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TITLE: Targeting the Lysosomal Acid Lipase A to "Stress Out" Ovarian Cancer

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CONTRACTING ORGANIZATION: University of Texas Health Science Center at San Antonio

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14. ABSTRACT

Ovarian cancer (OCa) is the deadliest of all gynecologic cancers in the United States. The overall survival for metastatic OCa is dismal (<20% 5-year survival) and has not changed significantly for decades. Intratumoral and intertumoral heterogeneity of OCa drives tumor resistance to standard therapies and represents a significant clinical challenge. We had identified a small molecule, ERX-41, that consistently enhances endoplasmic reticulum stress (ERS) and apoptosis in OCa cells. Our overarching hypothesis is that the high basal ERS in OCa represents a critical and targetable vulnerability. First-year experiments employing numerous established and primary ovarian cancer cells revealed that treatment with ERX-41 reduced cell viability, decreased colony formation, and increased apoptosis of OCa cells. The expression of LIPA is necessary for ERX-41's action because activity of ERX-41 was rendered inactive by LIPA knockout. Treatment of OCa cells with ERX-41 promoted ERS, as seen by increased sXBP1 splicing and increased ATF4 expression. Additionally, RT-qPCR analyses supported the activation of genes related to each of the three main UPR response stress pathways. Ongoing studies further examined mechanisms and validate the utility using preclinical models. Overall, the first-year data support the use of ERX-41 as a novel targeted therapy that specifically targets the ERS vulnerability in ovarian cancer.

15. SUBJECT TERMS
Ovarian cancer, LIPA, ER stress, UPR, ERX-41

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TABLE OF CONTENTS

1. Introduction

Ovarian cancer (OCa) is the deadliest of all gynecologic cancers in the United States. The overall survival for metastatic OCa is dismal (<20% 5-year survival) and has not changed significantly for several decades. Intratumoral and intertumoral heterogeneity of OCa at the molecular, cellular, and tissue levels drives tumor resistance to standard therapies and represents a significant clinical challenge. There is a desperate and unmet need for new therapeutic approaches to target fundamental and critical pathways in OCa that will effectively cause OCa cell death and improve survival of OCa patients.

OCa cells have a high growth rate and have a sustained and enhanced demand for de novo protein synthesis, folding and maturation. Since proper folding and post-translational maturation of most cellular proteins occurs in the endoplasmic reticulum (ER), OCa cells have a high basal level of ER stress (ERS). Several genome-scale genetic screens with short hairpin RNAs (shRNAs) identified components of the ERS and unfolded protein response (UPR) pathways as targets of vulnerability in OCa. While activation of UPR enables the OCa cells to compensate for higher levels of ERS and maintain OCa cell viability, sustained unresolved ERS can be lethal to OCa cells via induction of the CCAAT-enhancer binding protein homologous protein (CHOP) pathway and apoptosis. We thus reasoned that the enhanced basal level of ERS in OCa represents a critical vulnerability and drugs that further aggravate this already engaged system can exhaust the protective features of UPR and cause apoptosis.

We identified a small molecule, ERX-41, that enhances ERS and apoptosis in OCa cells. Using a whole genome CRISPR knockout screen, we identified lysosomal acid lipase A gene (LIPA) as critical for ERX-41 activity in inducing ERS and apoptosis. Depletion of LIPA abrogates the ability of ERX-41 to induce ERS or cell death in OCa cells. However, the precise molecular mechanism of how ERX-41 interaction with LIPA causes ERS and cell death is not known. Successful completion of these proposed studies will fill the knowledge gap as to how the novel therapeutic agent ERX-41 targets LIPA and causes ERS, and establish the preclinical data needed for further clinical translation.

The overarching hypothesis that the high basal ERS in OCa represents a critical and targetable vulnerability. The objective of this proposal is to conduct mechanistic studies that define how ERX-41 targeting of LIPA contributes to ERS in OCa models. Our studies will establish the preclinical rationale to enable rational lead optimization for drugs targeting LIPA and inducing ERS, and for advancing ERX-41 to clinical trials to treat OCa.

2. Keywords

Ovarian cancer, LIPA, ER stress, UPR, ERX-41

3. Accomplishments

3-1. What were the major goals of the project?

Aim 1. Determine the molecular mechanisms by which LIPA contribute to ERS in OCa.

Aim 2: Establish ERX-41 as a novel therapeutic using preclinical and patient derived OCa models *in vitro*, *in vivo* and *ex vivo*.

3-2. What was accomplished under these goals?

We have achieved great progress in the proposed experiments, as described below, over the first 12 months of the project, from May 1, 2022, to April 30, 2023, and we are on track to finish the remaining experiments.

Major Task 1: Determine the molecular mechanisms by which LIPA contribute to ERS in OCa.

Subtask 1: Define which UPR compensatory pathway is critical for ERX-41 mediated OCa cell death. (Timeline 1-24 months)

ERX-41 treatment promoted activation of genes activated by UPR pathway sensors (PERK, IRE1a, ATP6). For these experiments, we used three OCa cells (OCa39, OCa30, and OVCAR3). For RT-qPCR analysis, total RNA from OCa cells treated with or without ERX-41 was extracted. The results demonstrated that ERX-41 treatment promoted a considerable induction of the three UPR sensory pathways (PERK, IRE, and ATF6) as measured by their downstream target genes including XBP1, ATF3, DDIT3 (CHOP), ATF4, and HSPA5.

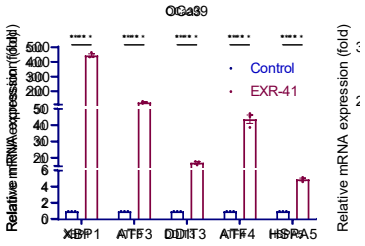

Figure 1. OCa cells were treated with ERX-41 (1 μM, 6h) and the status of ERS induced genes were measured by RT-qPCR (n=3). **p<0.01; ***p<0.001; ****p<0.0001

ERX-41 treatment enhances splicing of XBP1 in a time dependent manner. To examine the possibility that the IRE1a-XBP1 pathway contributes ERX-41 mediated cell death of OCa cells, we have treated three different OCa cells and profiled the splicing of XBP1 using agarose gel electrophoreses.

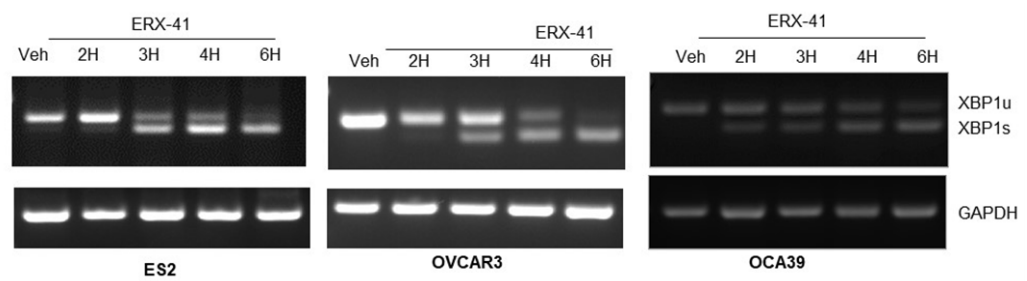

Figure 2. OCa cells were treated with ERX-41 for indicated time points and the status of splicing of XBP1, a well-established ER stress induced gene was measured by agarose gel electrophoresis (n=3)

Specifically, we have amplified the spliced and unspliced XBP1 mRNA using XBP1 primers. PCR products were separated on 2.5% agarose gel. GAPDH primers were used as control. Results showed time dependent splicing of XBP1 with earliest changes seen in 3h after ERX-41 treatment. Collectively, these studies suggest that ERX-41 treatment activates IRE1a-XBP signaling.

LIPA is needed for ERX-41 mediated splicing of XBP1. Our earlier studies using genome-wide CRISPR library screen identified LIPA as a potential target for ERX-41. To confirm the role of LIPA in ERX-41 mediated splicing of XBP1, we have utilized control and LIPA KO cells (Fig. 3A). In

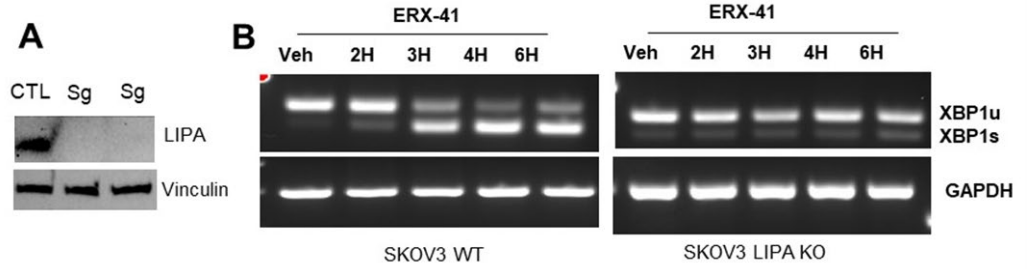

Figure 3. A. LIPA KO in SKOV3 cells was confirmed using Western blotting. B, SKOV3 WT and LIPA KO cells were treated with ERX-41 for indicated time points and the status of splicing of XBP1, a well-established ERS induced gene was measured by agarose gel electrophoresis (n=3).

control cells, we observed splicing of XBP1 in a time dependent manner upon ERX-41 treatment, while in LIPA KO, ERX41 treatment did not result in splicing of XBP1 (Fig. 3B). These results suggest that LIPA is critical for the ERX-41 mediated induction of stress.

ERX-41 treatment promotes activation of PERK downstream target ATF4. Here, we examined that ERX-41 treatment promotes activation PERK-ATF4 axis using Western blotting. The results showed that ERX-41 treatment of ovarian cancer cell line (OCa39) enhanced the expression of ATF4 in a time dependent manner (Fig. 4A). We also confirmed ATF4 activation using the second OCa model cell line (ES2). Further, we also observed increased expression of CHOP and phosphorylation of pEIF2a in ERX-41 treated cells (Fig. 4B). Collectively, these results imply that ERX-41 also activate PERK-ATF4 axis in addition to IRE1a-XBP signaling.

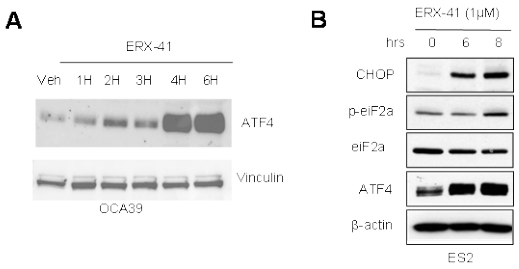

Figure 4. OCa cells were treated with ERX-41 for indicated time points and the status of the activation of UPR components in OCa cells analyzed by western blotting.

Multi-PI contributions: The above work was jointly performed by Dr. Vadlamudi and Dr. Raj labs. Dr. Raj lab generated CRISPR KO cells, provided needed ERX-41 and profiled the expression changes by ERX-41. Dr. Vadlamudi's lab conducted XBP1 splicing, Western, and RT-qPCR assays.

Subtask 2: Define ultra structurally how ERX-41 binding to LIPA causes ERS. (Timeline 13-36 months)

These studies will be initiated during second year.

Subtask 3: Define molecularly how ERX-41 binding to LIPA causes ERS. (Timeline 21-48 months)

These studies will be initiated during third year.

Subtask 4: Unbiased transcriptomic and proteomic approaches to understand how LIPA causes ERS in OCa cells. (Timeline 21-48 months)

These studies will be initiated end of second year.

Major Task 2. ERX-41 as a novel therapeutic using preclinical and patient derived OCa models *in vitro*, *in vivo* and *ex vivo*.

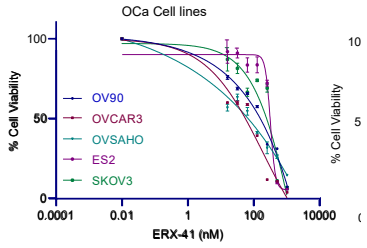

Figure 5. Effect of increasing doses of ERX-41 on the viability of OCa cells (ES2, SKOV3, OV90, OVSAHO, and OVCAR3), primary OCa cells (OCa30, OCa39, and OCa49) measured by MTT assay after seven days of ERX-41 treatment.

Subtask 1:
2.1.1 Test the utility of ERX-41 using *in vitro* biological assays. 2.1.2 Test the utility of ERX-41 using OCa orthotopic xenograft studies. 2.1.3. Test the utility of ERX- 41 using OCa Syngeneic models. 2.1.4. Test the utility of ERX-41 in combination with standard care chemotherapy. (Timeline 1-36 months)

ERX-41 reduced the cell viability of OCa cells. Here, we examined the efficacy of ERX-41 on cell viability of five established and three primary OCa cells. Treatment with ERX-41 resulted in a significant dose dependent reduction in the cell viability of OCa cells (IC_{50} of ~200 nM) (Fig. 5).

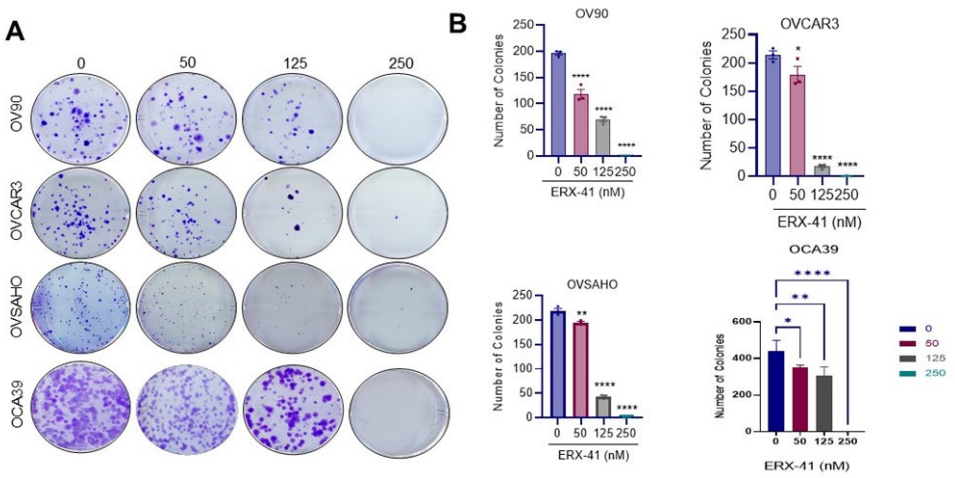

Figure 6. Effect of ERX 41 on the clonogenic colony formation following long-term treatment with indicated doses of ERX-41. *p<0.05; **p<0.01; ****p<0.0001

ERX-41 reduced clonogenic cells. Here, we examined the efficacy of the ERX-41 using four different OCa cells. In clonogenic survival assays, ERX-41 significantly reduced the colony formation ability of OV90, OVCAR3, OVSAHO, OCa39 cells in a dose dependent manner (Fig. 6).

However, ERX-41 failed to reduce colony formation ability of LIPA KO cells (Fig. 7). Collectively, these results suggest that LIPA is needed for ERX-41 mediated reduction in colony formation.

ERX-41 treatment induced apoptosis of OCa cells. The Annexin V assay was then used to determine if ERX-41 caused apoptosis in five distinct OCa cells. The death of OCa cells was markedly accelerated by ERX-41 treatment (Fig. 8). These findings collectively imply that ERX-41 promotes apoptosis.

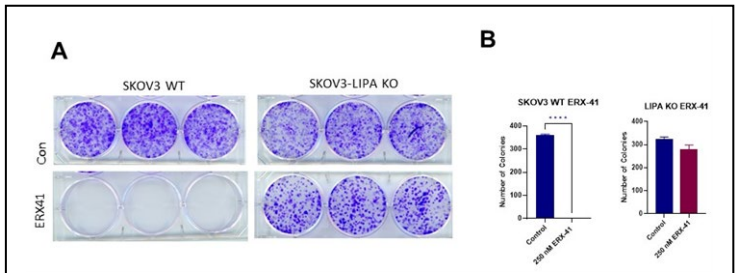

Figure 7. A, Effect of LIPA KO on the activity of ERX 41 in blocking the long-term colony formation. B, Quantitation of colonies were shown. ****p<0.0001

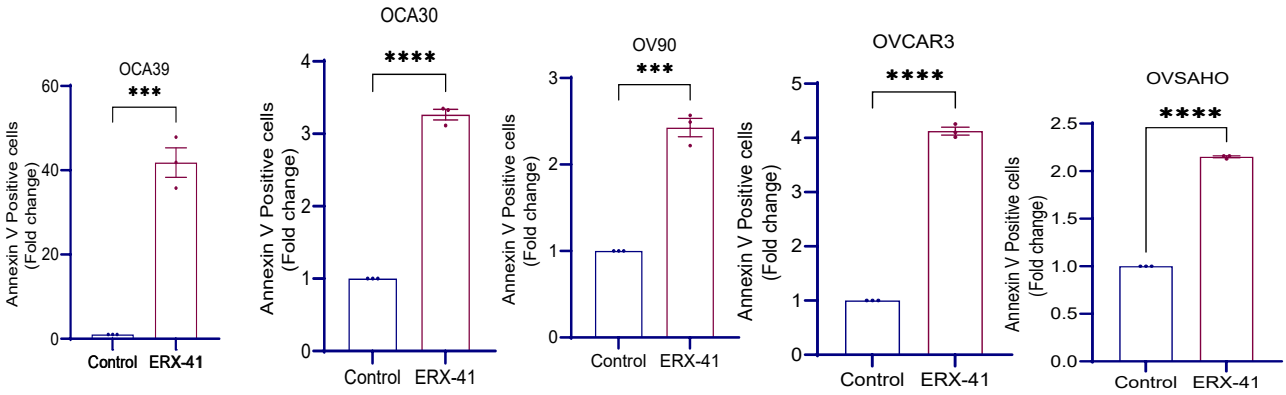

Figure 8. Effect of ERX-41 on apoptosis of OCa cells was measured using Annexin V Assays. ***p<0.001; ****p<0.0001

Multi-PI contributions: The above work was jointly performed by Dr. Vadlamudi and Dr. Raj labs. Dr. Raj lab provided ERX-41, established the initial IC_{50} using OCa cells and was involved in the design and analyses of assays conducted in Dr. Vadlamudi labs. Dr. Vadlamudi lab established the primary OCa cells, conducted cell viability, colony formation and apoptosis assays.

Subtask 2: Test the efficacy of ERX-41 in inducing ERS in OCa patient-derived organoids (PDOs) *in vitro*, patient derived explants (PDEs) *ex vivo*. (Timeline 1-36 months)

We have successfully procured tissues from ObGyn tissue core and frozen these tissues. These tissues will be used in the second and third year to establish organoids and to test the utility of ERX-41 for treating organoids.

Subtask 3: Test the efficacy of ERX-41 in preventing progression of OCa using PDXs *in vivo*. (Timeline 25-48 months)
These studies will be initiated during the third and fourth years.

3-3. What opportunities for training and professional development has the project provided?

Four students received training opportunities through this project during the first year: one graduate student, one post-baccalaureate student, one post-doctoral fellow, and one high school student. All the trainees/students received training in designing/analyzing research experiments, evaluating data, and conducting cancer research utilizing OCa model cells. Additionally, the post-doctoral fellow was given the chance to supervise graduate and undergraduate students, which gave him the chance to hone his mentoring abilities. The chance to assist high school kids was given to graduate students. All students took part in the weekly project meetings, and once a month they gave an oral presentation of their findings. Additionally, students and fellows were given numerous possibilities for professional growth, such as taking part in journal clubs, attending grand rounds, and attending an ethics seminar.

3-4. How were the results disseminated to communities of interest?
The results were presented as a poster presentation in April 2023 AACR annual meeting.

3-5. What **do you plan to do during the next reporting period to accomplish the goals?**

We will start performing the following tasks during second year:
Subtask 1: Define which UPR compensatory pathway is critical for ERX-41 mediated OCa cell death.
Subtask 2: Define ultra structurally how ERX-41 binding to LIPA causes ERS.
Subtask 3: Define molecularly how ERX-41 binding to LIPA causes ERS.
Subtask 2: (2.1.2) Test the utility of ERX-41 using OCa orthotopic xenograft studies.
Subtask 2: Test the efficacy of ERX-41 in inducing ERS in OCa patient-derived organoids (PDOs)

4. Impact

4-1. What was the impact on the development of the principal discipline(s) of the project?

Our findings showed that ERX-41 is very effective at reducing the viability of OCa cells. Additionally, ERX-41 potently stimulates ER stress in a variety of OCa cell subtypes, which results in cell death. Mechanistic investigations verified ERX-41's capacity to cause ERS by turning on several UPR arms. To fully comprehend ERX-41's potential, however, more preclinical, and mechanistic investigations that were suggested in years two through four need to be finished.

4-2. What was the impact on other disciplines?
Considering the expression of LIPA in multiple tumors, ERX-41 may have utility in treating other solid tumors such as endometrial cancer.

4-3. What was the impact on technology transfer?
Nothing to Report.

4-4. What was the impact on society beyond science and technology?

Nothing to Report.

5. Changes/Problems

5-1. Changes in approach and reasons for change.
Nothing to Report.

5-2. Actual or anticipated problems or delays and actions or plans to resolve them
None

5-3. Changes that had a significant impact on expenditures
Nothing to Report.

5-4. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report

6. Products

Collier AB, Viswanadhapalli S, Lee TK, Kassees K, Parra K, Sharma G, Reese T, Hsieh M, Liu X, Yang X, Ebrahimi B, Pratap UP, Gopalam R, Chen CY, Elmore ST, Sareddy GR, Kost ER, Ahn JA, Raj GV, Vadlamudi RK. Novel LIPA targeted therapy for treating ovarian cancer[abstract]. In: Proceedings of the 114th Annual Meeting of the American Association for Cancer Research; 2023 April 14-19; Orlando, FL. Philadelphia (PA): AACR; 2023. Abstract nr 3986.

7. Participants & Other Collaborating Organizations

Name	Project Role	Effort (mo.)	Site	Contribution to Project	Other Funding Support and Changes in the Reporting Period
Dr. Ratna Vadlamudi	Initiating PI	0.8	UTHSCSA	Experiment design and execution, project supervision, data analysis, data presentation	Active: RO1CA266970-01A1 (1.1 cm); R44CA250961 (0.26 cm); RO1CA262757 (0.8 cm); R01 CA267893 (0.62cm); R01CA269866 (1.2 cm); 1 I01 BX004545-01 (5 cm)
Dr. Suryavathi Viswanadhapalli	Co-investigator	1.2	UTHSCSA	Experiment design, data analysis and presentation	Active: 1R44CA250961 (0.48; R01 CA267893 93.7 cm); R01 CA262757 (1.32); R01 CA266970 (3.2); R01CA269866 (0.6cm)
Alondra Lee Rodriguez Sanchez	Research associate	6 cm	UTHSCSA	Experiment execution, data analysis and presentation	Active: None to report
Dr. Ganesh Raj	Partnering PI	0.9	UTSW	Experiment design, project supervision, data analysis and presentation	Active: 2P30CA142543-11(1.2 cm); 1R01-CA223828-01 (1.2 cm); W81XWH2110520 (0.6 cm); W81XWH-21-1-0687 (1.8 cm); 1R01CA262757-01A1 (1.8 cm)
Sharma Guarav	Research associate		UTSW	Experiment design, data analysis	None to report.

8. Special Reporting Requirements

Nothing to report

9. Appendices

PDF copy of the abstract attached.

Add to My Itinerary

Session PO.ET01.03 - Oncogenes and Tumor Suppressor Genes as Targets for Therapy 3

3986 / 9 - Novel LIPA targeted therapy for treating ovarian cancer

April 18, 2023, 9:00 AM - 12:30 PM | Section 19

Presenter/Authors

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Disclosures

A. B. Collier, None..
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G. Sharma, None..
T. Reese, None..
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X. Yang, None..
B. Ebrahimi, None..
U. P. Pratap, None..
R. Gopalam, None..
C. Chen, None..
S. T. Elmore, None..
G. R. Sareddy, None..
E. R. Kost, None.
J. Ahn,
EtiraRx Patent.
G. V. Raj,
EtiraRx Patent.
R. K. Vadlamudi,
EtiraRx Patent.

Abstract

BACKGROUND: Ovarian cancer (OCa) is the deadliest of all gynecologic cancers in the United States. Currently approved therapies have improved OCa survival for clinically localized disease, however, the majority (~90%) of patients with high-grade serous OCa (HGSOC) experience relapse with incurable metastases. There is a dire need for new therapeutic approaches. We hypothesized that the high basal endoplasmic reticulum stress (ERS) in OCa represents a critical and targetable vulnerability and may overcome the tumor heterogeneity. The objective of this project is to exploit increased ERS in ovarian cancer cells by engaging the novel target LIPA using the unique compound ERX-41.

METHODS: The utility of ERX-41 as a new therapy was evaluated using MTT and CellTiter-Glo Cell Viability Assays. We used multiple established and patient derived OCa cell lines. The effect of ERX-41 on the Cell viability of patient-derived organoids (PDO) was measured using CellTiter-Glo 3D Assay. Long term effects of ERX-41 on cell survival were measured using colony formation assays. Apoptosis was measured using Annexin V and Caspase-Glo® 3/7 Assays. Cell cycle analysis was analyzed by Flow Cytometry. Mechanistic studies were done using LIPA knockout (KO) cells, RT-qPCR, and western blotting. Status of LIPA in OCa was determined using TNMplot database. *In vivo* efficacy of ERX-41 was tested using both cell line derived (CDX) and patient derived (PDXs) xenografts.

RESULTS: TNM plot results showed that LIPA is highly expressed in OCa tumors compared to normal tissues and LIPA expression correlated with clinical grade. Kaplan-Meier plotter analyses of TCGA data revealed that LIPA expression is negatively correlated with overall survival in OCa patients. MTT and CellTitre-Glo assay results

showed that ERX-41 significantly reduced the cell viability of both established and primary OCa cells, and PDO's with an IC_{50} of ~500nM. ERX-41 treatment also significantly reduced the cell survival, increased S-phase arrest, and promoted apoptosis of OCa cells. A time course study revealed a robust and consistent induction of ERS markers (CHOP and sXBP1) in OCa cells by ERX-41 within 4h. Western blotting analyses also confirmed increased expression of ERS markers including CHOP, eIF2α, PERK, and ATF4 upon ERX-41 treatment confirming that ERX-41 induces ERS. In xenograft studies, ERX-41 treatment resulted in ~66% reduction of tumor volume measured by Xenogen-IVIS. Further, in studies using PDX tumors, treatment with ERX-41 resulted in a significant reduction (~60%) of tumor volume and tumor weight.

CONCLUSION: Collectively, our results suggest that ERX-41 is a novel therapeutic agent that targets the LIPA with a unique mechanism of action and implicate ERX-41 binding to LIPA induces ER stress, and apoptosis of OCa cells. Further molecular characterization of how ERX-41 binding to LIPA induces ER stress in OCa cells is ongoing.