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“Overcoming the Limitations of Endocrine Therapy for Ovarian Cancer”

PRINCIPAL INVESTIGATOR:

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CONTRACTING ORGANIZATION:

Dana-Farber Cancer Institute

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Epidemiological evidence suggests that steroid hormones may play a role in the pathogenesis of ovarian cancer. However, clinical trials utilizing endocrine therapies for the treatment of relapsed or recurrent ovarian cancer have largely failed to produce meaningful patient responses. Estrogen Receptor (ER) Alpha is a steroid hormone receptor that has been well characterized as a primary driver of both breast and endometrial cancers. However, despite the observation that the majority of high grade serous (HGSC) and endometrioid ovarian cancers express ER Alpha, the role of ER Alpha in ovarian cancer is not well understood. Our preliminary data have demonstrated that estradiol (E2) is sufficient to increase proliferation in the ER+ ovarian cancer cell line PEO1 and this increase in proliferation was attenuated by tamoxifen or fulvestrant. Furthermore, we found that E2 stimulates a transcriptome and cistrome in PEO1 cells that is distinct from that of breast cancer cells. We have also determined that the AP-1 transcription factor, FOSL2, is required for ER transcriptional activity and epigenetic remodeling at ER binding sites in ovarian cancer cells.

15. SUBJECT TERMS
Ovarian cancer, estrogen receptor, high grade serous cancer, endometrioid ovarian cancer, fulvestrant, tamoxifen, transcription

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1. Introduction

ERα is a ligand-dependent transcription factor that drives tumorigenesis in breast and endometrial cancers. ERα is the predominant isoform responsible for the pro-proliferative and anti-apoptotic effects of estrogen. Epidemiological evidence suggests that steroid hormones may play a role in the pathogenesis of ovarian cancer. Oral contraceptive use, pregnancy, and breastfeeding are associated with significantly decreased ovarian cancer risk while unopposed estrogen replacement therapy is associated with increased ovarian cancer risk. In addition, ERα is expressed in a substantial subset of ovarian cancers including 81% of high grade serous (HGSC) and 76% of endometrioid, 21% of mucinous and 20% of clear cell ovarian cancers. Preclinical studies investigating the role of ERα in ovarian cancer cell lines and mouse models have demonstrated that estrogen is sufficient to increase proliferation, invasion, migration, metastasis, and survival in a subset of models. While limited, clinical trials utilizing ER antagonists or aromatase inhibitors for the treatment of ovarian cancer have largely been disappointing. It is the aim of this proposal to decipher mechanisms underlying endocrine therapy resistance in ovarian cancer and to identify genetic targets that may be used in combination with endocrine therapy.

2. Keywords

Ovarian cancer, estrogen receptor, transcription, steroid hormone receptor, high grade serous ovarian cancer, endocrine therapy, fulvestrant, estrogen, estradiol

3. Accomplishments

Major Goals of Project:
Specific Aim 1: Determine estradiol-dependent essential gene networks and genetic sensitizers to ER antagonists through CRISPR/Cas9 genetic screens.
Specific Aim 2: Determine the mechanisms underlying ERα-dependent transcriptional activity in ovarian cancer.
Specific Aim 3: Determine the effects of combinatorial endocrine therapeutic approaches in HGSC PDX models.

Specific Aim 1: Determine estradiol-dependent essential gene networks and genetic sensitizers to ER antagonists through CRISPR/Cas9 genetic screens (~50% complete).

Results
CRISPR/Cas9 screening in ovarian cancer cells
In an effort to identify, genetic sensitizers to ER antagonists, we performed a whole-genome CRISPR screen in the presence and absence of the ER antagonist, fulvestrant. We performed this CRISPR screen in the ER+ HGSC cell line, PEO1. PEO1 cells are somewhat growth stimulated in response to the ER ligand, estradiol, and somewhat growth inhibited in response to fulvestrant (Figure 1).

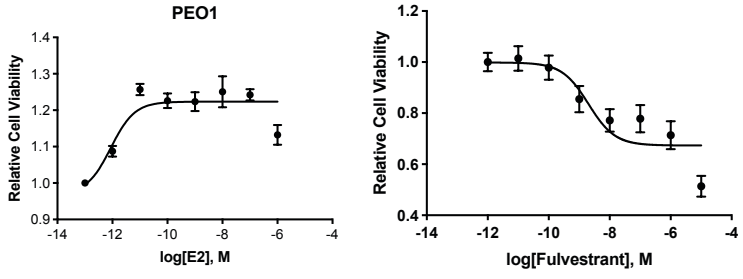

Figure 1. PEO1 cells are responsive to estradiol and fulvestrant.

The CRISPR screen revealed a number of common essential pathways that were negatively selected between the Vehicle and Fulvestrant treatment groups including the hallmark MYC, DNA repair, mTORC1 signaling, and P13K/Akt

signaling pathways. Additionally, in the Fulvestrant treatment group, we observed a strong expansion of positively selected genes whose pathways included hallmark Hedgehog signaling, epithelial mesenchymal transition, KRas signaling, and estrogen response pathways (Figure 2). Additionally, we identified that a number of the transcription factor, AP-1, family members were selected in the Fulvestrant treatment group but not the Vehicle treatment group. Specifically, FOSL1 and FOSL2 were the AP-1 family members that were the strongest negatively selected and positively selected, respectively (Figure 3). Notably, the most prominent ER transcriptional co-factors in breast cancer, FOXA1 and GATA3, are not essential in PEO1 cells. Furthermore, *ESR1* is essential in the Vehicle Control group but becomes more positively selected to where it is no longer essential in the Fulvestrant treatment group. Investigation into the roles of FOSL1 and FOSL2 on ER transcriptional activity is currently ongoing.

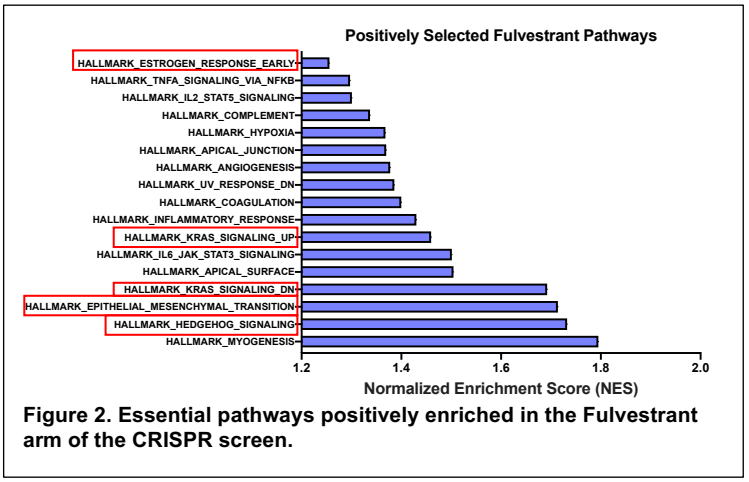

Figure 2. Essential pathways positively enriched in the Fulvestrant arm of the CRISPR screen.

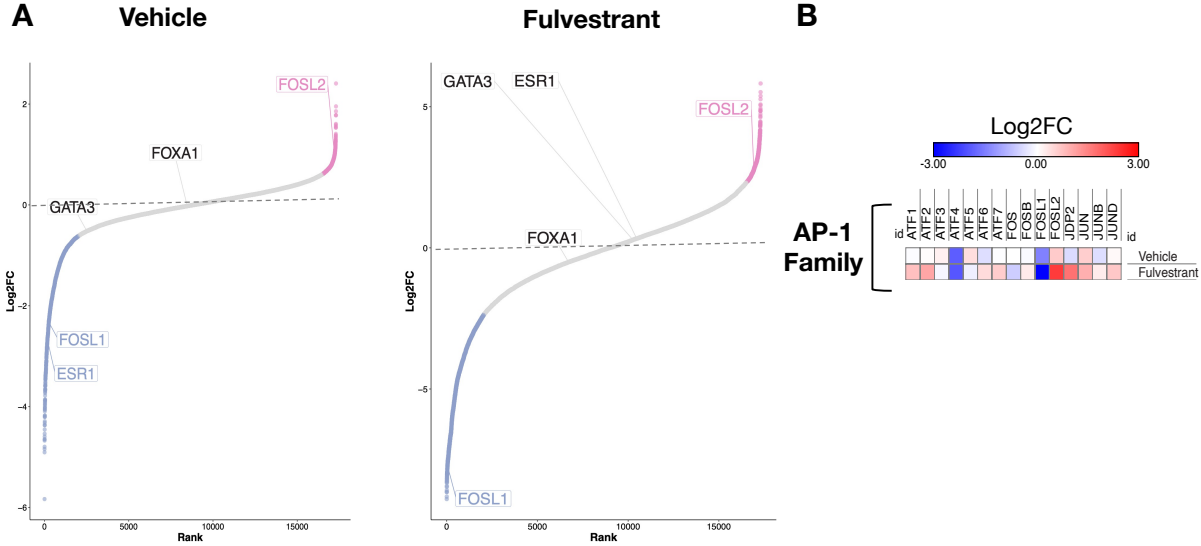

Figure 3. AP-1 family members are positively and negatively selected in the CRISPR Screen. A) Plot of essential genes in the Vehicle and Fulvestrant treatment groups, genes that are negatively selected are in blue and genes that are positively selected are in pink. B) Heatmap of AP-1 family members and their log2 Fold Change values between the Vehicle and Fulvestrant groups.

In order to identify genetic sensitizers to fulvestrant treatment, we performed a 9-square model analysis in which all genes from both treatment groups are plotted against each other (Figure 4). Genes that are negatively selected in the Fulvestrant treatment group but not the Vehicle treatment group are potential candidates for sensitizers to Fulvestrant. Through this analysis, we have identified DNA repair pathway genes, *XRCC1* and *PARP1*, that may be candidates for fulvestrant sensitization. The roles of these genes are currently ongoing.

Specific Aim 2: Determine the mechanisms underlying ERα-dependent transcriptional activity in ovarian cancer (~80% complete).

Transcriptome analysis of ER target genes in ovarian cancer cells

To identify the genes and pathways that are regulated by ER in ovarian cancer cells, we performed RNA-seq analysis in PEO1 cells. PEO1 cells were treated with 10 nM E2 for 24 hrs to identify the ERα regulated transcriptome. The majority (~66%) of differentially expressed genes in response to E2 were

downregulated (Figure 5). We then compared our ovarian cancer dataset to known published meta-analyses performed in MCF-7 cells. We found that the majority of differentially expressed genes in PEO1 cells did not overlap the MCF-7 dataset (Figure 6). These distinct genes were involved in non-canonical estrogen pathways such as epithelial to mesenchymal transition, WNT signaling, and VEGF signaling.

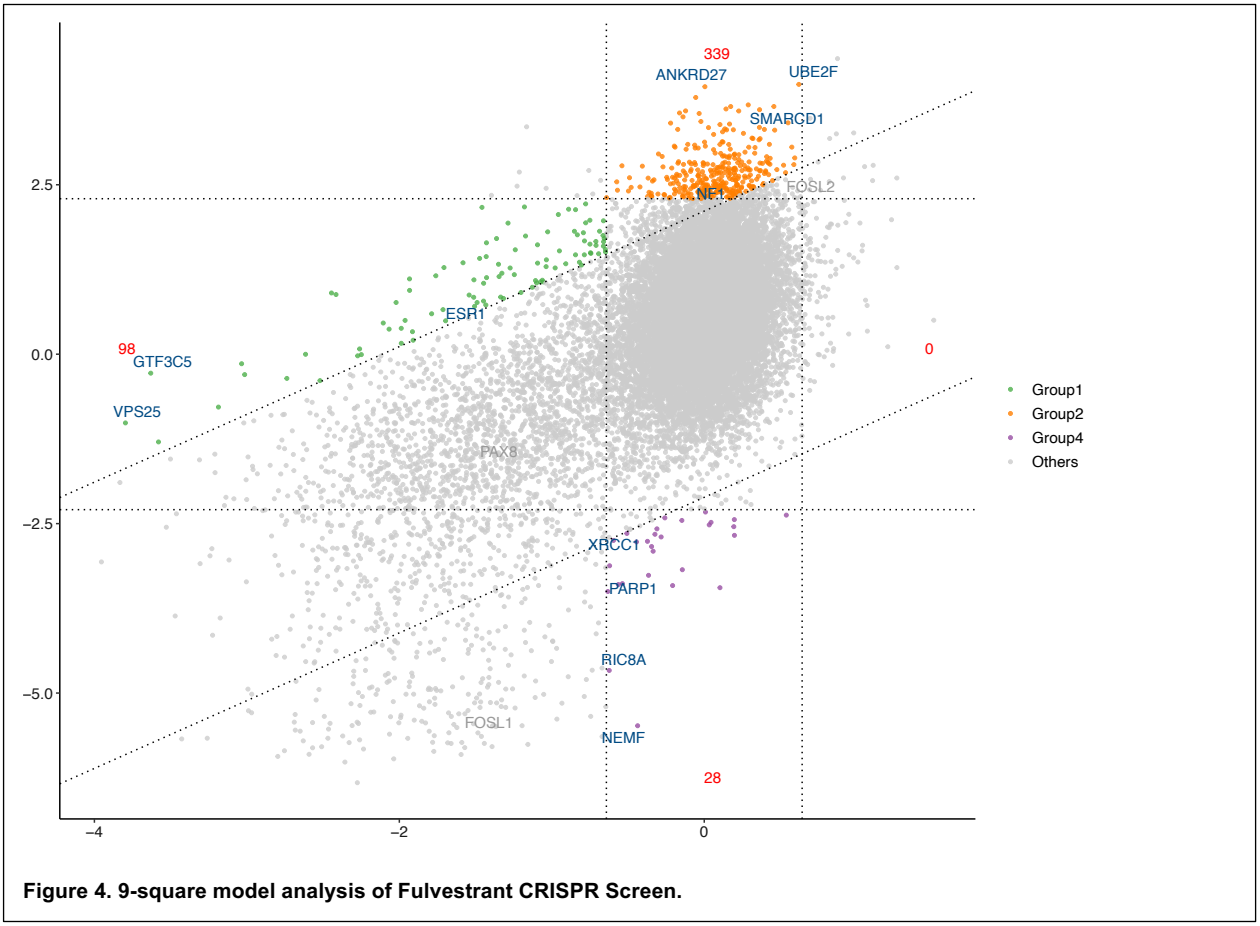

Figure 4. 9-square model analysis of Fulvestrant CRISPR Screen.

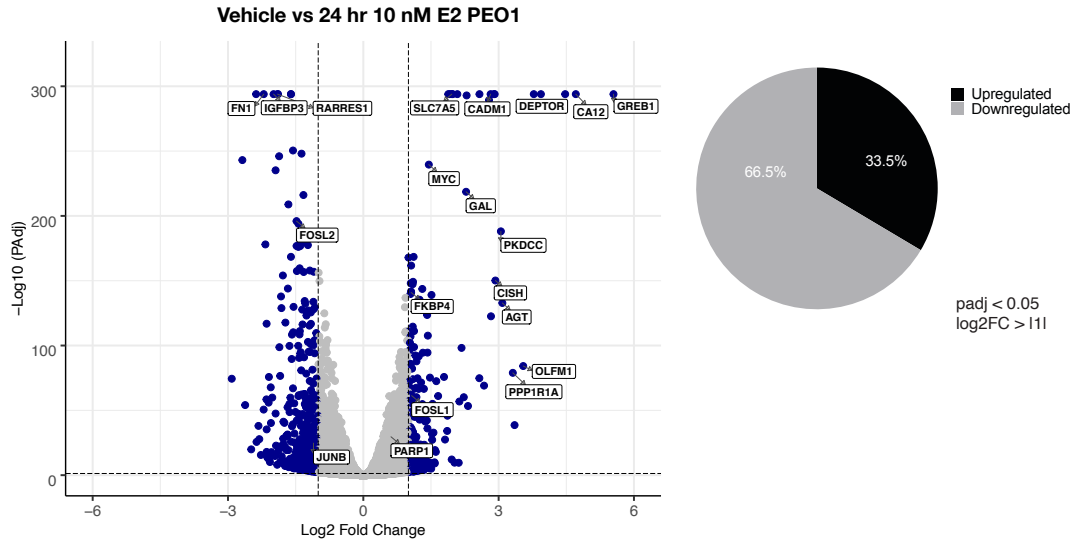

Figure 5. RNA-seq analysis of PEO1 cells treated with E2 for 24 hrs.

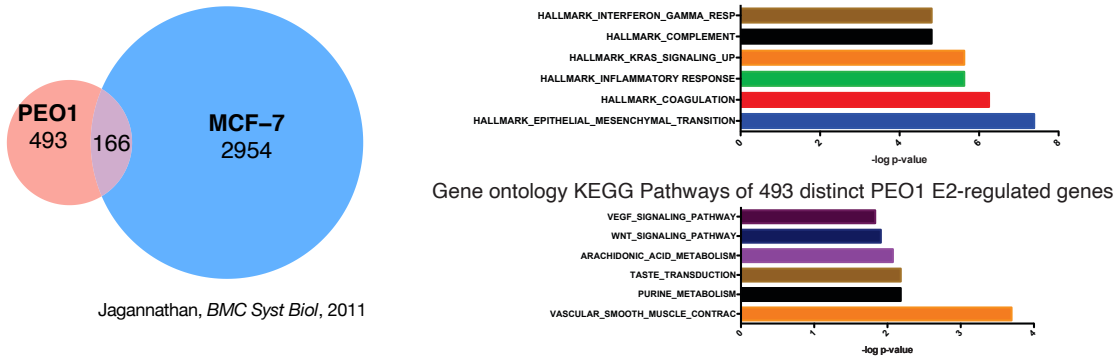

Figure 6. Comparison of PEO1 ER transcriptome to MCF-7 ER transcriptome. (left) Venn diagram showing the number of genes that overlap between the PEO1 and MCF-7 datasets. (right) Gene ontology analyses of the distinct PEO1 transcriptome.

Cistrome analysis of ER binding sites

To futher interrogate the mechanism of ERα transcriptional activity in ovarian cancer cells, we performed ChIP-seq in PEO1 cells treated with and without E2. We found that ERα is robustly recruited to its regulatory regions and demonstrates significantly increased binding in the presence of E2 (Figure 7A). Motif analysis was performed on the ERα binding sites and the top motifs all belonged to the AP-1 family of transcription factors. We additionally observed the canonical ER binding motif as among the top enriched motifs (Figure 7B). We then interrogated publicly available datasets to look for similarity between our PEO1 dataset and other previously published ChIP-seq datasets. The most similar dataset to our PEO1 dataset was a FOSL2 dataset, again indicating the potential importance of AP-1 transcription factors on ERα transcriptional activity (Figure 7C).

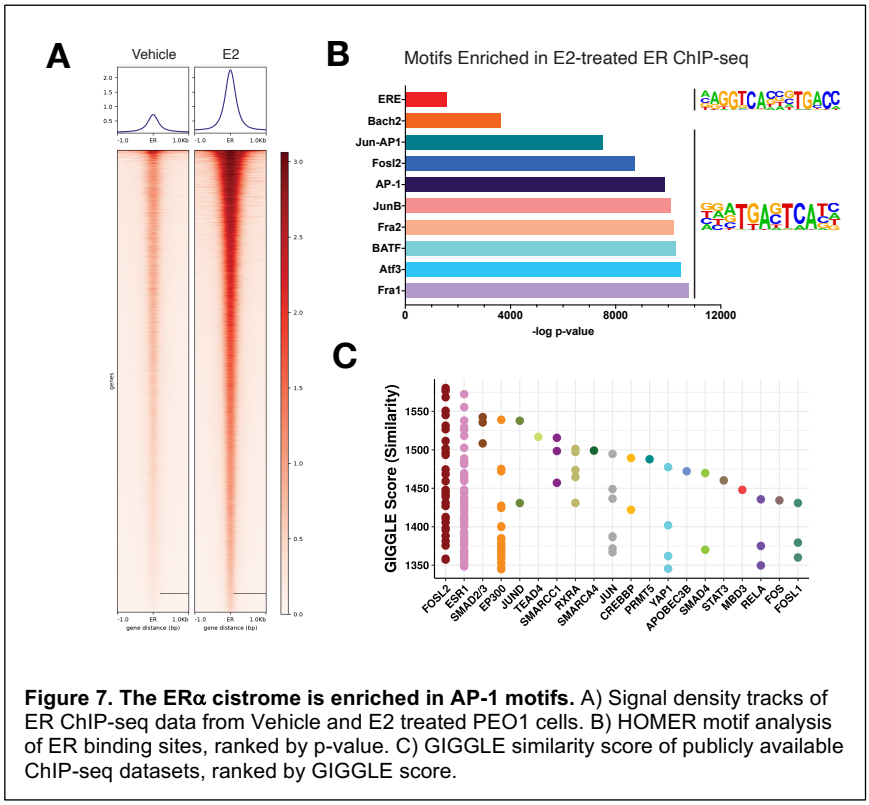

Figure 7. The ERα cistrome is enriched in AP-1 motifs. A) Signal density tracks of ER ChIP-seq data from Vehicle and E2 treated PEO1 cells. B) HOMER motif analysis of ER binding sites, ranked by p-value. C) GIGGLE similarity score of publicly available ChIP-seq datasets, ranked by GIGGLE score.

The role of FOSL2 in ERα-dependent transcription

The CRISPR screen and ERα ChIP-seq data indicated that FOSL2 may play a prominent role in regulating ERα activity. In order to investigate the dependence of ERα on FOSL2 activity, we prepared FOSL2 CRISPR knockout PEO1 cells and sgAAVS1 control knockout cells (Figure 8B). We then performed ERα ChIP-seq, H3K27ac ChIP-seq, and ATAC-seq on the sgAAVS1 and sgFOSL2 KO PEO1 cells (Figure 8A). We performed H3K27ac ChIP-seq to identify the active enhancers and we performed ATAC-seq to identify the open chromatin regions in an effort to determine if FOSL2 is required for chromatin remodeling at ER binding sites. We found that loss of FOSL2 resulted in altered ERα binding at approximately 25% of the total ER binding sites. These 25% of altered ERα binding

sites are both upregulated and downregulated in response to FOSL2 knockout. We overlaid these differential ERα binding sites with the corresponding H3K27ac and ATAC-seq data (Figure 8C). The signal aggregation plots of the ChIP-seq data revealed that loss of FOSL2 resulted in decreased H3K27ac signal and ATAC-seq signal at the ER downregulated peaks (Figure 8D). This data suggest that FOSL2 is required for ERα binding at a subset of sites, and induces epigenetic remodeling.

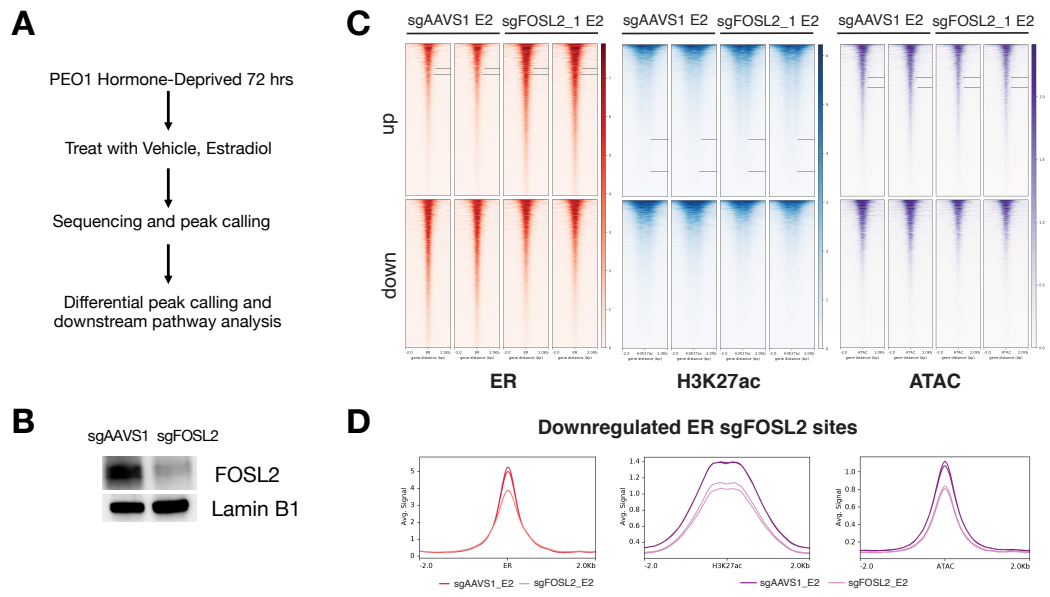

Figure 8. Loss of FOSL2 induces epigenetic remodeling at ER binding sites and alters the ER cistrome. A) Overview of ChIP-seq analyses. B) Western blot of sgAAVS1 and sgFOSL2 PEO1 cells immunoblotted for FOSL2 and LaminB1 expression. C) ChIP-seq signal intensity tracks of ER, H3K27ac, ATAC-seq FOSL2 ER differential peaks. D) Aggregation plot of ChIP-seq signals from ER, H3K27ac, and ATAC-seq.

We then performed gene set enrichment analysis of the sgFOSL2 differential ERα binding peaks to determine what genes and pathways are regulated by the ER/FOSL2 transcriptional complex. We identified that the Hallmark estrogen response early and late pathways were enriched among the sgFOSL2 differential ERα binding sites (Figure 9). Additionally, we observed that the epithelial to mesenchymal transition was also enriched in the sgFOSL2 differential ERα binding sites. The epithelial to mesenchymal transition was a pathway we identified from the PEO1 transcriptome analysis as being a non-classically regulated estrogen pathway. Altogether, these results indicate that ERα transcriptional activity is quite distinct in ovarian cancer cells. Our data indicates that ERα requires different cofactors for its transcriptional activity in ovarian cancer cells and may suggest a new mechanism for ERα action.

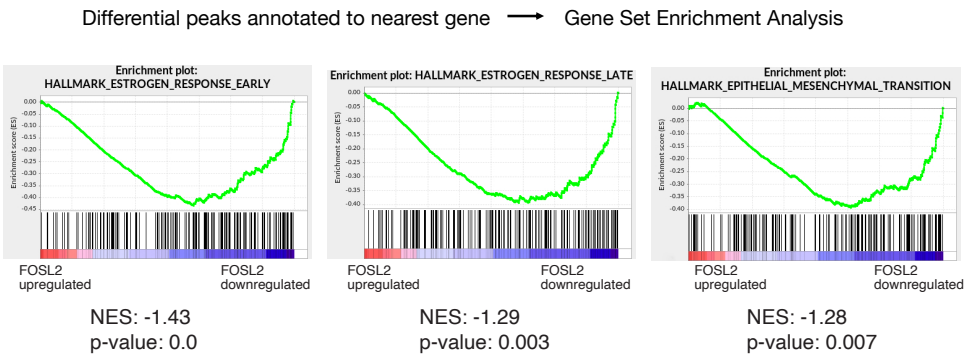

Figure 9. FOSL2 downregulated sites are associated with classical and non-classical estrogen regulated pathways.

<u>Specific Aim 3:</u> <u>Determine the effects of combinatorial endocrine therapeutic approaches in HGSC PDX models. (0% completed)</u>

We are currently awaiting validation of genetic targets from Specific Aim 1 before we begin the undertaking of testing in HGSC PDX models in mice.

<u>Future Plans</u>

In the next year, we hope to complete the validation from the CRISPR screen to identify genetic targets that may sensitize cells to fulvestrant. We would like to also complete another CRISPR screen in additional ovarian cancer cell lines. Furthermore, we will perform ER ChIP-seq in additional ovarian cancer cell lines to determine if AP-1 factors are required for ER activity in these additional models of ovarian cancer. Finally, after completing validation of genetic sensitizers to fulvestrant, we would like to begin testing of a combinatorial endocrine therapy in the HGSC PDX models.

Training Opportunities
Nothing to Report

Result Disseminated
Nothing to Report

4. Impact

Impact on the Development of Principle Discipline
Our proposal seeks to better understand the mechanisms behind endocrine insensitivity in ovarian cancer despite the high expression of ERα. We have begun to parse out these mechanisms of endocrine insensitivity through comprehensive CRISPR/Cas9 genetic screens. From this data, we will identify candidate genes that when inhibited, synergize with endocrine agents to further reduce ovarian cancer cell proliferation and tumor growth. Additionally, we have uncovered a novel mechanism by which the AP-1 transcription factor, FOSL2, governs ERα-dependent transcriptional activity. This understanding of the determinants of the ER transcriptional complex in ovarian cancer could provide additional points of therapeutic vulnerability to improve endocrine therapy. Endocrine therapies have been studied and used clinically for decades in the treatment of hormone- dependent breast cancers and gynecologic malignancies. ERα targeted therapies such as fulvestrant are highly selective for ERα and are generally well-tolerated and have few adverse effects. Furthermore, endocrine therapies can be taken long-term with few deleterious effects. In addition, novel ER antagonists are currently in clinical development for the treatment of breast cancer. These therapies could be rapidly translated into the clinic for the treatment of ovarian cancer as part of novel combinations. Our study could provide a new paradigm for treatment based on an understanding of the hormone dependence of ovarian cancer.

Impact on Other Disciplines, Technology Transfer, Society
Nothing to Report

5. Changes/Problems

Changes in Approach
Nothing to Report

Actual/anticipated problems or delays
We anticipate that the global COVID-19 pandemic will potentially result in delays in the acquisition of new data. Restrictions have been implemented by our institution regarding the number of researchers allowed in each lab at any one time, and therefore, we anticipate that experiments will be delayed. Additionally, because travel is currently discouraged and large gatherings are banned, we also anticipate that attendance and presentation at large meetings will also be affected in the coming year.

Changes that had a significant impact on expenditures
Nothing to Report

Significant changes in use of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report

6. Products

Conference Presentations:
Lee I. and Brown M. (June 2020). Characterizing the dependence of AP-1 transcription factors on Estrogen Receptor Alpha transcriptional activity and Fulvestrant sensitivity in ovarian cancer cells. AACR Annual Virtual Meeting II. (*Oral Presentation)*

Lee I. and Brown M. (February 2019). Overcoming the Limitations of Endocrine Therapy for Ovarian Cancer. 11th AACR-JCA Joint Conference on Breakthroughs in Cancer Research: From Biology to Precision Medicine. Maui, HI. *(Poster Presentation)*

Lee I. and Brown M. (February 2019). Overcoming the Limitations of Endocrine Therapy for Ovarian Cancer. Center for Functional Cancer Epigenetics Seminar. Dana Farber Cancer Institute, Boston, MA. (*Oral Presentation)*

Other Products
Nothing to Report

7. Participants & Other Collaborating Organizations

Individuals who have worked on project
Name: Myles Brown, MD
Project Role: PI
Nearest person month worked: 2
Contribution to Project: Dr. Brown is the PI on this project and has provided guidance and supervision for the project

Name: Theodore Saydah, BS
Project Role: Research Technician
Nearest person month worked: 5
Contribution to Project: Mr. Saydah has executed the experiments in support of this project.

Other organizations involved as partners
Organization Name: Harvard Medical School
Location of Organization: Boston, MA, USA
Partner's contribution to project: collaboration

Change to Active Support
Nothing to Report

8. Special Reporting Requirements

Nothing to Report

9. Appendices

Nothing to Report