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14. ABSTRACT
Triple-negative breast cancer (TNBC) is an overly aggressive breast cancer subtype that disproportionately affects African American women. Triple-negative breast cancer is characterized by a lack of expression of the estrogen and progesterone receptors as well as the absence of *HER2* amplification/mutation. *TP53* mutations are the only genetic correlate with poor clinical prognosis in this subtype. In our preliminary studies, we found that p53 mutations in TNBC often coincided with deletion/silencing of the *CDKN2A* locus that encodes both the ARF and INK4A tumor suppressors. Surprisingly, this genetic context was primarily present in African American women with TNBC. Concurrent loss of both p53 and *CDKN2A* function resulted in massive gains in proliferation and transformation of mouse and human mammary epithelial cells both in vitro and in vivo. These phenotypic tumor gains were the direct result of altered JAK1 (through loss of p53 and ARF) and CDK4 (through INK4A loss) activity. In this genetic context, JAK1 and CDK4 cooperate to stimulate breast tumor cell proliferation. ***For this reason, identifying these key growth-driving kinases is paramount to discovering novel combinatorial therapies for TNBC.*** In our year 1 report, we show that the JAK1 pathway is elevated in TNBC that also have loss of ARF and p53 function. Moreover, we have established the proliferation profiles of mouse and human TNBC cell lines that have been treated with JAK1 inhibitors.

15. SUBJECT TERMS
Breast cancer, ARF, INK4A, p53, metastasis, CDK4, mammary epithelial cells, patient-derived xenografts

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1. INTRODUCTION

Triple-negative breast cancer (TNBC) is an overly aggressive breast cancer subtype that disproportionately affects African American women. Triple-negative breast cancer is characterized by a lack of expression of the estrogen and progesterone receptors as well as the absence of *HER2* amplification/mutation. *TP53* mutations are the only genetic correlate with poor clinical prognosis in this subtype. In our preliminary studies, we found that p53 mutations in TNBC often coincided with deletion/silencing of the *CDKN2A* locus that encodes both the ARF and INK4A tumor suppressors. Surprisingly, this genetic context was primarily present in African American women with TNBC. Concurrent loss of both p53 and *CDKN2A* function resulted in massive gains in proliferation and transformation of mouse and human mammary epithelial cells both in vitro and in vivo. These phenotypic tumor gains were the direct result of altered JAK1 (through loss of p53 and ARF) and CDK4 (through INK4A loss) activity. In this genetic context, JAK1 and CDK4 cooperate to stimulate breast tumor cell proliferation. ***For this reason, identifying these key growth-driving kinases is paramount to discovering novel combinatorial therapies for TNBC.***

2. KEYWORDS

Breast cancer, ARF, INK4A, p53, metastasis, CDK4, mammary epithelial cells, patient-derived xenografts

3. ACCOMPLISHMENTS

Major Goals of the Project

There were two major goals for this first year of the grant proposal for Dr. Weber: 1) Establish a spontaneous TNBC mouse model, and 2) stain PDX TNBC tumors for each biomarker.

Goals Accomplished

We have not yet obtained ACURO approval, so we have not performed any mouse work as described. We anticipate receiving approval in April 2019. As such, we have focused out first year on Major Task 2 and major Task 3 and will describe the results we have obtained below. Growing evidence suggests that a significant portion of triple negative breast cancers is comprised of mammary stem cell progenitors and that these cells are capable of repopulating tumors following initial chemotherapies. Moreover, the breakdown of TNBC into smaller subgroups has complicated efforts to model the disease in mice. Fortunately, human patient derived tumor xenografts (PDXs) that have a high take rate in immune compromised mice are typically triple negative tumors. While this may seem opportune, these animals lack an intact immune system, embodying them with caveats when using them as exclusive pre-clinical models.

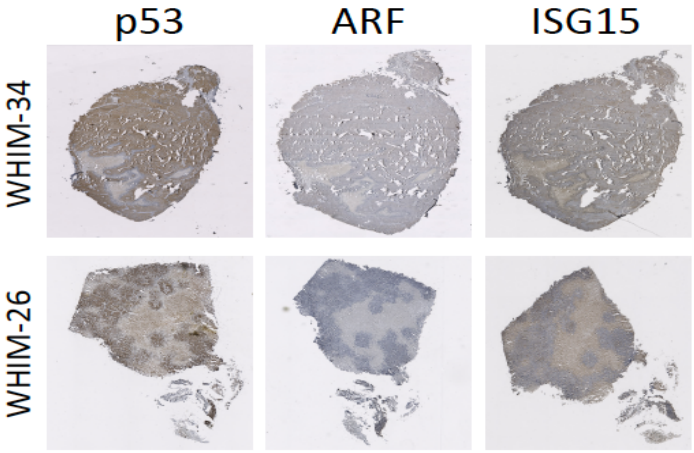

Figure 1. Immunohistochemical staining of human PDX mammary tumors in mice. Tumors from each designated WHIM PDX model were harvested 8 weeks after implantation in nude mice. Tumors were fixed and processed onto slides for analysis. Slides were stained with antibodies recognizing human p53, ARF, and ISG15. Antibodies were visualized with DAB chromogen.

However, PDX models generated at Washington University faithfully recapitulate the primary patient tumor even over multiple passages in vivo, suggesting that they are a

representative embodiment of the patient tumor.

In Major Task 2, Subtask 4, we had proposed to analyze 30 PDX tumors for p53, ARF, INK4A, phospho-STAT1 and ISG15 proteins via immunohistochemistry. In year 1, we have stained and analyzed 15 PDX tumors for p53, ARF and ISG15. As shown in Figure 1, a representative staining of two PDX tumors revealed that high p53 staining (indicative of mutant p53) and low ARF staining (indicative of ARF loss) correlated well with high ISG15 protein expression. This suggests that when ARF and p53 are functionally lost, the JAK1 pathway is elevated resulting in high ISG15 expression (which resides downstream of active JAK1/STAT1.

In Major Task 3, Subtask 1, we proposed to establish in vitro proliferation assays for established breast cancer cell lines. To do this, we cultured four established human breast cancer cell lines that we received commercially from ATCC: HCC1806, DT549, MDA-MB-231, and MDA-MB-157. Cells were initially cultured in varying amounts of fetal bovine serum without any supplements. Proliferation was measured as an increase in total cell number by counting subsequent cells on each day after the initial plating. Our results show that 10% serum is optimal for proliferation and that the HCC1806 cells have the most rapid proliferation rate of all four established cell lines (Figure 2).

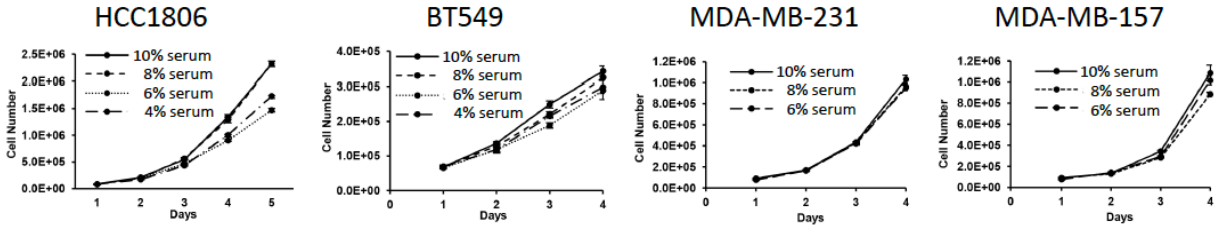

Figure 2. Establishing the proliferation rates of established human breast cancer cell lines. The indicated breast cancer cell lines were cultured in varying amounts of fetal bovine serum. Cells were initially plated at $1x10^5$ and subsequent cells were counted on plates for four or five consecutive days. All counting was performed in triplicate.

In major Task 3, Subtask 2, we proposed to treat established breast cancer cell lines with JAK1 and CDK4 inhibitors alone or in combination. We have begun these experiments. We plated equal numbers of four established human breast cancer cell lines and treated each cell line with varying concentrations of the selective JAK1 inhibitor, itacitinib. Cells remained in drug for three days and remaining cells on each plate were counted. Using this method, we were able to establish an IC50 for each cell line for itacitinib treatment (Figure 3). While the IC50 varies, the concentrations are all quite similar.

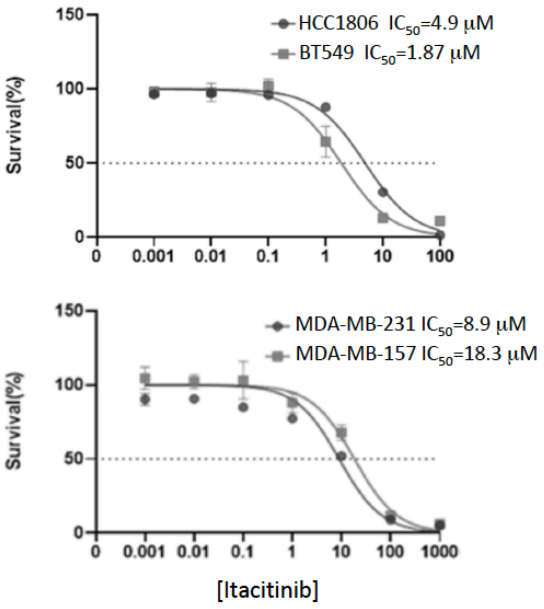

Figure 3. Tretament of breast cancer cells with itacitinib. The indicated breast cancer cell lines were cultured in varying concentrations of itacitinib (JAK1 inhibitor) for three days and then surviving cells were counted in triplicate.

Training Opportunities

Nothing to Report

Results Disseminated to the Community

I participated this past year in disseminating our initial findings to three independent groups of large donors to the American Cancer Society, including the CEO

of the ACS. These donors visited my laboratory at Washington University where I discussed the research in this grant proposal and how our results were moving the field of breast cancer research forward. We engaged in a question and answer session where the donors queried me on the clinical impact of this work. I anticipate doing this laboratory tour again next year and have already been asked by the American Cancer Society to do so. I also was the keynote speaker at the American Cancer Society Strides Against Breast Cancer event.

Plans for Next Reporting Period

In the next year, we will focus our attention on the in vivo mouse models. Specifically, we will generate the *TP53*$^{fl/fl}$/*CDKN2A*$^{fl/fl}$/K14-Cre and *TP53*$^{fl/fl}$/K14-Cre mice (Major Task 1, Subtask 1). We will also focus on harvesting mammary tumors from these mice (Major Task 1, Subtask 2 & 3) as described in our first major task. We will also continue experiments aimed at determining the expression of this pathway using additional TNBC patient derived xenografts from Caucasians and African Americans (Major Task 2, Subtask 4). We will also continue to treat TNBC cell lines with JAK1 and CDK4 inhibitors and measure proliferation and apoptosis (Major Task 3, Subtask 2).

4. IMPACT

Impact on Principal Discipline

Our current work will be incredibly impactful for those studying breast cancer aggression in vitro and in vivo. We have uncovered a novel pathway underlying the ability of breast cancer epithelial cells to proliferate at a high rate and readily form transformed colonies in soft agar. These are all hallmarks of aggressive tumors. In year 2, we will move these findings into a more relevant in vivo model system, hoping to underscore the importance of this pathway in tumor aggressiveness and metastasis.

Impact on Other Disciplines

Nothing to Report

Impact on Technology Transfer

Nothing to Report

Impact on Society

We have disseminated the data and ideals from this grant proposal to several groups in the St. Louis community. They were encouraged by our progress and excited about the future clinical impact our work might provide.

5. CHANGES/PROBLEMS

Changes in Approach

Nothing to Report

Anticipated Problems or Delays

We have had a delay in the generation of the mouse model due to our inability to get ACURO approval. However, we have now completed the long form of the ACURO and plan to submit it in April 2019. Animal work will begin once this is approved.

Changes in Human, Animal, Biohazards and/or Selective Agents

Nothing to Report

6. PRODUCTS

Publications, Conference Papers and Presentations

Nothing to Report

Internet Sites
Nothing to Report

Technologies or Techniques
Nothing to Report

Inventions, Patents and/or Licenses
Nothing to Report

7. PARTICIPANTS

Individuals That Have Worked on Project

Name:	Jason D. Weber
Project Role:	PI
Nearest person month worked:	1.2
Contribution to Project:	Dr. Weber served as the mentor for Dr. Kung in planning all experiments and overseeing the final data analysis.
Funding Support:	NIH R01CA190986

Name:	Shunqiang Li
Project Role:	Co-Investigator
Nearest person month worked:	0.6
Contribution to Project:	Dr. Li is growing all of the patient-derived tumors for both specific aims
Funding Support:	None

Name:	Pat Kung
Project Role:	Staff Scientist
Nearest person month worked:	12
Contribution to Project:	Dr. Kung was responsible for all of the in vitro assays in year 1 (Major Task 3) and the immunohistochemistry (Major Task 2).
Funding Support:	None

Changes in Active Other Support for PD/PI
Nothing to Report

Other Organizations Involved as Partners
Nothing to Report