data for specific criteria that was going to be evaluated. Briefly, data was checked for the gene expression levels of NR4A2 in: (A) cancer tissues and adjacent normal tissues in breast cancer patients (B), overall survival of patients (C), different subtypes of breast cancer (D), different tumor stages (E), in different cancer grades and (F), in the amount of positive lymph node. Graph Pad Prism was used for statistical analysis and data interpretation. A student t-test was performed to show the significances of results.

Next Generation Sequencing (NGS)

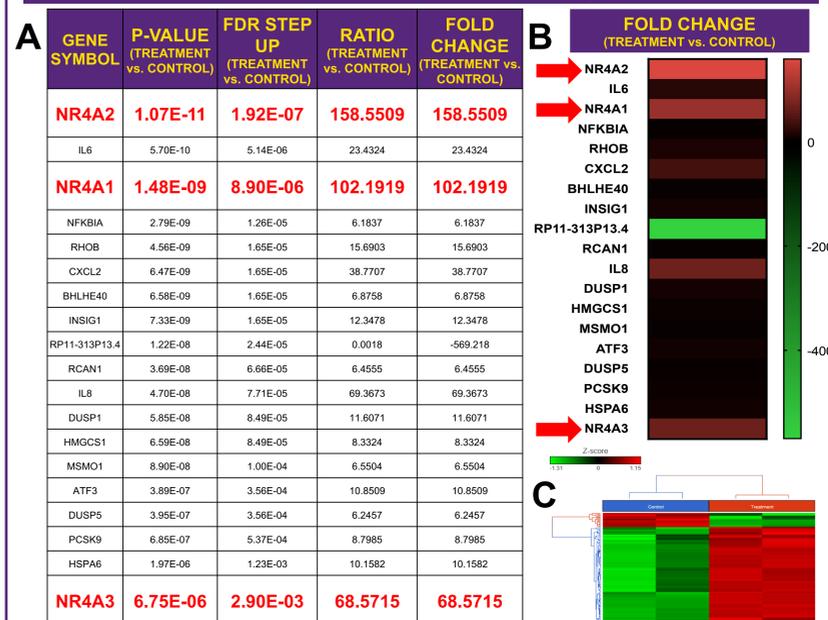


Figure 1. NGS data comparing the expression of genes in control vs. treatments. Experiment was done by Hassan Yousefi. **A)** A table showing 20 of the genes reported with corresponding fold change, highlighting that the NR4A family genes had highest fold change. **B)** A heat map used to interpret the changes that occurred in gene expression after treatment. **C)** A heat map illustrating the genes that were measured using NGS, done in duplicate.

NR4A2: An Overview

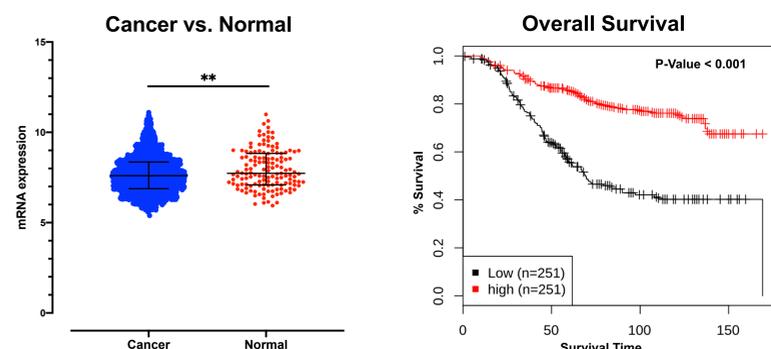


Figure 2. The expression of NR4A2 in cancer cells vs. normal cells. Graph shows that cancer cells have a significantly lower expression than normal cells with a p-value of 0.0038. Data was collected using METABRIC.

Figure 3. The overall survival of patients with high and low expression of NR4A2. Graph, provided by GENT2, showing that genes with low expression has a significantly lower survival than those with high expression (p-value of < 0.001). Data was divided by median expression.

RESULTS

NR4A2: Breast Cancer Subtypes

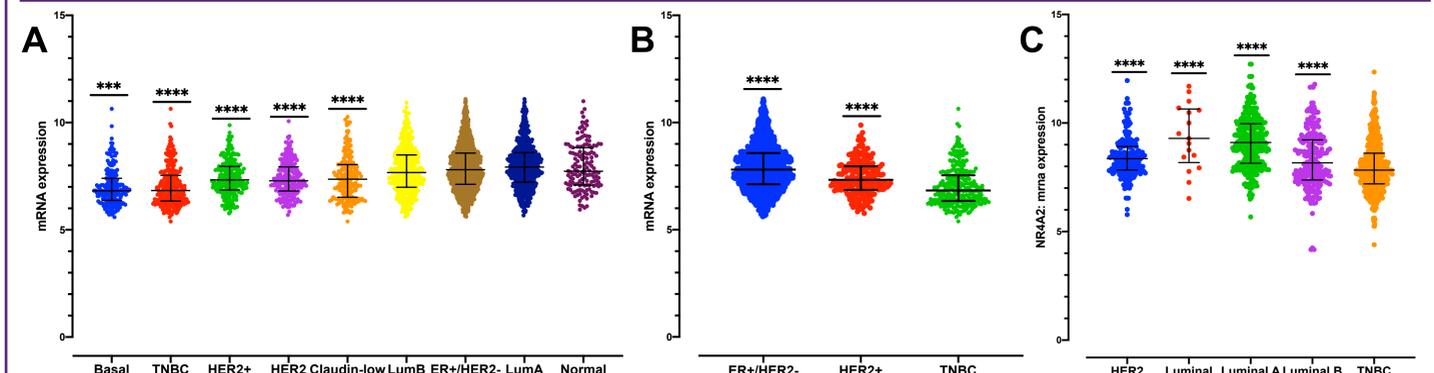


Figure 4. The gene expression of NR4A2 in different breast cancer subtypes. **A)** Gene expression in BC Subtypes compared to Normal cells using data from METABRIC. **B)** Gene expression in BC Subtypes showing that TNBC has a significantly lower expression of NR4A2 than other subtypes. **C)** Gene expression of Breast Cancer Subtypes using GENT2 to show consistency through different databases.

NR4A2: Cancer Classification

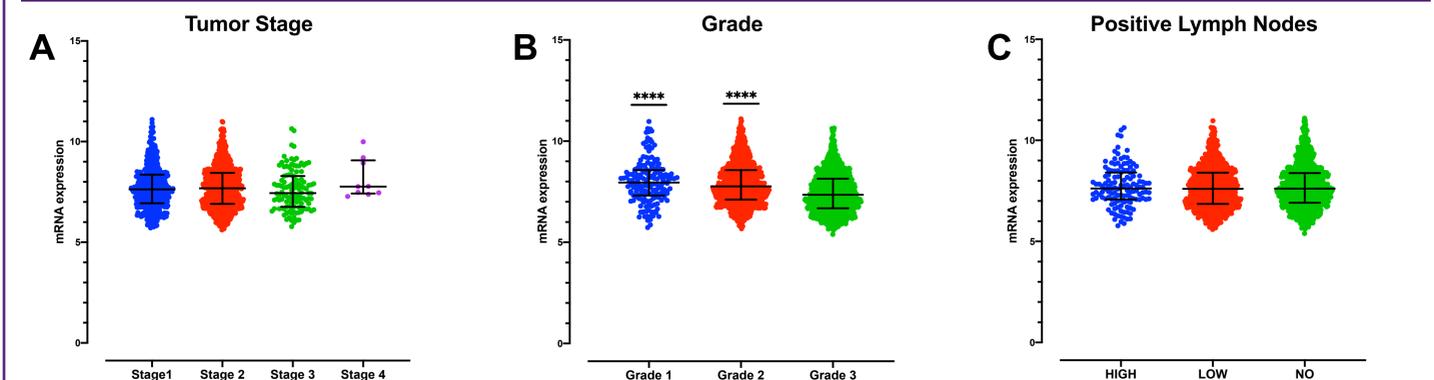


Figure 5. The gene expression of NR4A2 in Tumor Stage, Cancer Grades, and Amount of Positive Lymph Nodes. **A)** There was no significant difference in the gene expression between tumor stages **B)** Grade 3, which is more aggressive, had a significantly lower gene expression than other grades. **C)** Lymph nodes were categorized as low 1-7 nodes positive and high 8-23 nodes positive as defined from Broad Institute, and there was no significant difference.

CONCLUSION

Using bioinformatics and genomics we conclude that:

1. NR4A2 has a significantly lower gene expression in cancer cells than normal cells.
2. NR4A2 had the lowest expression in TNBC when compared to other subtypes.
3. Lower expression of NR4A2 led to a poor overall survival.
4. NR4A2 had the lowest expression in Grade 3, which is more aggressive compared to other grades.

Another drug, 6-Mercaptopurine (6-MP) has also been shown to increase the NR4A2 activity.

A combination of therapy with this NSC small molecule inhibitor and 6-MP could possibly lead to a significantly better prognosis for TNBC patients.

This research project was supported through the LSU Health Sciences Center, School of Medicine.