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TITLE: Hereditary X-Linked Tumor Suppressor Escapes Immune Control in Prostate Cancer

PRINCIPAL INVESTIGATOR: Kevin Eng, PhD

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Health Research-Roswell Park
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**14. ABSTRACT**

In our recent work, we isolated an X-linked signal predisposing men to prostate cancers in 2,700 families ascertained for hereditary ovarian cancers. We sequenced and found a candidate variant on Xq27.2 in MAGEC3 suggesting that HPCX may connect prostate and ovarian cancers. Our preliminary experiments show that, in a tetracycline inducible system, heterologous MAGEC3 may be associated with an anti-proliferative phenotype consistent with a tumor suppressor. We hypothesize that MAGEC3 is a heritable tumor suppressor that is silenced in prostate cancers leading to disease progression. Aim 1 is to confirm the connection between prostate and ovary families by segregation and germline X chromosome sequencing in family studies and in men with a relevant family history.

**15. SUBJECT TERMS**

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

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
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## 1) Introduction

In our recent work, we isolated an X-linked signal predisposing men to prostate cancers in 2,700 families ascertained for hereditary ovarian cancers. We sequenced and found a candidate variant on Xq27.2 in MAGEC3 suggesting that HPCX may connect prostate and ovarian cancers. Our preliminary experiments show that, in a tetracycline inducible system, heterologous MAGEC3 may be associated with an anti-proliferative phenotype consistent with a tumor suppressor. We hypothesize that MAGEC3 is a heritable tumor suppressor that is silenced in prostate cancers leading to disease progression. Aim 1 is to confirm the connection between prostate and ovary families by segregation and germline X chromosome sequencing in family studies and in men with a relevant family history.

## 2) Keywords

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

## 3) Accomplishments

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

| Specific Aim 1: to confirm the connection between prostate and ovary families by segregation and sequencing | Timeline | Progress |
|---|---|---|
| Sub Task 1. Develop RNA signature for MAGE expression | Years 1-2 | |
| Cohort development and RNA acquisition from biobank. Expected: N=30 | Year 1 | Complete |
| Develop BDR for the specific award (BDR: TBD) | Year 1 | Complete |
| Obtain ORSP (Roswell Park) approvals. Forward to HRPO for review. | Year 1 | Partially complete |
| Sequencing at Roswell Park | Year 1 | Delayed |
| Protein staining at Roswell Park | Year 1 | Delayed |
| Evaluate protein MAGE levels and RNAseq of matching samples | Year 1 | Delayed |
| Develop signature and estimate prediction properties | Year 1 | Partially complete |
| Sub Task 2. Execute sequencing and protein detection | Year 2 | |
| Develop new cohort from Roswell Park/PCAP biobank. Selection for: available tissue, family history Expected N=60-100 (depending on block availability) | Year 2 | Partially Complete |
| Develop BDR for the specific award (BDR: TBD) Obtain ORSP approvals. Forward to HRPO for review. | Year 2 | Not Started |
| Estimate MAGE silencing by RNA model. | Year 2 | Not Started |
| Sub Task 3. Cohort analysis using public datasets | Year 1-2 | |
| Identify relevant public datasets | Year 1 | Complete |
| Estimate MAGE silencing by RNA model. | Year 2 | Not Started |
| Sub Task 4. Develop manuscripts for publication | Year 2 | |
| Meta-analysis and development for publication. | Year 2 | Not Started |

*ORSP: Office of Research Subjects Protections, Roswell Park; GSR: Genomics shared resource, Roswell Park.

**What was accomplished under these goals?**

**Accomplished under sub task 1.**

Using the TCGA RNAseq data, we subset to cases that were submitted from Roswell Park for which we should have easy access to tissue. Using this data, we developed an RNA signature consistent with MAGEC3 expression. It remains to validate further develop this signature when the biospecimen acquisition is complete.

We have submitted a Biospecimen Data Request (BDR) to Roswell Park Office of Research Subjects Protection. This request has been approved and the research has been deemed non-human subjects research. We are working to complete the BDR for sub task 2 which is also expected to be determined to be non-human subjects research. We will then submit the combined BDR and non-human subjects determination to HRPO before proceeding with acquiring tissue and completing this sub aim.

**Accomplished under sub task 2**

Because of delays due to COVID19, we advanced the timeline for the second validation cohort selection. In conjunction with our co-investigators, we have developed a case/control cohort of men with radical prostatectomies occurring at Roswell Park. This cohort is selected for tissue availability and the use of androgen deprivation therapy prior to resection.

**Accomplished under sub task 3**

We have mined through the Gene Expression Omnibus and TCGA data sets for relevant public RNAseq data and organized these data for analysis.

**Accomplished under sub task 4.**

Nothing to report.

**Other achievements**

This award led to collaborations that produced a prostate cancer research paper with Dr. Krolewski and Dr. Kent Nastiuk at Roswell Park.

Pan C, Jaiswal Agrawal N, Zulia Y, Singh S, Sha K, Mohler JL, Eng KH, Chakkalakal JV, Krolewski JJ, Nastiuk KL. Prostate tumor-derived GDF11 accelerates androgen deprivation therapy-induced sarcopenia. JCI Insight. 2020 Mar 26;5(6). doi: 10.1172/jci.insight.127018. PubMed PMID: 32078585; PubMed Central PMCID: PMC7213789.

**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?**

In the next reporting period, we will finish submitting BDR paperwork to HRPO. Following a confirmation from HRPO, we will acquire tissue and clinical information. Immunohistochemistry staining and image analysis will proceed and informatic analysis and modeling will be performed.

It is expected that the next reporting period will also involve the development of manuscripts for publication.

**4) Impact**

**What was the impact on the development of the principal disciplines of the project?**
Nothing to report

**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**
Due to COVID19, our institution shut down research operations in March 2020 interrupting the planned pilot *in vivo* studies and terminating a number of cell culture projects. We are permitted to maintain a limited staff on site: in September 2020, we are at presently 50% operational staffing. Our institution reopened formally under the New York State phased reopening plan Phase IV. Because of the reopening and a continuing hiring freeze, our staffing level remains at 50% and we are reassessing the state of our planned grant activities. Access to our shared resource facilities has been limited during the shutdown and there is a backlog of work delaying research.

**Changes that had a significant impact on expenditures**
We had significant delays in hiring research staff. Under our institutional COVID19 policy, we were forced to discontinue the use of the research apprentice title series and to implement a reduction in work force.

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

Nothing to report.

**6) Products**

**Journal publications**

Pan C, Jaiswal Agrawal N, Zulia Y, Singh S, Sha K, Mohler JL, Eng KH, Chakkalakal JV, Krolewski JJ, Nastiuk KL. Prostate tumor-derived GDF11 accelerates androgen deprivation therapy-induced sarcopenia. JCI Insight. 2020 Mar 26;5(6). doi: 10.1172/jci.insight.127018. PubMed PMID: 32078585; PubMed Central PMCID: PMC7213789.

**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, 1.5 Calendar Months

- Dr. Eng oversaw the project and managed cohort development and design; he managed data for the project.
-

Michalis Mastri, PhD. Affiliate Member. 5.25 Calendar Months.

- Dr. Mastri reviewed records, optimized MAGEC3 IHC staining and conducted informatics experiments. He executed the modeling work.

Gurkamal Chatta, MD. Co-investigator. 1.2 Calendar Months

- Dr. Chatta assisted with the development of the clinical and validation cohorts.

John Krolewski, MD PhD. Co-investigator. 0.6 Calendar Months.

- Dr. Krolewski assisted in the development of the clinical and validation cohorts.

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

Previously pending awards were activated during this period.

| | Level of support |
|---|---|
| DOD award W81XWH-19-1-0378 (PI: Krolewksi) | 8.0% |

| | |
|---|---|
| NIH award R01 CA247362-01A1 (PI: Knudsen) | 5.0% |
| NIH award R01 CA247771 (PI: Lovell/Abrams) | 1.9% |

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

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

**9) Appendices**

None