Illuminating the Molecular Determinants of Therapeutic Response in Triple Negative Breast Cancer with NanoString® 3D Biology™ Technology

Douglas Hinerfeld[1] and Justin Balko[2]

[1]NanoString Technologies, Seattle, WA.
[2]Vanderbilt University Medical Center

Triple Negative Breast Cancer (TNBC) is a particularly aggressive form of breast cancer with limited therapeutic options. Patients with TNBC have a high incidence of distant recurrence and death compared to other forms of breast cancer. As compared to many other cancer types, TNBC has no approved molecularly-targeted therapies, leaving chemotherapy as the prominent treatment. The aggressiveness of TNBC and the limited approved drugs necessitates the identification of additional therapies to improve clinical outcome.

Uncontrolled activation of the RAS/MAPK pathway is known to be central to the initiation and progression of cancers, including non-small cell lung cancer and melanoma. Activation of these pathways is typically a result of oncogenic activating mutations in genes such as BRAF, EGFR, and KRAS. Mutations in these genes result in sensitivity to small molecule inhibitors of the RAS/MAPK pathway making these a good biomarker to predict the effectiveness of molecularly-targeted therapies. TNBC tumors, however, rarely harbor such somatic activating mutations. In some cases, aberrant activation of this pathway can also result from reduced expression of negative regulators of the pathway, including neurofibromin (NF1) and dual specificity phosphatase-4 gene (DUSP4). Increased activity of the mitogenic RAS/MAPK pathway in chemotherapy-resistant TNBC, either by activating mutations or loss of negative regulators, suggests that inhibitors of this pathway may be efficacious.

To test this hypothesis, TNBC patient derived xenograft (PDX) Models were treated with adjuvant chemotherapy (AC) followed by docetaxel (T) with or without the addition of a MEK inhibitor (MEKi). PDX Models A and B are derived from the same patient's primary and subsequent chemotherapy-resistant tumor, respectively. PDX Models C and D are derived from two independent TNBC patient tumors. The changes in tumor growth following treatment of the PDF Models are shown in Figure 1.

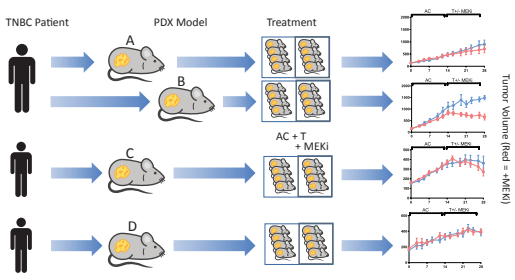

FIGURE 1
Eight PDX mice from each model were treated with AC, followed by the addition of docetaxol (T) at 14 days. Four of the animals from each model were treated with a MEK inhibitor (MEKi) at 14 days. Tumor size was monitored throughout the treatment.

The chemotherapy resistant PDX Model B uniquely responds to the addition of MEKi, suggesting aberrant activation of the RAS/MAPK pathway. To elucidate the molecular mechanisms of the differential therapeutic response to MEKi, two FFPE sections from each animal were subjected to molecular analysis using the Vantage 3D™ RNA MAPK Panel and Vantage 3D Protein Solid Tumor Panel. This assay profiles the expression of 192 genes and 26 total and phosphoproteins that are primarily associated with the RAS/MAPK pathway (Figure 2).

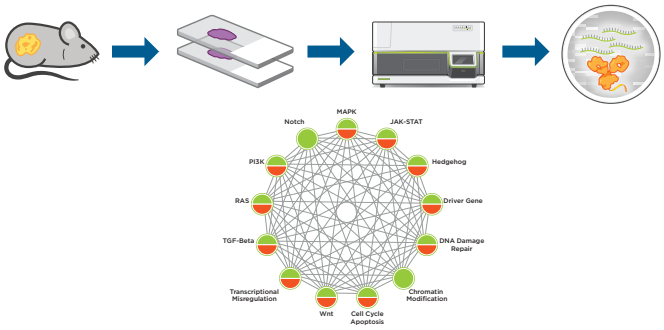

FIGURE 2
Starting from two 5 µm FFPE sections, the expression of 192 RNA and 26 proteins and phosphoproteins were measured using the Vantage 3D™ RNA MAPK Panel and Vantage 3D Protein Solid Tumor Panel.

Analysis of the RNA and protein expression data demonstrates the close correlation in expression within each of the four Models (Figure 3). The PDX Model B from the chemo-resistant tumor shows a unique molecular response to the MEKi, just as it showed a unique therapeutic response (Figure 1). Interestingly, the molecular profile of the MEKi treated PDX Model B closely correlates with the primary tumor sample from the same patient (PDX A). This finding suggests that activation of the RAS/MAPK pathway was the cause of resistance and inhibition of this pathway drives the tumor back towards its primary state.

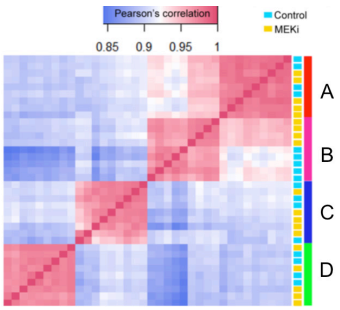

FIGURE 3
Pearson correlation of the gene and protein expression across all samples. The vertical colored bars show the close correlation of the data within each of the PDX Models. The PDX Models that were treated with (Yellow) or without (Blue) the MEKi are denoted.

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To further understand the molecular determinants of therapeutic response to the MEKi in PDX Model B, genes and proteins that were differentially expressed between the control and treated PDX Models were identified. Downregulation of critical signaling phospho-proteins ribosomal protein S6, ERK 1/2, and MEK were detected (Figure 4A). Phospho-ribosomal protein S6 and phospho-ERK 1/2 were validated by western blot (Figure 4B). Expanding on this comparison, NanoString nSolver™ Advanced Analysis Software was utilized to reveal changes in molecular pathways in response to MEKi treatment (Figure 5). Notably, downregulated pathways include RAS/MAPK and PI3K while upregulated pathways include those involved in cell cycle control and apoptosis.

A

Protein	Fold Change	P-value
EGF Receptor	0.689	1.15E-05
pS6	**0.551**	**1.30E-05**
pAMPKa	0.754	0.000256
p4E-BP1	0.613	0.000277
pERK1/2	**0.278**	**0.00132**
pMEK1/2	0.717	0.00205

B

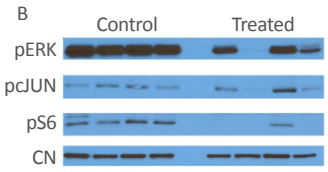

FIGURE 4
Reduced expression of proteins and phospho-proteins detected by NanoString (A) and validated by Western blot (B).

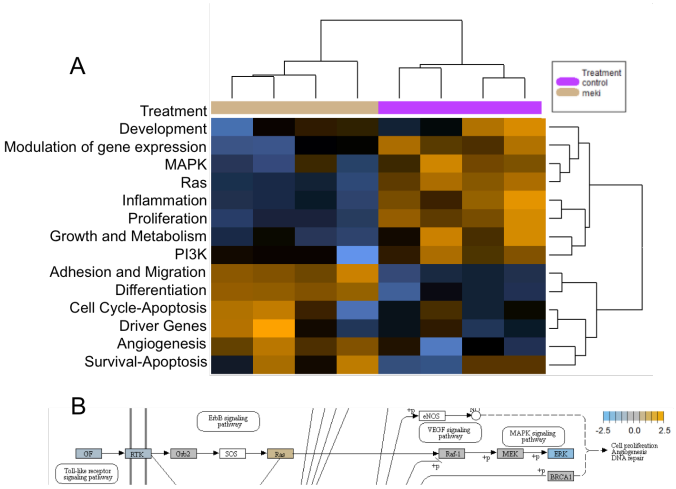

FIGURE 5
Pathway analysis of PDX Model B after MEKi treatment using nSolver Advanced Analysis reveals significantly altered pathways.

Understanding the mechanisms of therapeutic sensitivity and resistance can potentially lead to the discovery of prognostic biomarkers and additional cancer drug targets. Overcoming the dearth of therapeutic options for TNBC is critical to improving clinical outcome. This study demonstrates the power of PDX models and the integrated analysis of RNA, protein, and phosho-protein to gain insights into therapeutic efficacy and the molecular mechanisms that underlie response to combination therapies.

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NanoString Technologies, Inc.
530 Fairview Avenue North
Seattle, Washington 98109
T (888) 358-6266
F (206) 378-6288
nanostring.com
info@nanostring.com

Sales Contacts
United States **us.sales@nanostring.com**
EMEA: **europe.sales@nanostring.com**
Asia Pacific & Japan **apac.sales@nanostring.com**
Other Regions **info@nanostring.com**

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