An Organoid Model System to Study Resistance Mechanisms, Predictive Biomarkers, and New Strategies to Overcome Therapeutic Resistance in Early-Stage Triple-Negative Breast Cancer

Tam Binh Bu1, Denise Wolf1, Kaitlin Moore1, Isaac Harris1, Pravin Phadaster1, Christina Yau1, Lamorna Brown-Swiggart1, Laura Esserman1, Jean-PhilippeCoppe1, Julia Wulfkuhle1, Emanuel Petricoin4, Michael Campbell1, I-SPY2 investigators, Laura Selfors2, Deborah Dillon2, Beth Overmoyer2, Filipa Lynch2, Laura van’t Veer2, Jennifer Rosenbuth1
1UCSF, San Francisco, CA; 2Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA; 3University of Rochester, Rochester, NY; 4George Mason University, Fairfax, VA.

BACKGROUND

- Organoids: promising technology used for growing breast cancer cells, but the extent to which it can model treatment resistance is largely unknown.
- This research: using patient-derived organoid cultures in the context of computational analyses of large molecular and clinical datasets (I-SPY2) to study resistance mechanisms, biomarkers, and alternative treatment strategies in early-stage triple-negative breast cancer (TNBC).

METHODS & DATA

- An organoid biobank, enriched for inflammatory breast cancer (IBC), was established from organoids derived from breast tumor samples, digested to the organoid level using collagenase, and grown in three-dimensional culture using a basement membrane extract and a fully-defined organoid medium (1).
- Next, previously analyzed I-SPY2 gene expression and protein biomarkers associated with resistance (identified in pre-treatment patient tumors) were explored to determine if they were present in organoids propagated from breast cancer post-treatment residual disease (2,3).
- Bulk RNA sequenced data of 11 TNBC organoids were normalized and merged with the TCGA dataset (4) to analyze expression of TNBC subtypes (5-7) and I-SPY2 gene expression biomarkers in a larger context.
- Immunofluorescence analysis of key biomarkers (from I-SPY2 RPPA analysis) was performed, using breast cancer cell lines as controls.
- A high-throughput 386 anti-cancer drug screen was performed (with and without cisplatin) in a tumor organoid modeling resistance to cisplatin. The most promising compounds were selected for subsequent synergy analysis.
- High-throughput kinase activity-mapping assays (HT-KAM, or kinase assay) in this organoid model are in progress, with the goal of identifying (drugable) kinase mediators of cisplatin sensitivity and resistance (8).

RESULTS

- Top 50 most differentially expressed genes (largest standard deviation) across tumor organoids.
  - Highly expressed genes in normal-like subgroup of TNBC organoids: related to the extracellular matrix (MMAC1, FAP, BRF1).
  - Highly expressed genes in basal-like subgroup: basal cytokeratins (KRT5, KRT14, KRT17).
- IBC cases present in all 3 subsets.

Table 1: TNBC organoids are characterized predominantly by either normal-like/luminal androgen receptor or basal-like features

Table 2: RPPA biomarker analysis by I-SPY2 investigators highlights potential markers of resistance to veliparib-carboplatin (VC) (3)

- Ranking of 386 compounds based on their synergistic activity with carboplatin in TORG40.
  - AUC of inhibitory concentration with compounds and without carboxiplatin were used to rank the compounds and select top hits of therapeutic interest based on percent reduction in AUC with the addition of carboplatin (with or without cisplatin).

CONCLUSIONS

- Therapeutic resistance in residual disease tumor organoid cultures can be studied to identify new strategies for TNBC.
- Tumor organoids modeling drug resistance states are a useful context for developing strategies for TNBC and can be used for compound testing.
- A pipeline is being developed to propagate residual tumors from patients enrolled in I-SPY2 into organoid cultures to create a broader platform for preclinical drug screening and therapeutic response.

FUTURE DIRECTIONS

- Preliminary Blas Synergy and Kinome Assay Data
  - A Preliminary Blas Synergy Score analysis of carboplatin and ABT-263 (analyzed using SynergyFinder package in R).
    - Future results could help select promising, and clinically relevant dose combinations to guide future research, including organoid-based mechanistic studies.
  - Blas assay data from a pilot kinase assay (8) comparing cancer cell lines to patient derived TORG40 organoids highlight new potentially drugable kinase targets to overcome resistance to carboplatin, including FGFR3, MLK3 and MAP4K3.

Long-term goal:
- Leverage organoid model system to enable faster, more successful drug discovery studies and find new treatment regimens for resistant disease.

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