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Defining High-Risk Precursor Signaling to Advance Breast Cancer Risk Assessment and Prevention

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14. ABSTRACT

Making a major impact on the incidence and lethality of breast cancer will require a detailed understanding of the earliest tissue changes that ultimately drive the process of breast cancer development. There is no substitute for the ability to define and understand the early, pre-malignant changes as they occur in women who are breast cancer-predisposed. One group of women at high breast cancer risk (up to 80% lifetime breast cancer risk) are those who have inherited mutations in the BRCA1 and BRCA2 genes. Currently, the only way these women can eliminate their risk is to undergo bilateral mastectomy before developing cancer. We have established an IRB-approved protocol that allows us to collect and analyze a portion of this tissue. Here, we propose detailed functional and molecular analysis of these tissues in order to reveal critical early steps in breast cancer development. We will then test how reversing these changes can prevent breast cancer in well-established animal models. These studies are likely to lead directly to clinical trials of new approaches to prevent breast cancer.

15. SUBJECT TERMS
Breast cancer; BRCA1/2; cancer prevention; paracrine signaling

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1. INTRODUCTION:

Making a major impact on breast cancer will require a detailed understanding of the earliest tissue changes. One group of women at high breast cancer risk are those with BRCA1 and BRCA2 mutations. Currently, the only way these women can eliminate their risk is to undergo bilateral mastectomy before developing cancer. Here, we propose detailed functional and molecular analysis of these tissues in order to reveal critical early steps in breast cancer development. We will then test how reversing these changes can prevent breast cancer in well-established animal models. These studies should lead directly to clinical trials of new approaches to prevent breast cancer.

2. KEYWORDS:

Breast cancer; BRCA1/2; cancer prevention; paracrine signaling

3. ACCOMPLISHMENTS:

Aim 1: Functional analysis of progenitor and stem cells in high-risk tissues.
Major Task 1Functional quantitation (100% Completed in Months 1-24)
Major Task 2 Functional analysis (100% Completed in Months 1-12)
Major Task 3 Signaling Analysis (100% Completed in Months 1-12)

Aim 2: Discover and validate new pathways activated in cancer-predisposed tissues.
Major Task 1Transcriptomics (100% Completed in Months 1-24)
Major Task 2 Sub-clonal genomics (100% Completed in Months 1-24)
Major Task 3 Integrated bioinformatics (30% Completed in Months 1-24)

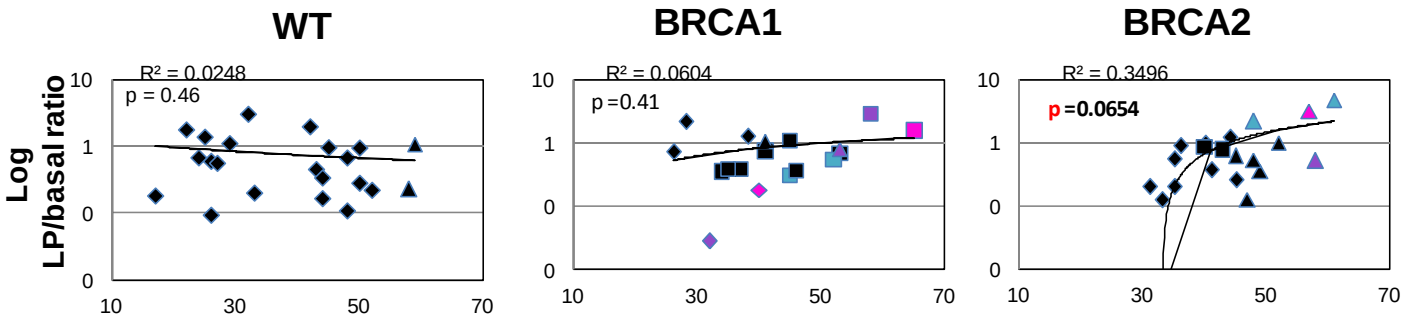
Aim 3: Block abnormal signaling in vitro and in vivo
Major Task 1Reverse abnormal signaling (30% Completed in Months 1-24)
Major Task 2 In vivo cancer models (In Process)

What was accomplished under these goals?
NB: We are pleased to share the remarkable accomplishments under this award to date. In order to facilitate review, accomplishments are now organized and enumerated based on tasks corresponding to the original SOW for this award. Accomplishments are supported by tables and figures to facilitate review.

Specific Aim 1: Functional analysis of progenitor and stem cells in high-risk tissues.

Major Task 1: Quantitation of LP (Luminal Progenitor) and basal stem cell (MASC) populations

A. Quantitation of LP and basal stem cell (MASC) populations



We have continued to add patients to the cohorts between months 12 and 24. (This reporting period). Latest data on all current cohorts are demonstrated above.

Methods and accomplishments (Figure 1 above):
Method: Primary mammary tissues from each patient underwent enzymatic digestion, followed by specific antibody labeling and FACS as described in detail in the original proposal.

Accomplishments: Shown is the ratio of LP to basal (MASC) population for each patient. We accomplished the novel and remarkable finding that there is a near significant age-associated increase in ratio of LP/to basal cells specifically in BRCA2 carriers. This finding supports the hypothesis of a deregulation of the LP population in BRCA2 carriers.

Diamond= premenopausal, no HRT; Square= Post menopausal, +HRT
Triangle= Post menopausal, no HRT; Pink= breast cancer; neoadjuvant chemo
Purple= breast cancer; no neoadjuvant chemo; Teal= Ovarian cancer; prior chemo

B. CFU and mammosphere assays for functional quantitation
Previously reported

Major Task 2: Functional analysis of LP and basal stem cell (MASC) populations

A. CFU assays in presence and absence of growth factors.
Previously Reported.

B. Assessment of bi-lineage differentiation
Previously Reported.

Accomplishments: These experiments show for the first time decreased basal-like differentiation in LP cells from BRCA2 mutation carriers. These findings are consistent with the hypothesis that not only cell numbers but also cell fate is deregulated in the BRCA2 LP population.

Major Task 3: Analyze signaling in high-risk tissues in vivo.

IHC to analyze signaling in high risk tissues and controls in vivo
Previously reported.

Specific Aim 2: Discover and validate new pathways activated in cancer-predisposed tissues.
Major Task 1: Transcriptomic analysis in LP and MASC cells
Methods and accomplishments: (Figure 2 below, carried out during months 1-24).

Methods: We have now carried out additional RNA sequencing on RNA extracted from primary FACS-sorted mammary epithelial cells during months 12-24. Additional comprehensive genomic analyses have been carried out, from control (N=7), BRCA1 carriers (N=5) and BRCA2 carriers (N=5) to date. In each case, basal cells, LP cells, mature luminal cells and stromal cells were analyzed separately.

Accomplishments: The most biologically and clinically significant finding to date (Figure 2) is that, unlike BRCA1-mutant mammary epithelia, BRCA2 mutant epithelia exhibit highly suppressed NF-KB signaling. The second important observation (Figure 3) is a cell cycle stress ("G1/G2") RNA profile in BRCA2 mammary LP cells (See Below).

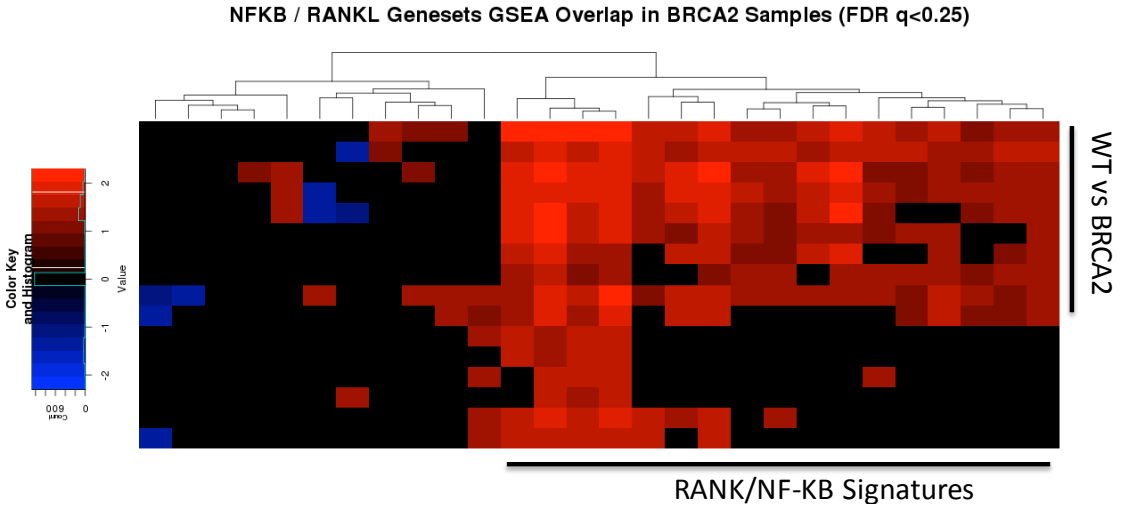


Figure 2 (above) Legend: Above is shown a heat map of NFkB (right) and other gene expression signatures. Each column represents one signature. Rows represent comparisons of wild-type (WT) vs. BRCA2 mutant tissues from each compartment (Basal, LP, ML, stromal) using different gene expression normalization strategies. Red color indicates downregulation of NF-kB signature in BRCA2 vs WT samples in each case.

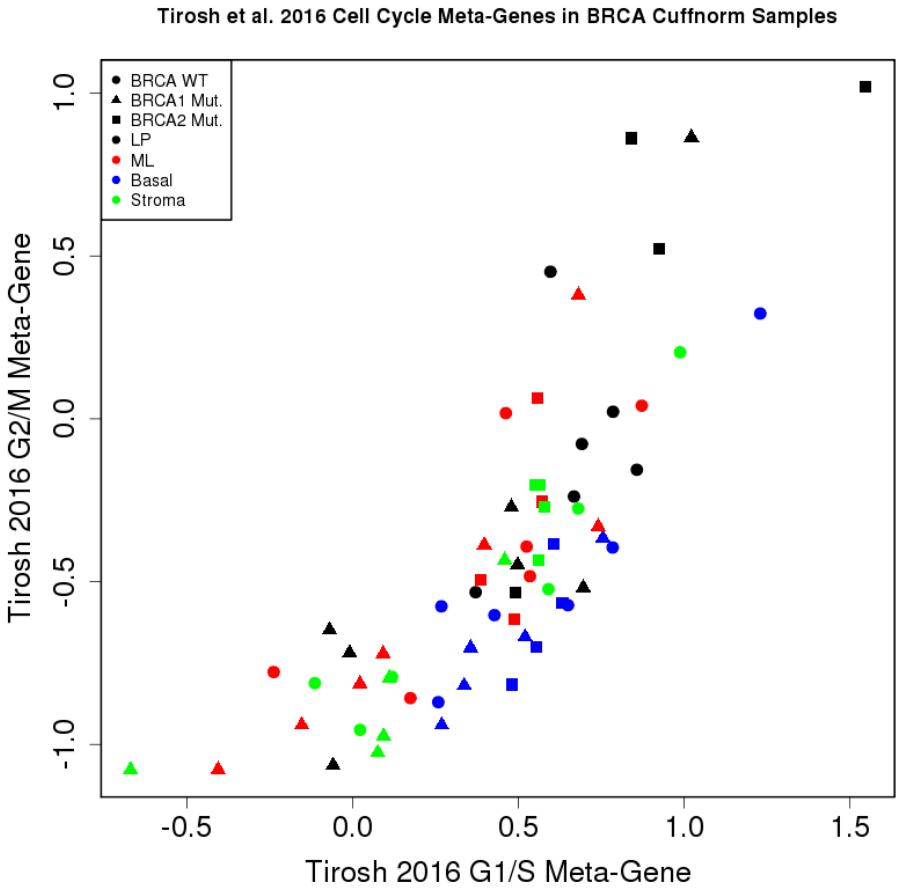
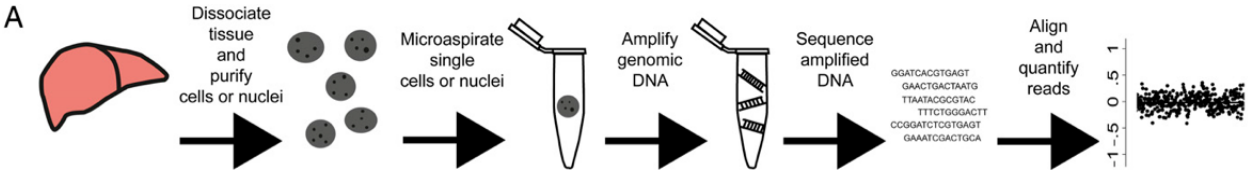


Figure 3. Cell cycle analysis using RNAsequencing data. Y-axis shows G2/M expression signature strength of each sample, while X-axis shows G1/S expression strength. Each point on the graph represents one cell population from one patient. Black squares are BRCA2 LP cells, which cluster in upper right quadrant, indicating cell cycle stress gene expression pattern.

Major Task 2. Sub-clonal genomic analysis in LP and MASCs

Methods: We carried out low-coverage whole-genome sequencing on individual LP and Basal/MASC cells from primary tissues of BRCA2 carriers during months 12-24. Bioinformatic analysis was carried out to determine genomic quantification and copy number. The experimental protocol is shown below:



Accomplishments: these analyses provided an unprecedented window in genomic aberrations in early breast cancer pathogenesis. In brief, we showed that 30% of LP cells from BRCA2 carriers demonstrate significant chromosomal aneuploidy, compared to <5% of LP cells from wild-type tissues. This is the first demonstration of such aneuploidy in normal tissues in the setting of BRCA1/2 mutation.

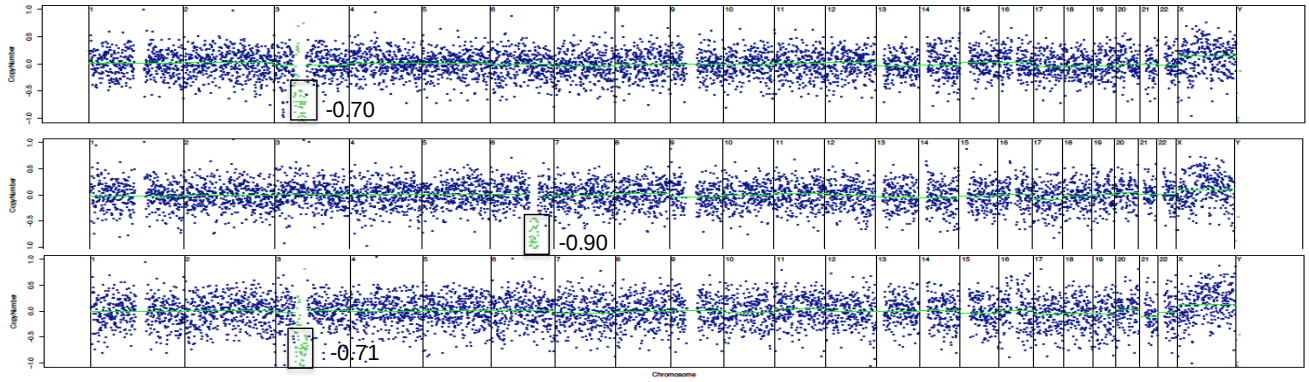


Figure 4. Whole-genome analysis of cells from a BRCA2 carrier. In the above figure each horizontal row is one cell (3 cells shown), while each box within the row represents an individual chromosome in that cells. Normal DNA content is indicated by dots (sequencing reads) aligned horizontally in the center of each box. Loss of a portion of the chromosome is evidenced by dots below the center line, which are shown in green. Thus, each cell shown has on major sub-chromosomal loss involving a different chromosome.

Major Task 3: Integrated bioinformatics of expression/mutation (ONGOING)

Methods: Together with our computational biologists, we have integrated the genomic findings with the gene expression data to identify signatures associated with the aneuploid state we discovered.

Accomplishments: Current comprehensive integrated analysis shows a gene expression signature of G2/M stress that is likely a reflection of the aneuploid state we demonstrated through sub-

clonal genomic analysis. This stress signature may prove to be an “achilles heel” of the BRCA2 mutant tissue that can be exploited to eliminate abnormal, aneuploid cells that are the precursors of breast cancer in this genetic context.

Specific Aim 3: Block abnormal signaling in vitro and in vivo

Major Task 1. Reverse abnormal LP signaling in vitro (ONGOING)

Methods: Given our observation of aneuploidy and G2/M stress in BRCA2-mutant cells from patients, we sought to exploit this abnormal DNA damage phenotype as a potential cancer prevention strategy. Accordingly, we treated primary epithelia in 3-dimensional culture with an inhibitor of Poly-ADP Ribose Polymerase (PARP) inhibitor (Figure 5).

Accomplishments: These experiments demonstrate the sensitivity of BRCA2 primary cells exhibiting cell cycle stress (Fig. 3) and aneuploidy (Fig. 4) to PARP inhibition. These experiments thus provide proof-of-principle for use of this and related agents to eliminate breast cancer precursor cells as a prevention strategy for breast cancer in young women.

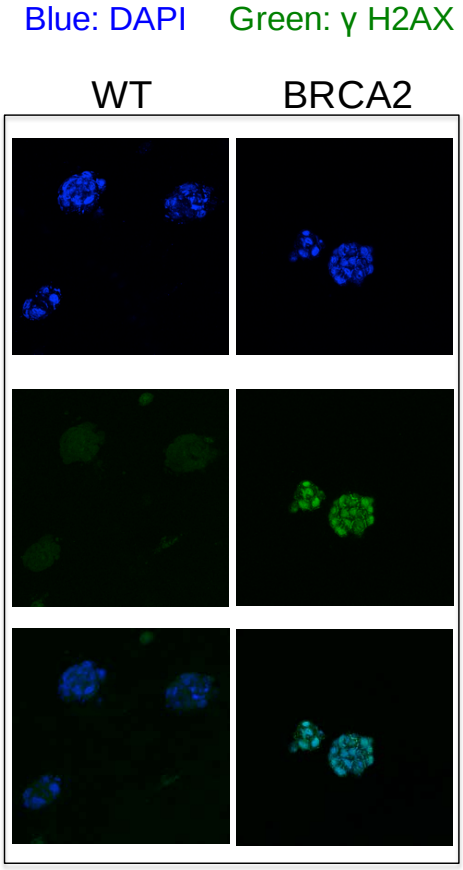


Figure 5. Sensitivity of patient-derived BRCA2-mutant primary epithelia to PARP inhibition. DAPI staining (top) indicates nuclei, whereas gamma-H2AX staining (middle) indicates double –strand DNA breaks. The staining of BRCA2-mutant but not non-mutant (WT) cells indicates sensitivity of the non-malignant, mutant cells to this agent.

Major Task 2: Inhibit abnormal signaling in vivo cancer models
(IN PROCESS). Note that animal experiments are planned in months are temporarily delayed due to capacity issues in our institutional animal facility. We anticipate commencement of these studies in months 24-36.

What opportunities for training and professional development has the project provided?

Mihriban Karaayvaz, PhD is providing ongoing training to Devika Salunke, a research assistant in the laboratory who is developing professional skills in preparation for graduate school.

Dr. Karaayvaz herself was able to attend a bioinformatics course, as well as multiple conferences concerning topics related to the area of the proposal.

How were the results disseminated to communities of interest?

1. Presentation at meeting of Consumer Advocates.
2. Presentation in joint laboratory meeting, MGH Center for Cancer Research 10/2016.
3. Presentation at Scientific Advisory Board Meeting of the Mass General Hospital 4/2016.
4. Presentation at the Harvard Cancer Center Breast/Ovarian Cancer Retreat 3/2017.
5. Presentation at the MGH Cancer Center Annual Retreat 10/2016.

Plan to complete Aim 2, including additional integrated bioinformatic analysis of RNAseq and genomic data. Furthermore, we will continue testing of functional pathways and hypotheses gleaned from the bioinformatic analysis.

These experiments will set the stage for ongoing experiments in Aim 3, to unveil unanticipated synthetic lethalities in BRCA1 and BRCA2 mutant non-malignant cells. These studies will set the stage for in vivo tests of blocking these pathways in order to credential new opportunities for prevention of breast cancers in young women.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

New types of techniques for human breast tissue analysis described in the figures above, and functional characterization are being developed through this project, and these will allow other investigators to explore related questions in breast cancer biology.

New findings have provided unprecedented view into early cancer pathogenesis in humans.

What was the impact on other disciplines?

None to report at this time.

What was the impact on technology transfer?

None to report to date, but this work is expected to lead directly to application and development of new technology for cancer prevention and clinical trials.

What was the impact on society beyond science and technology?

Use of donated tissue proves the value of this approach for scientific advances that benefit patients. This concept will be disseminated through the results of this research.

5. CHANGES/PROBLEMS:

None to report

Actual or anticipated problems or delays and actions or plans to resolve them

Delays in implementation of animal experiments as described above due to institutional limitations, which are anticipated to be temporary.

Changes that had a significant impact on expenditures

None.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

None.

Significant changes in use of biohazards and/or select agents

None.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

None to date.

Books or other non-periodical, one-time publications.

None to date.

Other publications, conference papers and presentations.

1. Presentation in joint laboratory meeting, MGH Center for Cancer Research.
2. Presentation at Scientific Advisory Board Meeting of the Mass General Hospital
3. Presentation at the Harvard Cancer Center Breast/Ovarian Cancer Retreat.
4. Presentation at the MGH Cancer Center Annual Retreat.

- **Website(s) or other Internet site(s)**

None to date.

- **Technologies or techniques**

New types of techniques for human breast tissue analysis and functional characterization are being developed through this project, and these will allow other investigators to explore related questions in breast cancer biology.

- **Inventions, patent applications, and/or licenses**

None to date.

Other Products

New datasets of gene expression in normal and mutated human breast tissue will be a valuable resource and will be publicly available once results of the study are published.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Leif Ellisen*
Project Role:	*PI*
Nearest person month worked:	*1*
Contribution to Project:	*No change*
Funding Support:	*No change*
Name:	*Devika Salunke*
Project Role:	*Research Assistant*
Nearest person month worked:	*12*
Contribution to Project:	*No change*
Funding Support:	*No change*
Name:	*Kenneth Ross*
Project Role:	*Bioinformatician*
Nearest person month worked:	*1*
Contribution to Project:	*No change*
Funding Support:	*No change*
Name:	*Mihriban Karaayvaz*
Project Role:	*Research Fellow*
Nearest person month worked:	*6*
Contribution to Project:	*No change*
Funding Support:	*No change*

previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

No change in active support for PI/key personnel.

What other organizations were involved as partners?

The project is conducted at the Mass General Hospital.
A portion of cell sorting is conducted at the Ragon Institute of MGH, MIT and Harvard.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: N/A