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14. ABSTRACT
The objective of this proposed study is to investigate a role for HER2/3 activation in MutLdefective ER+ breast cancer progression and resistance to endocrine therapy. By targeting HER2/3 signaling and key nodes of adaptive kinome response, we aim to significantly improve patient disease-specific survival.

15. SUBJECT TERMS
HER2 inhibitors, endocrine treatment resistance, growth factor signaling, ER+ breast cancer, DNA damage repair, mismatch repair

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1. INTRODUCTION

More than 70% of breast cancer is estrogen receptor positive (ER+) and is treated with endocrine therapy, which targets the ER-pathway. While the majority of patients respond to treatment, ~30% of patients are resistant. This resistant subset is a significant contributor to the >40,000 breast cancer-related deaths that occur every year in the US. Activation of HER signaling has been previously suggested to induce endocrine therapy resistance. The HER family of tyrosine kinase receptors consists of EGFR, HER2, HER3 and HER4, and they are all known oncogenes and growth promoters. However, clinical trials incorporating drugs targeting EGFR/HER2 and/or downstream signaling pathways (PI3K/AKT/mTOR) into endocrine treatment regimens have obtained mixed results. This failure is potentially explained by a lack of predictive biomarkers to demarcate patients most likely to benefit from such targeted therapies.

Recently, we identified that loss of mismatch repair (MMR), specifically of the MutL complex consisting of MLH1, PMS1 and PMS2 genes, causes endocrine therapy resistance in ER+ breast cancer cells. To identify more efficacious, preferably cytotoxic therapeutic targets in MutL-defective ER+ breast tumors, we performed a proteomics screen on MCF7 cells stably engineered to downregulate MLH1, PMS1 or PMS2 (shMLH1, shPMS1, shPMS2) collectively termed shMutL cells. The proteomic response of these cells to the endocrine therapy, fulvestrant (an ER degrader), differed from that of control (shLuc) MCF7 cells in one important, druggable way: shMutL cells upregulated HER2/3 signaling. This finding is completely novel and presents a unique opportunity to exploit existing HER inhibitors to successfully treat endocrine therapy resistant ER+ breast cancer patients using rational drug combinations.

While HER2/3 inhibitors have been recommended for endocrine therapy resistant ER+ breast tumors in the past, clinical trials suggest that only an undefined subset of patients respond to this treatment, indicating a critical need for stratification based on predictive biomarkers. In the proposed study, we will investigate a role for MutL in predicting response to HER2/3 inhibitors in up to 30% of endocrine therapy resistant ER+/HER2- breast cancer.

2. KEYWORDS

HER2 inhibitors, endocrine treatment resistance, growth factor signaling, ER+ breast cancer, DNA damage repair, mismatch repair

3. ACCOMPLISHMENTS

What were the major goals of the project?

Major goals of the project were to (a) Validate activation of HER2/3 signaling in MutL-defective ER+/HER2- breast cancer cells (b) Investigate HER2/3 activation and signaling mechanisms in MutL-deficient ER+ breast cancer (c) Test efficacy of HER inhibition in decreasing MutL-defective ER+ breast cancer growth on endocrine treatment.

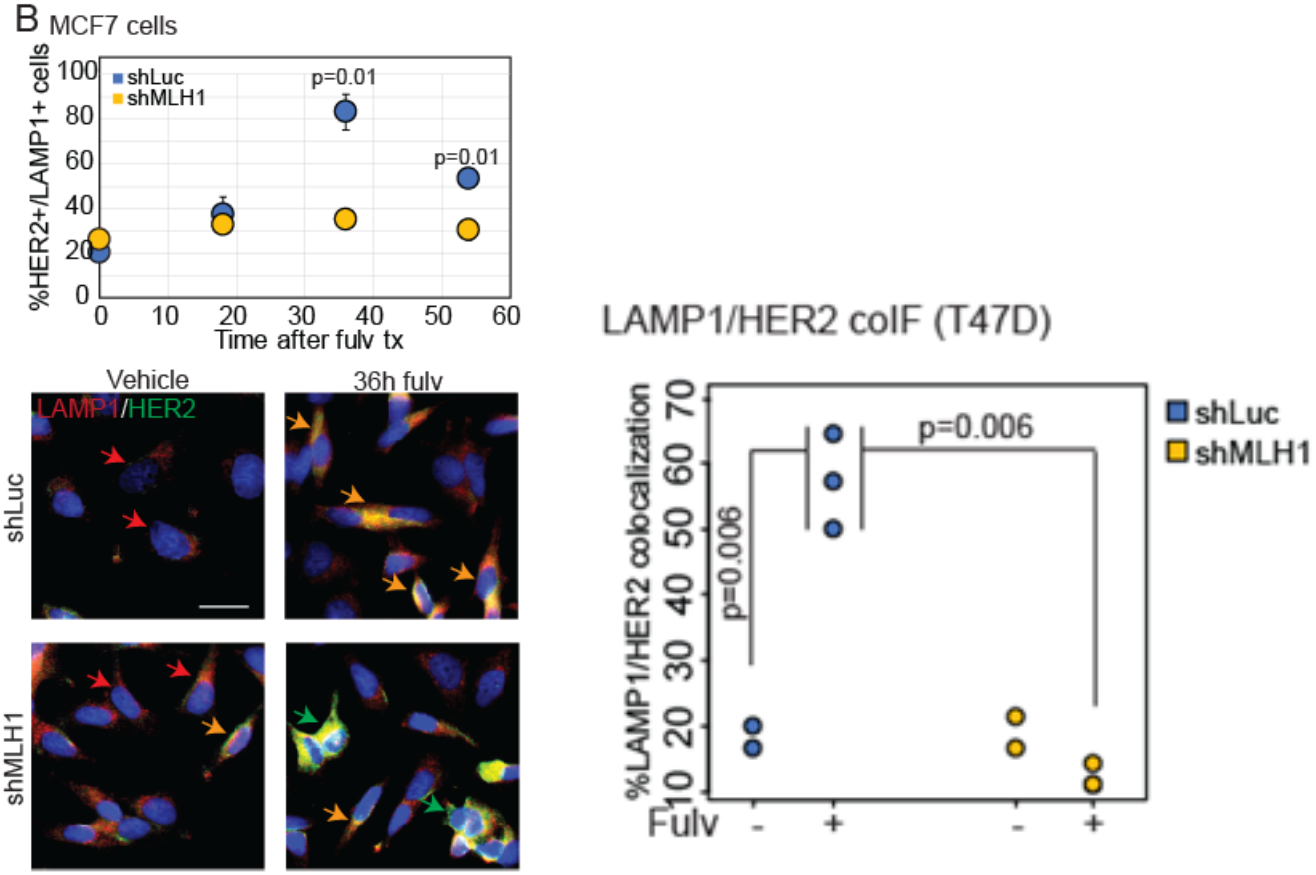
What was accomplished under these goals?

In year 3, milestone#5 was achieved in that a co-corresponding manuscript describing efficacy of HER2 inhibitors in MutL- ER+ breast cancer cells was submitted and has been accepted for publication in *Nature Communications.* In response to reviewer comments, we conducted additional experiments demonstrating a role for autophagy and protein degradation in the upregulation of HER2 in the shMLH1 cells (see Fig 1-3).

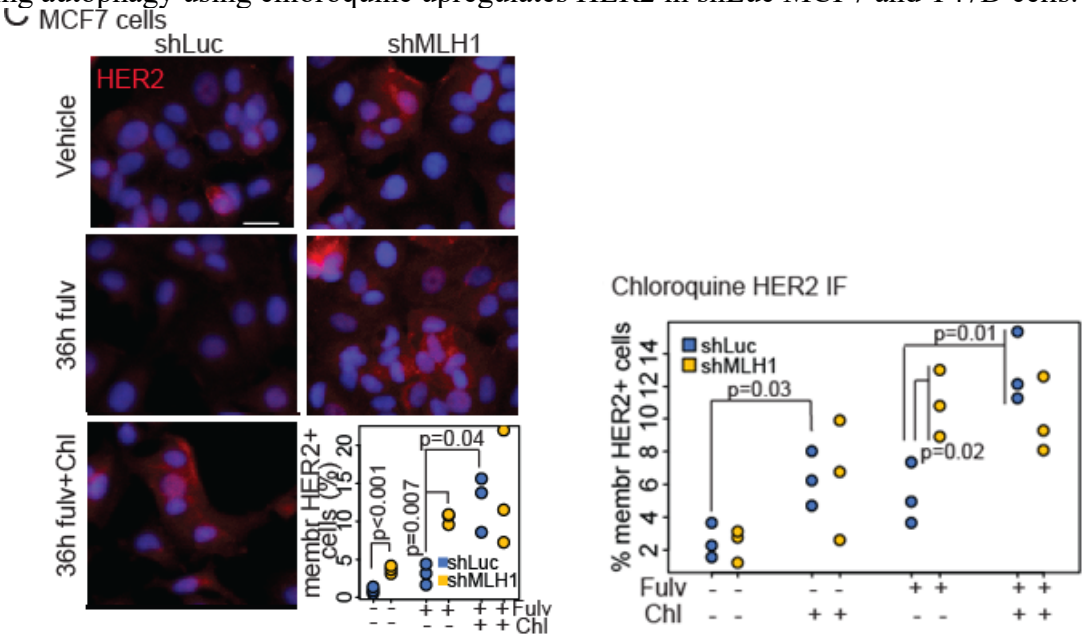
In terms of proposed experiments, Aim 1 Major Task 1 and Aim 2 Major Task 5 have been completed and the results included in the manuscript mentioned above. Aim 1 Major Task 2 is in the final phase of completion with

our collaborator Dr. Fusco completing the IHC optimization on patient tumor samples currently. We anticipate completing this task and writing up a manuscript describing these results in the next few months. For Aim 2 Major Task 3, we have validated the upregulation of BTC, NRG-1, NRG-2 and HB-EGF in both MCF7 and T47D shMLH1 cells. We are currently conducting experiments testing the sufficiency and necessity of these ligands for mediating the endocrine treatment resistance observed in shMLH1 cells. We are also working on creating HER2 and HER4 cDNA expressing shLuc cells, and stably downregulating HER2 and HER4 in shMLH1 cells to demonstrate the sufficiency and necessity of this heterodimerization for endocrine treatment resistance and sustained HER2 activation in shMLH1 cells. These results will be completed over the next four month and a manuscript describing these results will be submitted for peer reviewed publication.

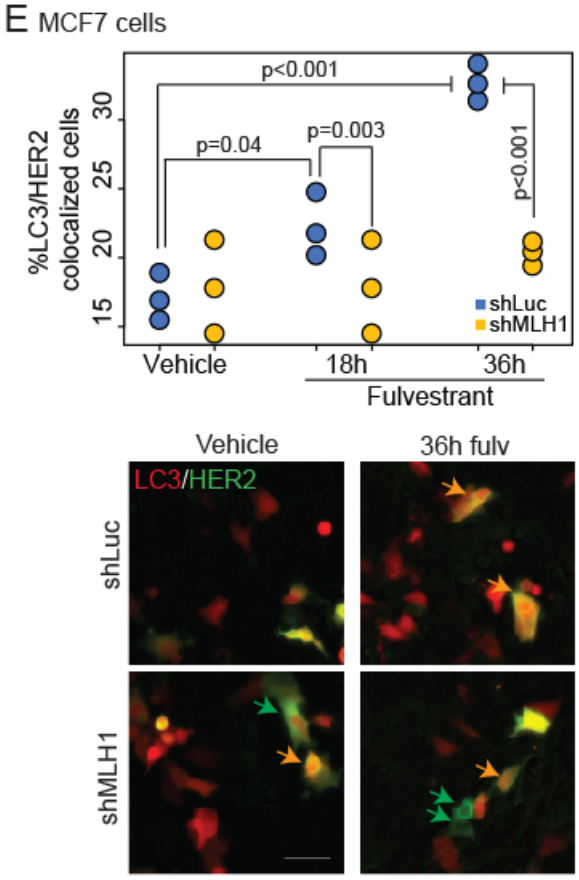
(Fig 1) LAMP1 colocalizes with HER2 in shLuc, but not in shMLH1 cells with fulvestrant treatment in MCF7 and T47D cells.



(Fig 2) Inhibiting autophagy using chloroquine upregulates HER2 in shLuc MCF7 and T47D cells.



(Fig 3) Live cell imaging shows colocalization of GFP-tagged HER2 with autophagosomes in shLuc MCF7 cells treated with fulvestrant, but not in shMLH1 counterparts.



In progress: (1) Necessity and sufficiency of HER ligands for HER2 activation, HER2/HER4 heterodimerization and endocrine treatment resistant growth of shMLH1 cells
(2) Necessity of HER2 and HER4 for endocrine treatment resistant growth in vitro and in vivo
(3) Sufficiency of HER2 and HER4 for endocrine treatment resistant growth in vitro

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

Ongoing collaborations with Drs. Fusco and Venetis resulted in an invited lecture at the University of Milan and a second review that is under peer review currently. It also resulted in an invitation to Dr. Haricharan to serve as guest editor for a special edition on DNA repair in breast cancer for Frontiers in Oncology.
The collaboration with Dr. Shaio has resulted in the submission of a DoD Breakthrough Level 3 application which was invited for full submission, but ultimately missed funding. This application has been resubmitted for consideration in 2021.
Dr. Haricharan also presented work funded by this DoD grant at SABCS in a spotlight session. This led to her being invited to submit a review on HER2 and DDR in breast cancer for publication in Endocrinology.
Dr. Haricharan also served as a reviewer for two DoD Breakthrough panels in 2020.

The Sanford Burnham Prebys Medical Discovery Institute (SBP) Office of Education, Training & International Services (OETIS) oversees and coordinates an annual individual development planning (IDP) process for all postdocs at the Institute. The focus of the IDP process at SBP is the career goal of the postdoc; identification of what skills, knowledge, and accomplishments will be necessary for the postdoc to obtain a desired independent position following training; and identification of training and professional development opportunities that are available for the postdoc to obtain the necessary skills and knowledge. OETIS provides guidance and advising to both postdocs and PIs throughout the postdoc's training with respect to developing IDPs and preparing for a successful transition to independence post-training. OETIS also maintains webpages containing comprehensive resources on career path identification, career planning, and creating an IDP that can be utilized in conjunction with the formal annual IDP process.
Dr. Haricharan and Dr. Mazumder participated in the IDP Process this past year.

How were the results disseminated to communities of interest?

Dr. Haricharan has presented this work at the SABCS in a Spotlights Session, and has been invited to write a review highlighting this work for publication in Endocrinology. She will also be presenting some of this work at two SBP-sponsored events intended for a lay audience in 2021.

What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period, functional experiments with testing sufficiency and necessity of identified ligands and HER2/HER4 will be conducted. These will constitute a second manuscript for submission. Additionally, IHC for MLH1 and HER2 in patient tumor samples will be completed with Dr. Fusco at the Institute of Milan and put together as a third manuscript.

4. IMPACT

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

We have demonstrated a novel mechanism by which HER2 is activated in DNA repair defective ER+ breast cancer cells with potential to change therapy in the clinic.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Breast cancer patients with MMR-defective ER+ disease might now be candidates for HER2 inhibitor therapy. An ongoing collaboration aims to conduct a proof of concept clinical trial to test this therapeutic hypothesis.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change.

Nothing to Report.

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

There has been a brief halt due to the COVID-19 outbreak in completing IHC on HER2 and HER4, but we anticipate completing this experiment in 2021 with no anticipated hurdles.

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

We have submitted an amendment to our IACUC protocol for ACURO review to test the role of HER2 in metastasis in MLH1- ER+ breast tumors. We had an ongoing experiment under this IACUC protocol and decided to complete a pilot survival surgery experiment with the same mice in order to reduce harm and distress to animals.

6. PRODUCTS

Publications, conference papers, and presentations.

1. SABCS Spotlight Session presenter (2020).

2. Punturi, N., Seker, S., Devarakonda, V., Mazumder, A., Kalra, R., Chen, C.H., Li, S., Primeau, T., Ellis, M.J., Kavuri, S.M., Haricharan, S. "Mismatch repair deficiency predicts response to HER2 blockade in HER2- negative breast cancer." *Nature Communications* (2021). Accepted for Publication. Acknowledgement of federal support yes.

3. Mazumder, A., Jimenez, A., Ellsworth, R.E., Freedland, S., George, S., Bainbridge, M., Haricharan, S. "Race specific differences in DNA damage repair dysregulation in breast cancer and association with outcome." bioRxiv (2020).

Website(s) or other Internet site(s).

Nothing to Report.

Technologies or techniques.

Nothing to Report.

Inventions, patent applications, and/or licenses.

1. Invention Disclosure 21-007, IDENTIFICATION OF PROGNOSTIC BIOMARKERS OF POOR OUTCOME SPECIFIC TO AFRICAN AMERICAN BREAST CANCER PATIENTS

2. Provisional Patent Application No. 63/106,777, filed 10/28/20202 - PROGNOSTIC BIOMARKERS FOR BREAST CANCER

Other products.

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Svasti Haricharan, PI – No change

Aloran Mazumder, Postdoctoral Associate – No change

Nindo Punturi, Research Assistant – No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Baylor College of Medicine, Dr. Kavuri, Partnering PI

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report.

9. APPENDICES

Nothing to Report.