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TITLE: Contribution of interstitial deletion of 21q22.2-3 *per se* to prostate cancer progression in tumors harboring TMPRSS2-ERG translocations

PRINCIPAL INVESTIGATOR: Yan Dong

CONTRACTING ORGANIZATION: Tulane University
New Orleans, LA 70112

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Yan Dong

E-Mail: ydong@tulane.edu

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Tulane University

6823 Saint Charles Ave
New Orleans, LA 70118-5665

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14. ABSTRACT

TMPRSS2-ERG gene fusions are present in close to 50% of human prostate cancers. Approximately half of the fusions are generated through an interstitial deletion between these two genes, and the other half through an interchromosomal insertion. The deletion class is in general more aggressive than the insertion class. The purpose of this study is to understand the mechanism underlying the aggressive phenotype of the deletion class of TMPRSS2-ERG-positive prostate cancers. We identified two potential tumor suppressor genes in this deleted region. The loss of these two genes may work together with TMPRSS2-ERG to drive prostate cancer progression. Understanding the function of the interstitial deletion may provide markers to distinguish aggressive from indolent disease and will facilitate rational design of therapeutic approaches to improve the treatment of this appreciable group of patients.

15. SUBJECT TERMS
TMPRSS2-ERG fusion; prostate cancer; tumor suppressor

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Table of Contents

Page

1. **Introduction……. 4**
2. **Keywords……. 4**
3. **Accomplishments……..……... 4**
4. **Impact……...…… 7**
5. **Changes/Problems...….…… 8**
6. **Products…….……..…..……. 9**
7. **Participants & Other Collaborating Organizations…… 9**
8. **Special Reporting Requirements…… 10**
9. **Appendices…… 11**

INTRODUCTION:

TMPRSS2-ERG gene fusions are present in close to 50% of human prostate cancers [1]. Approximately half of the fusions are generated through an interstitial deletion between these two genes (referred to as Edel), and the other half through an interchromosomal insertion [1]. The Edel subtype is in general more aggressive than the insertion subtype [1]. The purpose of this study is to understand the mechanism underlying the aggressive phenotype of the Edel tumors.

KEYWORDS:

TMPRSS2-ERG fusion; prostate cancer; tumor suppressor

ACCOMPLISHMENTS:

What were the major goals of the project?

Task 1. To determine the biological effects of knocking down individual interstitial genes, either alone or in concert with TMPRSS2-ERG fusion.

50% completed

Task 2. To delineate the biological effects of overexpressing individual interstitial genes in Edel subtype of fusion-positive prostate cancer cells, and to evaluate the involvement of the interstitial-gene RNA *per se* in the action.

40% completed

What was accomplished under these goals?

Task 1. To determine the biological effects of knocking down individual interstitial genes, either alone or in concert with TMPRSS2-ERG fusion.

The interstitial region between the TMPRSS2 and ERG genes encompasses 27 genes, including 16 coding, 9 long non-coding RNA (lncRNA), and 2 microRNA (miRNA) genes. We obtained the shRNA constructs for all the 16 coding genes and 4 lncRNAs (LINC00114, BRWD1-IT2, C21ORF88, and LINC00323) because of their commercial availability. To expedite the study, we first grouped these genes into 4 groups (5 genes/group) according to their proximity on the chromosome. Pooled shRNA vectors for each group of genes were transfected into VCaP cells, and the knockdown efficiencies were assessed by qRT-PCR analysis. Unfortunately, we could not achieve efficient knockdown of the genes with the pooled shRNA vectors. We therefore had to knock down the genes individually, and have successfully knocked down the expression of IGSF5, FAM3B, BACE2, and PCP4 genes (Fig. 1).

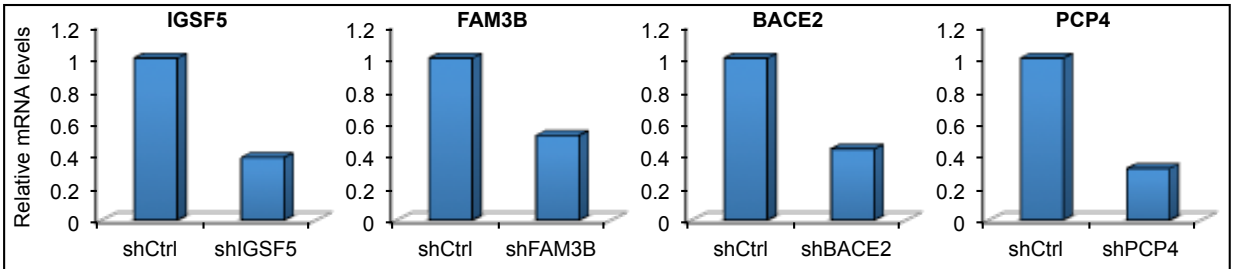

Fig. 1. Interstitial gene knockdown. VCaP cells were infected with lentivirus encoding either the control shRNA (shCtrl) or shRNA for the indicated gene. Knockdown efficiency was assessed by qRT-PCR analysis.

We then evaluated the effect of the knockdown on cell growth by the SRB assay. As shown in Fig. 2, both knockdown of IGSF5 and FAM3B enhanced the growth of VCaP cells, indicating the growth suppressive activity of IGSF5 and FAM3B. One critical property of cancer cells is their ability to form colonies. We assessed the ability of VCaP cells to form colonies after knockdown of IGSF5 or FAM3B. The knockdown cells showed an increased ability to form colonies compared with the control cells (Fig. 3). Together, our data indicated a tumor suppressive function of IGSF5 and FAM3B, loss of which in the Edel tumors may contribute to the aggressive phenotype of these tumors.

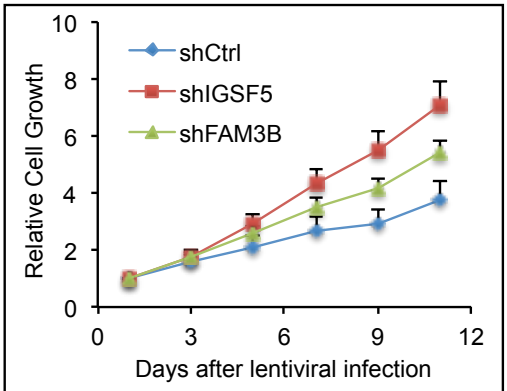

Fig. 2. Knockdown of IGSF5 and FAM3B genes stimulated the growth of VCaP cells. VCaP cells were infected with lentivirus encoding either the control shRNA (shCtrl) or shRNA for the indicated gene. Cell growth was assessed by the SRB assay.

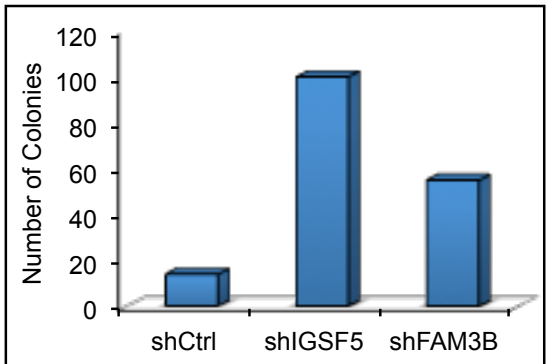

Fig. 3. Knockdown of IGSF5 and FAM3B genes increased the ability of VCaP cells to form colonies. VCaP cells infected with lentivirus encoding either the control shRNA (shCtrl) or shRNA for the indicated gene were subjected to colony formation assay.

We proceeded to use the transwell invasion assay to assess the effect of IGSF5 and FAM3B knockdown on cell invasion and migration. However, we have been having difficulty in getting the transwell invasion assay to work for VCaP cells. As shown in Fig. 4, LNCaP cells, but not VCaP cells, can migrate to the membrane in the lower chamber of the transwell and can also invade through the Matrigel. We are currently trying different experimental conditions to work out the transwell invasion assay for VCaP cells. We have also wanted to use the *in vitro* wound-healing assay to assess the effect of IGSF5 and FAM3B knockdown on cell migration. Unfortunately, the wound gap that we created had an uneven width. We are trying to use different forces to scratches the culture surface to resolve this issue.

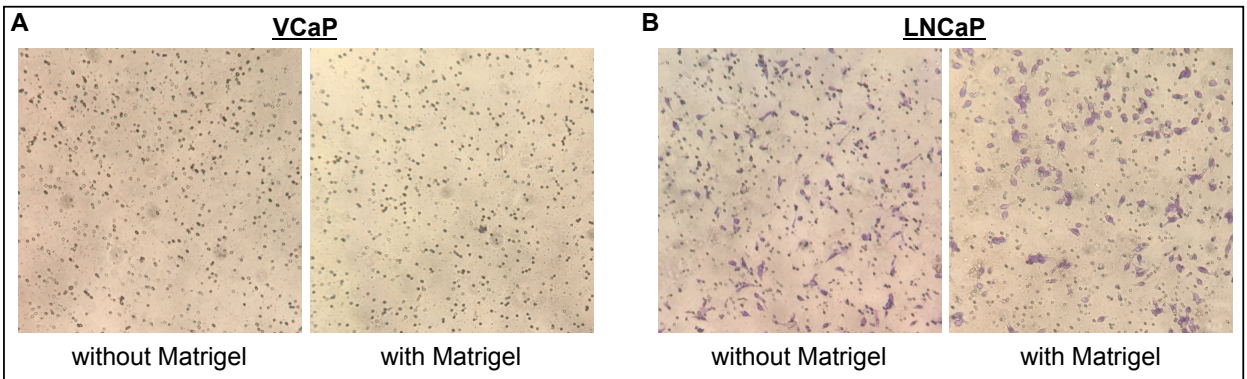

Fig. 4. Transwell invasion assay without Matrigel or with Matrigel to assess the migration and invasion abilities of VCaP (A) and LNCaP (B) cells, respectively. Migrated and invaded cells were stained purple with crystal violet, and the numerous small, round and dark colored dots are the pores of the membranes.

We are also in the process of knocking down the IGSF5 and FAM3B genes in the fusion-negative LAPC-4 cells to determine whether loss of these genes without cooperation with overexpressed ERG is sufficient to elicit a biological effect.

Task 2. To delineate the biological effects of overexpressing individual interstitial genes in Edel subtype of fusion-positive prostate cancer cells, and to evaluate the involvement of the interstitial-gene RNA *per se* in the action.

We purchased the expression constructs of IGSF5 and FAM3B, and subcloned their coding sequences into the pLVX lentiviral vector. We also PCR amplified the 3'-UTR regions of IGSF5 and FAM3B from a cDNA sample of the VCaP cells, cloned the PCR products separately into a TA vector, and subcloned the PCR products from the TA vector to the pLVX lentiviral construct (Fig. 5). Transient transfection of the cDNA and the 3'-UTR pLVX constructs into the 293T cells showed that the levels of the coding

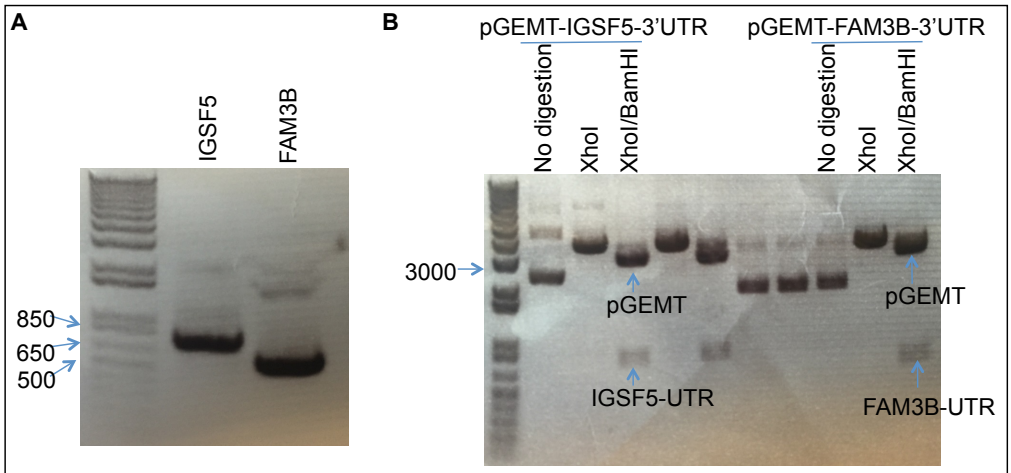

Fig. 5. Cloning of IGSF5 and FAM3B 3'-UTR lentiviral constructs. A, PCR amplified the 3'-UTR regions of IGSF5 and FAM3B from a cDNA sample of the VCaP cells. B, The PCR products were cloned separately into the pGEMT vector. The 3'-UTRs were digested out of the pGEMT constructs using XhoI and BamHI, and subcloned into the pLVX lentiviral construct.

regions were >100 fold of that in the control cells and the levels of the 3'-UTRs were >3 fold of that in the control cells.

We have obtained the NCI-H660 cells from ATCC, but the cells have not been growing well since arrival. We will communicate with ATCC to resolve this issue. Once resolved, we will infect NCI-H660 cells with the lentivirus encoding the coding or 3'-UTR of IGSF5 and FAM3B and assess the impact on cell growth, colony formation, migration, and invasion.

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.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The deletion class of TMPRSS2-ERG-positive prostate cancers is in general more aggressive than the insertion class. However, our understanding of how the deletion leads to the aggressive behavior of this class of prostate cancers is still limited. In the current study, we identified two potential tumor suppressor genes in this deleted region. The loss of these two genes may work together with TMPRSS2-ERG to drive prostate cancer progression. Understanding how gene deletions contribute to the aggressive behavior of the deletion class of TMPRSS2-ERG-positive prostate cancers may provide markers to identify prostate cancers that are better left untreated, thereby preventing overtreatment and improving the quality of life of these patients. In addition, a better understanding of the biology of this class of prostate cancers will facilitate rational design of therapeutic approaches to improve the treatment of this appreciable group of patients. Much effort has been devoted to the development of therapeutics against ERG because TMPRSS2-ERG fusions lead to high expression of the ERG oncogene. Our data indicate that ERG inhibitors as single agents may have a relatively low efficacy in patients with the deletion class of TMPRSS2-ERG-positive prostate cancers. By elucidating how these prostate cancers progress, our research may suggest which pathways could be additional relevant therapeutic targets.

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.

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:

The problems that we encountered are as follows.

1. We have been having difficulty in getting the transwell invasion assay to work for VCaP cells. We are currently trying different experimental conditions, e.g., changing the chemo-attractant, the digestion method to detach the cells, to work out the transwell invasion assay for VCaP cells.
2. The wound gap that we created in the *in vitro* wound-healing assay had an uneven width. We are trying to use different forces to scratches the culture surface to resolve this issue.
3. The NCI-H660 cells have not been growing well since arrival. We will communicate with ATCC to resolve this issue.

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.

PRODUCTS:

Publications, conference papers, and presentations:

Nothing to Report.

Website(s) or other Internet site(s):

Nothing to Report.

Technologies or techniques:

Nothing to Report.

Inventions, patent applications, and/or licenses:

Nothing to Report.

Other Products:

Lentiviral construct of IGSF5 coding region
Lentiviral construct of IGSF5 3'-UTR
Lentiviral construct of FAM3B coding region
Lentiviral construct of FAM3B 3'-UTR

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Yan Dong	Subing Cao
Project Role	PI	Postdoctoral Fellow
Researcher Identifier	N/A	N/A
Nearest Person month worked	0.6	12
Contribution to Project	Supervise Dr. Cao to conduct the experiments and prepare the results for progress report	Performed all the experiments
Funding Support	N/A	N/A

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

The PI has obtained the following supports:

NCI/NIH, 1 R01 CA188609-01 (role: PI) 09/01/14 – 08/31/19
Level (%) of effort: 25%
Title: An inevitable mechanism of resistance to androgen-directed therapy
The major goals of this project are to determine the mechanism by which androgen-directed therapies increase the expression of androgen receptor splice variants and the mechanism by which androgen receptor splice variants regulate target-gene expression.
Specific Aims:
Aim 1: Delineate the mechanism of AR-V induction following androgen-directed therapies.
Aim 2: Elucidate the role of dimerization in AR-V regulation of target-gene expression.
Aim 3: Correlate AR-V expression in circulating tumor cells with abiraterone/enzalutamide response.
Grant Management Specialist: Tracie Mcgraw
Email: mcgrawth@mail.nih.gov
(301) 631-3020 phone
301-451-5391 fax
No overlap

Department of Defense, W81XWH-15-1-0439 (role: PI) 09/01/15 – 08/31/18
Level (%) of effort: 10%
Title: A combination approach for treatment of castration-resistant prostate cancer
The major goal of this project is to investigate the preclinical efficacy of 20(S)-protopanaxadiol-aglycone in improving the therapeutic outcome of abiraterone, enzalutamide, and cabazitaxel.
Specific Aims:
Aim 1: Delineate the mechanism of AR-V upregulation by taxanes.
Aim 2: Elucidate the role of disrupting AR-FL and AR-V dimerization in mediating PPD downregulation of AR-FL and AR-Vs.
Aim 3: Establish the potential of using PPD, in comparison with the AR N-terminal-domain-targeting drug EPI-001, to improve the efficacies of abiraterone, enzalutamide, and cabazitaxel and to inhibit therapy resistance
Contracting Officer: JANET P KUHNS
US Army Medical Research Acquisition Activity
Phone: 301-619- 2827
Email: janet.p.kuhns.civ@mail.mil
No overlap

What other organizations were involved as partners?

Nothing to Report.

SPECIAL REPORTING REQUIREMENTS:

none

APPENDICES:

None

References:

1. Tomlins, S.A., et al., *ETS gene fusions in prostate cancer: from discovery to daily clinical practice.* Eur.Urol., 2009. **56**(2): p. 275-286.