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

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13. SUPPLEMENTARY NOTES

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.

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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?

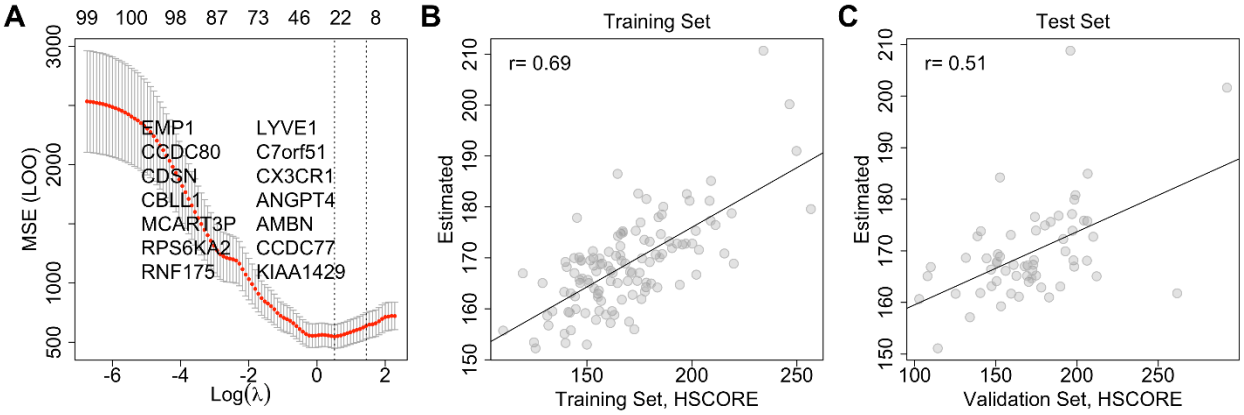
Specific Aim 1: to confirm the connection between prostate and ovary families by segregation and sequencing	Timeline	Progress
Sub Task 1. Develop RNA signature for MAGE expression	Years 1-2	
Cohort development and RNA acquisition from biobank. Expected: N=30	Year 1	Complete
Develop BDR for the specific award (BDR: 101818)	Year 1	Complete
Obtain ORSP (Roswell Park) approvals. Forward to HRPO for review.	Year 1	Complete
Sequencing at Roswell Park	Year 1	Complete
Protein staining at Roswell Park	Year 1	Complete
Evaluate protein MAGE levels and RNAseq of matching samples	Year 1	Complete
Develop signature and estimate prediction properties	Year 1	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	Complete
Develop BDR for the specific award (BDR: 101818) Obtain ORSP approvals. Forward to HRPO for review.	Year 2	Complete
Estimate MAGE silencing by RNA model.	Year 2	In Progress
Sub Task 3. Cohort analysis using public datasets	Year 1-2	
Identify relevant public datasets	Year 1	In Progress
Estimate MAGE silencing by RNA model.	Year 2	In progress
Sub Task 4. Develop manuscripts for publication	Year 2	
Meta-analysis and development for publication.	Year 2	In Progress

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

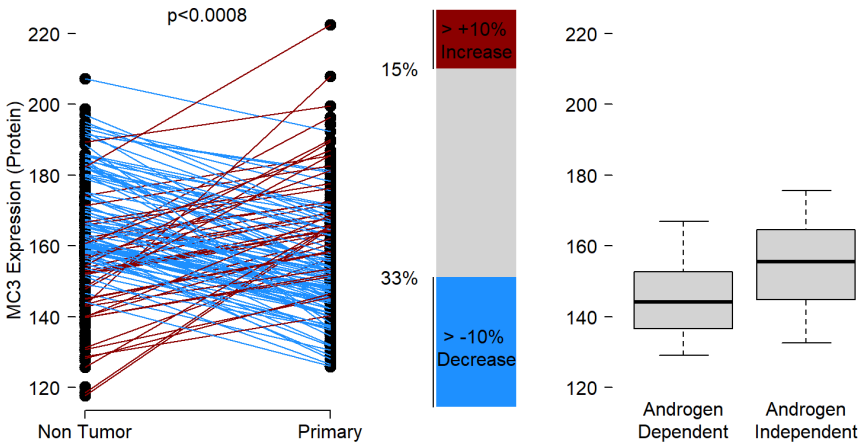
What was accomplished under these goals?

Accomplished under sub task 1.

We have completed the model fitting and validation of an elastic net (lasso) type regression model for predicting protein MAGEC3 levels. To build the model, we used N=180 cases contributed by Roswell Park to the TCGA project. We cored and arrayed on a tissue microarray matched to bulk RNA sequencing data from TCGA. One-third of the data (N=60) was withheld as a testing set and two-thirds (N=120) was used to train the model using leave out one (LOO) cross validation. The 1 standard error rule was used to select a tuning parameter close the minimizer of the LOO mean squared error but favoring a more parsimonious model. Features were pre-screened using a Sure Independence Screening procedure. The resulting fit had strong correlation (r=0.69) between true and estimated values of MAGEC3 protein in the training set and nearly equal correlation in the testing set (r=0.51).



Preliminary analysis of cases arrayed on tissue microarray slides stained in this task include N=263 paired non-tumor and primary samples. In these cases, MAGEC3 expression levels predominantly decrease (33% of tumors drop by over 10% of their non-tumor levels, paired t-test $p<0.0008$). Among tumors later shown to be androgen dependent and androgen independent based on response to androgen deprivation therapy, MAGEC3 levels rebound (two-sample t, $p=0.0003$) to their non-tumor levels ($p=0.08$).

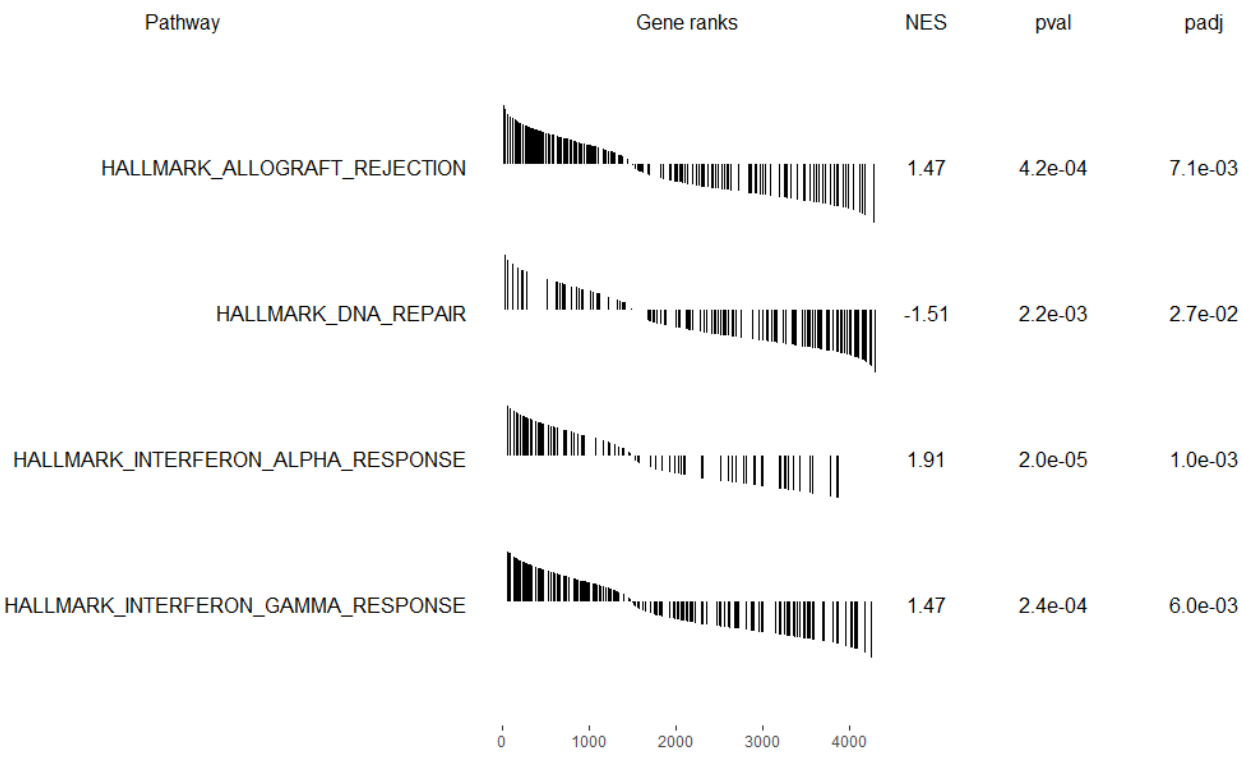


Arrays contained N=167 self-identified white men and N=112 self-identified black men. Protein levels of MAGEC3 were consistently higher in non-tumor (p=0.0003) and primary tumor (p-0.002) in black men. We previously described a population-level allele preference for the familial cancer associated MAGEC3 SNP. Among HAPMAP populations, European ancestry individuals are more likely to carry the A allele and Asian and African ancestry individuals are more likely to carry the G allele. We therefore conjectured that the expression of MAGEC3 in G allele carriers is likely to be higher than in A allele carriers.

Accomplished under sub task 2

We completed FFPE-based RNA acquisition and library preparation for N=43 (N=86 total) matched case/control samples from Roswell Park. Cases were defined as men who received ADT therapy prior to prostatectomy. Controls were case-matched (age, year of diagnosis) for no neo-adjuvant ADT prior to prostatectomy.

In non-ADT treated cases, the level of expression was comparable to tumor cases reviewed in sub task 1. In matched cases, predicted MAGEC3 levels were higher after ADT treatment (21.4%, paired t-test p=0.007) suggesting that MAGE expression is responsive to the castrate environment. Other MsigDB Hallmark pathways included a positive association with Allograft Rejection and the two interferon pathways; a negative association with DNA repair pathway.



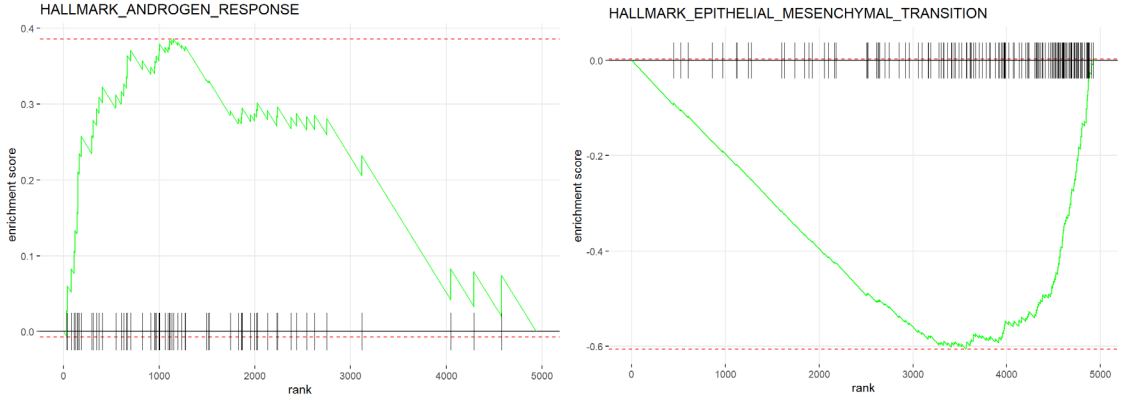
Accomplished under sub task 3

We continue to search for public datasets where we can apply the RNA-feature predictor for MAGEC3 protein to study prostate cancer biology. Current analyses show the predictor is robust to pre-processed TCGA data as well as Illumina whole transcriptome RNAsequencing data and FFPE capture kit derived libraries.

Biologic results suggest that MAGEC3 is responsive to ADT consistent with our findings in Sub Tasks 1 and 2.

TCGA Prostate Adenocarcinoma (PRAD)

- We observed positive correlation between MAGEC3 and Androgen response element containing genes TMPRSS2 (r=0.219, p<1e-6) and PSA(KLK3) (r=0.207, p<1.0e-6).



- GSEA analyses suggest that MAGEC3 expression is associated with the AR response pathway and anti-correlated with the EMT pathway.

GSE111177: Sharma NV, Pellegrini KL, Ouellet V, Giuste FO et al. Identification of the Transcription Factor Relationships Associated with Androgen Deprivation Therapy Response and Metastatic Progression in Prostate Cancer. Cancers (Basel) 2018 Oct 11;10(10). PMID: 30314329

- Data comprise whole transcriptome analysis of 20 patient-matched Pre-ADT biopsies and 20 Post-ADT prostatectomy specimens.
- Predicted MAGEC3 levels rise following ADT (+21.8%, t-test p=0.0295).
- We are performing differential correlation analyses to study whether MAGEC3 networks change in the context of castration. This is likely to be significant due the observation that TMPRSS2, PSA, and AR all appear to have weak correlation with MAGEC3 in untreated tumors but a strong negative correlation after ADT. This is consistent in the current data as MAGEC3 levels rise and the ARE containing genes' expression falls.
- Reconciling this observation with the TCGA/PRAD data is an important next step.

GSE126078: Labrecque MP, Coleman IM, Brown LG, True LD et al. Molecular profiling stratifies diverse phenotypes of treatment-refractory metastatic castration-resistant prostate cancer. J Clin Invest 2019 Jul 30;129(10):4492-4505. PMID: 31361600

- Data comprise RNA sequencing of human prostate tumor cell lines, patient-derived xenograft (PDX) models, and metastatic castration-resistant prostate cancer (mCRPC) tumors.
- MAGEC3 levels appear to be associated with the neuroendocrine positive/AR negative classification described in this paper. (Linear regression, score test p=0.0316)
- Among the cell line data, it is predicted that PC-3 cells will express a significantly lower level of MAGEC3 than other cell lines (PacMet AR-null and C4-2) (Linear regression, score test p=0.0001).
- Accounting for metastatic sites, the average level of CRPC metastatic tumors was 160.95 consistent with the androgen independent level observed in sub task 1.

GSE147250: Nyquist MD, Corella A, Coleman I, De Sarkar N et al. Combined TP53 and RB1 Loss Promotes Prostate Cancer Resistance to a Spectrum of Therapeutics and Confers Vulnerability to Replication Stress. Cell Rep 2020 May 26;31(8):107669. PMID: 32460015

- Data comprise RNA sequencing of human prostate tumor cell lines and metastatic castration-resistant prostate cancer (mCRPC) tumors. The mCRPC tumors are duplicated in GSE126078.
- MAGEC3 levels were positively correlated with PSA in cell lines (r=0.447, p=0.028) but anti-correlated in tissue samples (r=-0.264, p=0.0017) suggesting a cell-dependent effect may exist. TMPRSS2 correlations were similar but not significant.
- MAGEC3 effects were significantly lower in cells with functional p53 (-5.6 points, linear regression, score test p=0.0064). There was no effect due to treatment with enzalutamide. LnCap cells with a double knock out of Rb1 and p53 had slightly, but statistically significant elevated levels of MAGEC3 (+2.8 points, p=0.021).

Accomplished under sub task 4.

We documented the development of the RNA signature using a pan-cancer approach in

- Ellegate J, Mastri M, Isenhart E, Krolewski JJ, Chatta G, Kauffman E, Moffit M, Eng KH. MAGEC3 is a prognostic biomarker in ovarian and kidney cancers. medRxiv; 2021. DOI: 10.1101/2021.04.30.21256427.

A manuscript describing the results in this award and specifically in prostate cancers is under development.

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?

It is expected that the next reporting period will develop 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
Staffing and split shifts continue to be a challenge during COVID19. We have increased the use of asynchronous meetings and video calling to coordinate projects.

We are carefully monitoring backorders and supply chain for laboratory supplies needed to complete sequencing projects.

Changes that had a significant impact on expenditures
Nothing new to report in this period.

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

Nothing to report.

6) Products

Journal publications
Nothing to report

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

Praveen Kumar, PhD. Postdoctoral Fellow. 6.0 Calendar Months.
- Dr. Kumar validated antibodies and assisted with modeling and data analysis.

Michalis Mastri, PhD. Affiliate Member. 12.0 Calendar Months.
- Dr. Mastri conducted bioinformatics processing and analyses.

Emily Isenhart. Graduate Student 12.0 Calendar Months.
- Ms. Isenhart scored TMA slides and conducted image analysis.

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?

Nothing to report.

What other organizations were involved as partners?
Nothing to report

8) Special reporting requirements
Nothing to report

9) Appendices

None