AWARD NUMBER: W81XWH-18-1-0134

TITLE: Novel Tumor Suppressor Gene in Hereditary X-Linked Ovarian Cancers

PRINCIPAL INVESTIGATOR: Kevin Eng, PhD

CONTRACTING ORGANIZATION: Health Research, Inc., Buffalo, NY

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TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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**5b. GRANT NUMBER**
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**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Health Research, Inc.
Health Research-Roswell Park
Cancer Inst
Elm and Carlton St
Buffalo NY 14263-0001

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Development Command
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Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

Our previous work has identified an ovarian cancer risk locus on the X-chromosome and likely within the gene MAGEC3. The overall goal of this proposal is forward the idea that MAGEC3 is a tumor suppressor gene and to determine the scope and impact of the mechanism. Aim 1 is focused on studying the mechanism of MAGEC3 silencing in clinical samples. Aim 2 uses cell lines to study the function of MAGEC3 and Aim 3 will confirm our findings in mouse xenograft models. We determined that candidate tumor suppressor MAGEC3 is highly likely to be transiently expressed and cell cycle regulated with tight epigenetically-related expression of the protein increasing the confidence that it is a tumor suppressor gene. This result was achieved through the engineering of multiple cell lines with inducible MAGEC3 expression and transgene tags that will enable continuing RNA and protein level analyses. Single cell level expression analyses confirmed cell cycle association and flow cytometry protein-level analysis also supports these findings.

**15. SUBJECT TERMS**

Cancer antigen, cancer genetics, DNA repair, genetic epidemiology, ovarian cancer, tumor suppressor gene.

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRDC |
| Unclassified | Unclassified | Unclassified | Unclassified | 10 | 19b. TELEPHONE NUMBER *(include area code)* |

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## 1) Introduction

Our previous work has identified an ovarian cancer risk locus on the X-chromosome and likely within the gene MAGEC3. The overall goal of this proposal is forward the idea that MAGEC3 is a tumor suppressor gene and to determine the scope and impact of the mechanism. Aim 1 is focused on studying the mechanism of MAGEC3 silencing in clinical samples. Aim 2 uses cell lines to study the function of MAGEC3 and Aim 3 will confirm our findings in mouse xenograft models. We determined that candidate tumor suppressor MAGEC3 is highly likely to be transiently expressed and cell cycle regulated with tight epigenetically-related expression of the protein increasing the confidence that it is a tumor suppressor gene. This result was achieved through the engineering of multiple cell lines with inducible MAGEC3 expression and transgene tags that will enable continuing RNA and protein level analyses. Single cell level expression analyses confirmed cell cycle association and flow cytometry protein-level analysis also supports these findings.

## 2) Keywords

Cancer antigen, cancer genetics, DNA repair, genetic epidemiology, ovarian cancer, tumor suppressor gene.

## 3) Accomplishments

### What were the major goals of the project?

| Specific Aim 1 | Timeline | Progress |
|---|---|---|
| **Major Task 1 is to analyze selected familial cases from FOCR and RPCI Biobanks.** | | |
| Local IRB Approval: CIC95-27. | 0 | Complete |
| Sub Task 1. Conduct germline/somatic WES, X chromosome sequencing, RNA sequencing, Methylation. | 1-8 | Complete |
| Sub Task 2. Conduct genetic analyses. | 8-12 | Complete |
| **Major Task 2 is to analyze sporadic cases from the RPCI biobank.** | | |
| Sub Task 1. Receive and qualify WGS data from APOLLO collaborators. | 1-3 | Complete |
| Sub Task 2. Conduct germline/somatic WES, X chromosome sequencing, RNA sequencing, Methylation. | 1-6 | Complete |
| Sub Task 3. Conduct genetic analyses. | 6-10 | Complete |
| Sub Task 4. Correlative studies with clinical and pathological variables and outcomes. | 8-12 | Complete |
| **Specific Aim 2** | | |
| **Major Task 3 is to characterize ovarian cancer cell line phenotypes in response to MAGEC3** | | |
| Sub Task 1. Construct MAGEC3 shRNA knockdown lines | 13-18 | Complete |
| Sub Task 2. Construct lentiviral MAGEC3 lines | 13-18 | Complete |

| | | |
|---|---|---|
| Sub Task 3. Characterize cell lines' proliferative phenotype and MAGEC3 expression. Perform statistical analyses. | 18-30 | Partial – in progress |
| **Specific Aim 3** | | |
| **Major Task 4 is to assess the tumorigenic potential of modified MAGEC3 cell lines** | | |
| Sub Task 1. Obtain IACUC Approval | 18-21 | Complete |
| Sub Task 2. Pilot intrabursal study. | 21-24 | Complete |
| Sub Task 3. Perform xenograft studies, isolate and collect tumors. Conduct statistical analyses. | 24-36 | Partial – In progress |
| **Major Task 5 is to prepare manuscripts for submission** | | Complete |

**What was accomplished under these goals?**

**Accomplished under major task 1.**

Major Task 1 was completed in previous reporting period.

**Accomplished under major task 2**

Major Task 2 was completed in previous reporting period.

**Accomplished under major task 3**

Subtask 3 was not completed at the time of Dr. Eng's departure and no further updates are available.

**Accomplished under major task 4**

Subtask 3 was not completed at the time of Dr. Eng's departure and no further updates are available.

**Accomplishments under major task 5**
One publication is in print (Cancers (Basel) . 2022 Jan 30;14(3):731. doi: 10.3390/cancers14030731. Loss of MAGEC3 Expression Is Associated with Prognosis in Advanced Ovarian Cancers James Ellegate Jr 1, Michalis Mastri 1, Emily Isenhart 1, John J Krolewski 1, Gurkamal Chatta 2, Eric Kauffman 3, Melissa Moffitt 4, Kevin H Eng 1 5.

One publication is still under review (Combined BRCA2 and MAGEC3 expression predict outcome in Advanced Ovarian Cancers Emmanuel B. Omole1, Iqbal Aijaz1, James Ellegate Jr1, Emily Isenhart1,2, Mohamed M. Desouki3, Michalis Mastri1, Kristen Humphrey1, Emily M. Dougherty1, Spencer R. Rosario2, Kent L. Nastiuk1,4, Joyce E. Ohm1* and Kevin H. Eng1,2)

**Other achievements**
Nothing to report.

**What opportunities for training and professional development has the project provided?**
Nothing to report.

**How were the results disseminated to communities of interest?**
Nothing to report.

**What do you plan to do during the next reporting period to accomplish the goals?**
Nothing to report.

**4) Impact**

**What was the impact on the development of the principal disciplines of the project?**
We have determined that MAGEC3 silencing does not occur at the DNA level. This has refocused our studies on the regulation of protein MAGEC3.

We have preliminary evidence that MAGEC3 modulates DNA repair capabilities in multiple cell lines which introduces the potential for augmenting PARP inhibition therapy.

In a retrospective cohort study, we determined that, unlike other MAGE family members, the MAGEC3 protein is normally expressed in ovarian tissue but is lost in half of ovarian cancers. Similar to other predisposition genes like BRCA2, survival modeling suggests that expression

loss is associated with favorable progression-free survival and continued expression is associated with response to platinum therapy. Using transcriptomic modeling, we predicted that MAGEC3 expression is associated with stress related cell cycle stalling and DNA repair pathway expression.

We reported that MAGEC3 was difficult to measure by bulk RNA sequencing and subsequently developed an RNA-based linear model to predict MAGEC3 protein levels learned from labelled data. The accuracy of the predictor was evaluated in a validation set and subsequently applied to ovarian cancer patients within the TCGA pan-cancer dataset. Using the predicted scores, we were able to validate the results found for cases with directly measured MAGEC3 scoring. The ability to accurately predict protein levels based off an RNA predictor greatly increased the sample size and impact of our results.

**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?**
Nothing to report

**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**
Dr. Eng left Roswell Park Cancer Institute January 22,2022

**Changes that had a significant impact on expenditures**
Nothing to report.

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

Nothing to report.

**6) Products**

**Journal publications**
One article has been published:

Cancers (Basel) . 2022 Jan 30;14(3):731. doi: 10.3390/cancers14030731. Loss of MAGEC3 Expression Is Associated with Prognosis in Advanced Ovarian Cancers James Ellegate Jr 1, Michalis Mastri 1, Emily Isenhart 1, John J Krolewski 1, Gurkamal Chatta 2, Eric Kauffman 3, Melissa Moffitt 4, Kevin H Eng 1 5

And one publication is under review:

Combined BRCA2 and MAGEC3 expression predict outcome in Advanced Ovarian Cancers
Emmanuel B. Omole1, Iqbal Aijaz1, James Ellegate Jr1, Emily Isenhart1,2, Mohamed M. Desouki3, Michalis Mastri1, Kristen Humphrey1, Emily M. Dougherty1, Spencer R. Rosario2, Kent L. Nastiuk1,4, Joyce E. Ohm1* and Kevin H. Eng1,2

**Websites or other internet sites**
Nothing to report

**Technologies or techniques**
Nothing to report

**Inventions, patent applications and or licenses**
Nothing to report

**7) Participants and other collaborating organizations**

**What individuals worked on the project?**

Kevin Eng, PhD. PI, no change

Iqbal Aijaz, PhD. Postdoctoral Researcher. 8.4 Calendar Months.

- Dr. Aijaz is responsible for the design and execution of bench experiments and development of inducible cell lines.

John Krolewski, MD PhD. Co-investigator. 0.7 Calendar Months.

- Dr. Krolewski assisted in the development and execution of the IACUC protocol and the animal models.

**Has there been a change in the active other support of the PI or senior/key personnel since the last reporting period?**
Nothing to report

**What other organizations were involved as partners?**
Nothing to report

**8) Special reporting requirements**
Nothing to report

**9) Appendices**

Award Chart

# OC170368: Novel Tumor Suppressor Gene in Hereditary X-linked Ovarian Cancers

**PI:** Kevin Eng, Roswell Park, NY

**Budget:** $773,100

**Topic Area:** OCRP

**Mechanism:** Funding Opportunity

**Research Area(s):** Ovarian Cancer, Cancer genetics

**Award Status:** 15 May 2018-14 May 2021

## Study Goals:

To strengthen the evidence for or against heritable ovarian cancer X-linkage in an candidate locus and to conduct detailed functional studies of the candidate gene.

## Specific Aims:

Aim 1 is to determine whether MAGEC3 expression is silenced at the RNA or DNA level using deeper sequencing in familial ovarian cancers seen in women with an affected paternal grandmother from the FOCR, consistent with the TSG hypothesis.

Aim 2 is to test the hypothesis that restoration of MAGEC3 expression can suppress the tumor phenotype *in vitro*.

Aim 3 is to test the hypothesis that orthotopic mouse models can recapitulate the tumor suppressor effects of MAGEC3.

## Key Accomplishments and Outcomes:

**Publications: none to date**

**Patents: none to date**

**Funding Obtained: none to date**