DAXX is a Novel Predictive Biomarker of Response to Anti-Notch plus Endocrine Therapy to Target Cancer Stem Cells in ER-Positive Breast Cancer

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Abstract

Background:

- Notch promotes cancer stem cells (CSC) and resistance to endocrine therapy (ET) in ER+ breast cancer.
- A pre-surgical window study using ET plus a γ-secretase inhibitor (GSI MK-0752) in ER+ breast cancer identified novel Notch genes.
- Global RNA expression profiling showed many genes to be downregulated by ET + GSI.
- Of the few genes (DAXX, NOXA, and LFNG) increased by ET plus the GSI, DAXX was the only gene required for GSI-mediated blockade of CSC survival of two distinct ER+ breast cancer cell lines (MCF-7 and BT474).
- Goals were to determine if DAXX is a novel target gene of Notch1, a bonafide cancer stem cell suppressor, predictive biomarker of anti-tumor efficacy of the GSI MK-0752, and if DAXX expression is associated with response to ET in the neoadjuvant setting.

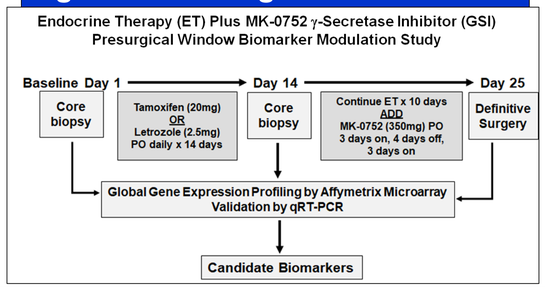
Methods:

- RNA expression profiling was performed using Affymetrix microarray
- Enrichment of Notch1 to CSL-regulatory elements was conducted using ChIP-PCR
- Mammosphere forming efficiency (MFE) measured CSC survival in MCF-7 and BT474 when DAXX was knocked down.
- Tumor xenograft studies were performed using MCF-7 cells expressing DAXX (DAXX+) and depleted for DAXX (DAXX-). Mice were treated with estrogen, estrogen deprivation, to mimic the use of an aromatase inhibitor, 75mg/kg GSI MK-0752, or estrogen deprivation plus MK-0752 for 80 days.
- DAXX and Ki67 protein expressions were measured by IHC and blindly scored in 35 matched untreated and ET or chemotherapy treated human ER+ tumors.

Results:

- **Figure 1: Schema of Pre-surgical Window Trial in 20 patients with ER+ Breast Cancer.**
- **Figure 2: Global Gene Expression Profiling for ER+ breast Tumors at Baseline, after ET, and after ET + GSI.**
- **Figure 3: Notch1 Chromatin Immunoprecipitation-PCR for Candidate Genes**
- **Figure 4: Mammosphere forming efficiency to assess the role of DAXX in anti-CSC efficacy of the GSI.**
- **Figure 5: Tumor Xenograft study to determine if DAXX is predictive of GSI efficacy in vivo.**
- **Figure 6: Association of DAXX RNA and Protein Expression in ER+ Breast Tumors and outcomes.**

Figure 1: Presurgical Window Trial



Results

Figure 2: Global Expression Profiling

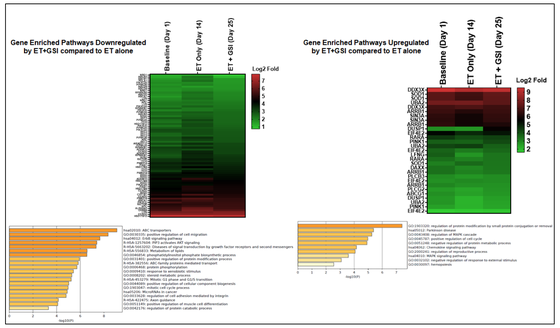


Figure 3: Notch1 ChIP-PCR

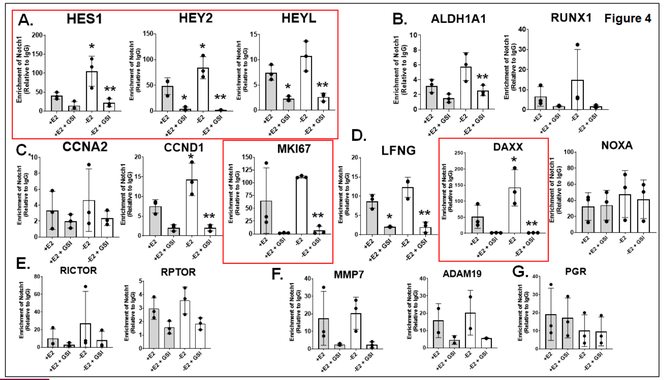
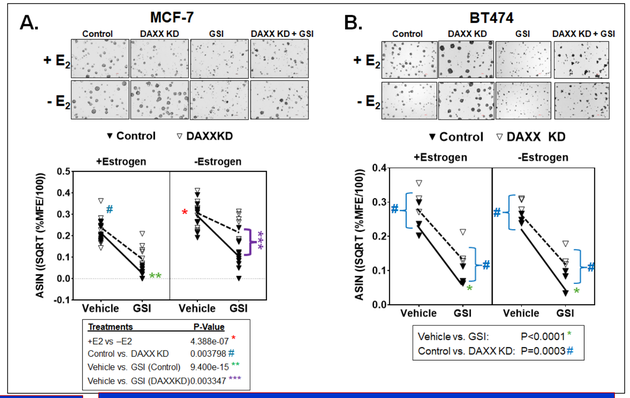


Figure 4: Anti-CSC Efficacy of GSI is Dependent on DAXX



Treatments	P-Value
+E2 vs –E2	4.388e-07 *
Control vs. DAXX KD	0.003798 #
Vehicle vs. GSI (Control)	9.400e-15 **
Vehicle vs. GSI (DAXXKD)	0.003347 ***

Vehicle vs. GSI:	P<0.0001 *
Control vs. DAXX KD:	P=0.0003 #

Figure 5: DAXX Is Required for ET + GSI Efficacy

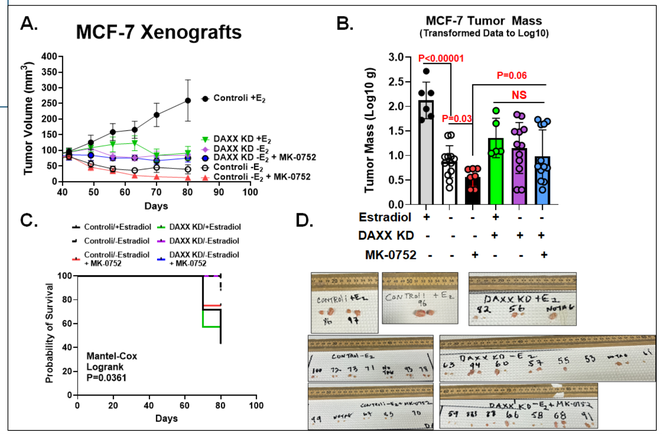
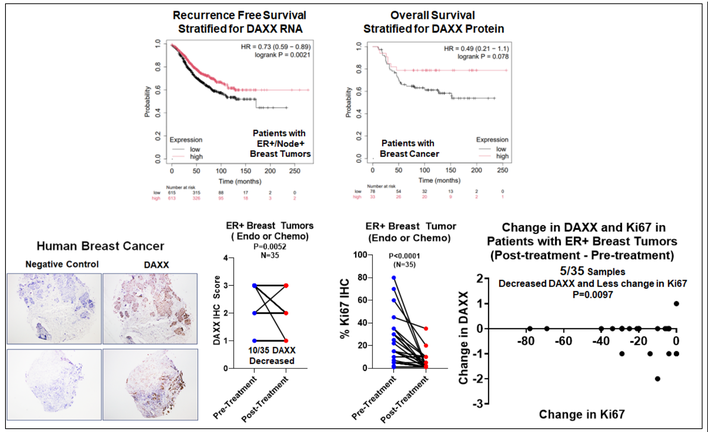


Figure 6: DAXX Is Associated with Better Outcomes



Conclusions

This translational project demonstrates for the first time that GSI-induced DAXX expression was necessary for the anti-CSC and anti-tumor efficacy of the GSI. DAXX is a potent CSC inhibitor and possible predictive biomarker of response to ET or ET plus the GSI MK-0752. High DAXX expression correlates with increased response to ET as measured by Ki67. Testing of anti-Notch or other agents that increase DAXX should be a future clinical trial priority.

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