#405 A novel panel of 325 biomarkers is part of a large interconnected network representing multiple cell signaling pathways and allowing development of predictive tests for oncology drugs

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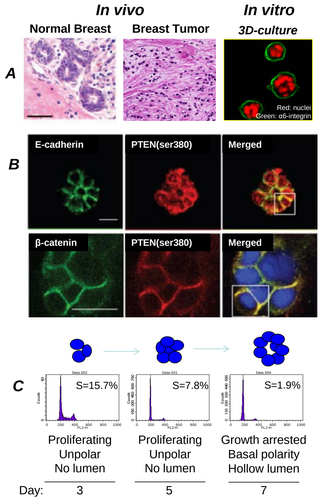
Introduction:

For over a century pathologists have understood that there is a relationship between the microscopic structure of a tumor and clinical outcome. Even today, physicians use a tumor grading system based on the relative preservation of normal tissue and cell structures as a guide for treatment. Our recapitulation of a non-malignant breast epithelial cell differentiation and organizational program *in vitro* allowed differentiated cells to form multicellular acini-like structures with defined lumens and tight junctions with specific localizations of cadherin and integrins[1,2]. In contrast, cells from breast cancers displayed a general loss of structure under the same conditions. Microarray analysis of these acini-like structures generated a list of genes which exhibited significant changes in expression during this developmental program. This list, **BA325**, contains 325 genes of which 251 are novel and not present in 8 other cancer based gene expression panels such as FoundationOne or Prosigna (PAM50) and 32 of the genes have significant drug associations. We report below some of the pathways identified in BA325 and highlight several examples of possible interaction with processes important for cancer therapies including immunotherapy. We also developed a unique series of algorithms to identify the best combination of BA325 biomarkers to reliably predict a specific end-point such as a patient's response to neoadjuvant chemotherapy, MRI phenotypes, tumor burden, among others with the goal of increasing response rates and directly patients away from ineffective treatments.

Figure 1: ***in vitro* generated acini from non-malignant mammary epithelial cells grown in three dimensional culture show characteristic appearance and polarized localization**.
A. The first two images show standard H&E staining of normal breast tissue and a breast tumor. The normal tissue shows well defined ducts with normal nuclei while the tumor has a disorganized structure with distorted cells and nuclei. The far right image shows fluorescent staining of *in vitro* generated acini with red nuclei and polarized expression of α6 integrin around the periphery of the structure. **B**. Immunofluorescent staining of E-cadherin and β-catenin show colocalization with PTEN limited to points of cell-cell contacts. **C**. DNA content analysis by flow cytometry shows a shift from a proliferative state on left, to a quiescent state on the right, correlating with acini formation of non-malignant cells.



Objectives:

- Investigate cell-signaling pathways and drug associations to inform about oncology drug response and sensitivity / resistance mechanisms
- Apply novel algorithm pipeline to create high quality gene classifiers / prediction models agnostic of technology platform
- Study putative oncology drug associations with implications for structural formation, motility, metastasis and antigen presentation

Methods::

The Qiagen Ingenuity Pathway Analysis, GeneGo, and DAVID programs and manual curation were used to identify pathways and drug interactions containing significant overlap with BA325. A proprietary algorithm pipeline using a combination of logistic regression methods and recursive ranking processes was developed to collect the most predictive genes from BA325 for a wide variety of outcome measurements.

References

1. Fournier, et al., 2006, Cancer Research 66:7095-7102
2. Fournier, et al., 2006, Cancer Research 69:4545–4552

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Results:

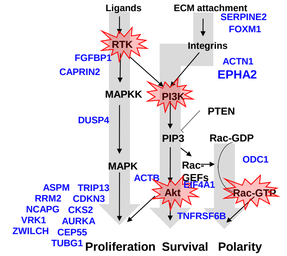
Table 1: BA325 offers a unique insight into tumor cell biology

- 251 of the 325 genes are unique to BA325
- Allows qualitative and quantitative measures of a tumor's retention of a normal developmental program

Oncology Gene Panels	Total number of genes	Genes in common	Percent of BA325
FoundationOne	343	8	2%
NS_cancer_immune	770	15	5%
NS_cancer_path	770	31	9%
NS_cancer_prog	770	20	6%
Illum_PanCancer	1386	42	13%
OncotypeDX	21	3	1%
Prosigna	50	7	2%
Mammaprint	70	9	3%
NS Nanostring Illum Illumina			

Table 1

Figure 2: Examples of significant pathway associations relevant for oncology drug discovery and targeted treatments



BA325 genes in blue surround druggable targets outlined in red. 32 BA325 genes are currently druggable

Diverse interconnected pathways provide robust information about mechanisms of drug sensitivity and resistance

- Cell Cycle Control of Chromosomal Replication (p=8.1E-14),
- Polo-like-kinase and HSP90 complex (p=6.3E-07),
- G2/M DNA Damage Checkpoint (p=6.4E-07),
- Integrin Signaling (p=3E-05),
- Integrin Linked Kinase
- Signaling (p=4.51E-05),
- BRCA1 DNA Damage Response (p=1.75E-04),
- Estrogen Mediated S-phase entry (p=4.24E-04),
- Regulation of Actin Based Motility by RHO (p=4.55E-04),
- Adherins Junction Remodeling (p=5.6E-04),
- Actin Cytoskeleton Signaling (p=7.25E-04),
- Mismatch Repair (p=1.6E-03), RAN Signaling (p=1.64E-03),
- Protein Ubiquitination (p=1.78E-03)
- Cholesterol Biosynthesis (p=4.8E-03).

Figure 3: Concentration of BA325 genes in cell cycle control of chromosomal replication

- Qiagen IPA predicted p=8.1E-14
- BA325 genes are surrounded in purple, multiple genes in complexes are listed next to the complex.
- Genes in bold and * are unique to BA325
- Compact pathway with few branches with 6 of 14 BA325 genes being unique

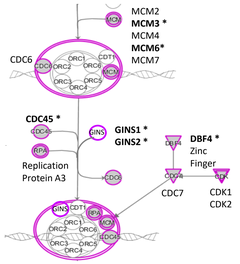
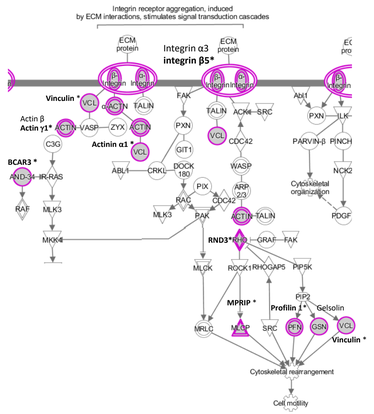


Figure 4: Complex, branching pathways for Integrin signaling

- Qiagen IPA predicted p=3E-05
- 8 out of 11 BA325 genes are unique
- Connections to many other pathways involved in signal transduction, motility and tight junctions
- BCAR3 involved in resistance to anti-estrogen therapy



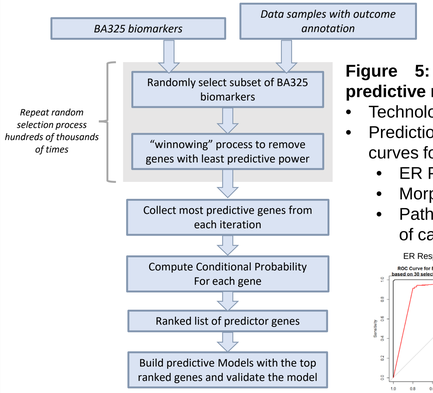
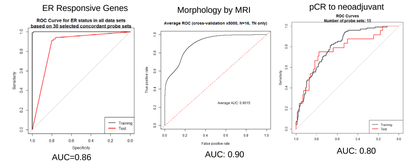
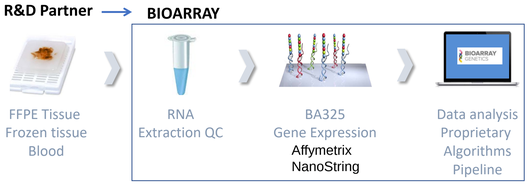


Figure 5: Novel Algorithm creates high quality predictive models.

- Technology platform agnostic
- Prediction of multiple end-points with quality ROC curves for diverse outcome predictions:
  - ER Pathway activation
  - Morphology by MRI
  - Pathological complete response (pCR) to standard of care neo-adjuvant (AC-T or T-FAC)



Positioning BA325 for R&D



Conclusions:

The BA325 oncology gene panel in combination with a novel algorithm pipeline provide actionable information for Drug Discovery and Development, prediction of clinical outcomes, and rationale for selection of patients for clinical trials and companion diagnostics. BA325 is quite different from other cancer-focused gene panels and exhibits membership in a variety of pathways expected to impact tumor development and treatment. BA325 should find great utility in future companion diagnostics and allow a more robust pathway-based understanding of drug activity and resistance.